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Uterine glands: biological roles in conceptus implantation, uterine receptivity, and decidualization

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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 uterine receptivity, blastocyst/conceptus survival and implantation, and stromal cell decidualization. Studies with the ovine uterine gland knockout (UGKO) model support a primary role for uterine glands and, by inference, their secretions present in uterine luminal fluid histrotroph for conceptus survival and development. In rodents, studies with mutant and progesterone-induced UGKO mice found that uterine glands and their secretions are unequivocally required for establishment of uterine receptivity and blastocyst implantation and also may influence blastocyst trophectoderm activation and stromal cell decidualization in the uterus. Similarly in humans, histotroph from uterine glands appears critical for blastocyst implantation, uterine receptivity, and conceptus nutrition during the first trimester and uterine glands likely have a role in stromal cell decidualization. An increased understanding of uterine gland biology is important for diagnosis, prevention and treatment of fertility problems, particularly infertility and recurrent pregnancy loss, in domestic animals and humans.

Keywords

uterus; glands; blastocyst; implantation; decidualization; pregnancy

Introduction

Although the organogenetic development and differentiation of most female reproductive tract organs are complete at birth, the uterus is not fully developed or differentiated at birth, and establishment of tissue-specific histoarchitecture is only completed postnatally in laboratory rodents, domestic animals, and humans (Bartol *et al*., 1999, Gray *et al*., 2001a). The process of postnatal radial patterning morphogenesis establishes the three classic histological elements of the uterine wall, including the: (1) endometrium; (2) myometrium, which consists of an inner circular layer and an outer longitudinal layer of oriented smooth muscle; and (3) perimetrium. Morphogenetic events common to postnatal morphogenesis of

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uteri include: (1) organization and stratification of endometrial stroma; (2) differentiation and growth of the myometrium; and (3) coordinated development of uterine glands. The glandular epithelium (GE) begins to develop as invaginations of luminal epithelium (LE) that progressively invade the stroma, ultimately resulting in an extensive network of epithelial glands that extend to the myometrium. Several recent reviews focus on the biology of uterine gland differentiation and development (Cooke *et al*., 2013, Spencer *et al*., 2012, Spencer *et al*., 2005, Zhang *et al*., 2013). The purpose here is to provide an overview of how uterine glands and histotroph have established or potential biological roles in conceptus survival and development, uterine receptivity, blastocyst implantation and stromal cell decidualization.

Uterine glands: a historical perspective

While empirical evidence that uterine glands are required to support pregnancy was provided only recently (Cooke *et al*., 2012, Filant and Spencer, 2013b, Gray *et al*., 2001c), the idea that uterine secretions are essential to the success of pregnancy developed over the last 500 years (see (Cooke *et al*., 2013)). Hippocrates (460-370 BC) and his students argued that the fetus was nourished *per os* by sucking on maternal cotyledons or ‘uterine paps’. For centuries thereafter it was envisioned that, as the uterus grew with pregnancy, pressure of the reproductive tract against the breasts would result in milk being pumped directly into uterine arteries. Aristotle (384-322 BC) argued against this notion on anatomical grounds; however, drawings by both Leonardo da Vinci (1452–1519 AD) and Andreas Vesalius (1514–1564 AD) still showed arteries connecting the breasts and reproductive tract. William Harvey (1578–1657 AD) was among the first to recognize conceptus nourishment by substances within the uterus (‘vicar of the breasts’), much as the neonate is nourished by milk. Walter Needham (1631–1691) refuted Hippocratic theory, arguing that the substance that could be squeezed from uterine tissues was distinct from lymph and important in fetal nutrition. Needham is credited with naming this substance ‘uterine milk’ (Amoroso, 1952, Needham, 1959). In the late 19th century, von Hoffman observed, relative to the human placenta, that “*fetal villi in the placenta do not float naked in the maternal blood, but are surrounded by cells whose function it is to secrete a special fluid serving for nutrition of the fetus, and called uterine milk”* (von Hoffman, 1884). According to Amoroso (Amoroso, 1952), the term embryotrophe, coined in the late 19th century, was used to describe all available material supplied to the conceptus *in utero*. In the early 20th century, the terms hemotroph and histotroph were coined to describe those substances essential for support of conceptus and fetal development supplied via the uterus either: (1) directly from blood (hemotroph); or (2) from the uterine endometrium (histotroph) (Bazer, 1975). Direct evidence of the essential nature of histotroph came with the demonstration in sheep and mice that uterine glands are unequivocally required for female fertility (Cooke *et al*., 2012, Filant and Spencer, 2013b, Gray *et al*., 2001c).

Uterine glands and early pregnancy in sheep

Ruminants (cattle, goats, and sheep) have a bicornuate uterus with a small common corpus and single cervix (Spencer *et al*., 2012). The endometrium in adult sheep and cattle has a large number of aglandular caruncular areas, which are dense stromal areas covered by a

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simple LE, and intercaruncular areas. The intercaruncular areas of the endometrium contain many hundreds of glands in a cross-section of the uterine wall. Caruncular areas are the sites of superficial implantation and placentation. In synepitheliochorial placentation found in ruminants, fusion 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.

Early pregnancy events

Establishment of pregnancy in domestic ruminants (i.e., sheep, cattle, goats) begins at the conceptus stage and includes pregnancy recognition signaling, implantation, and placentation (Spencer *et al*., 2004, Spencer *et al*., 2008). 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 trophectoderm. 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) (Guillomot, 1995, Hue *et al*., 2012). The ovoid conceptus of about 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 elongating conceptus begins the process of implantation and placentation. Conceptus elongation involves exponential increases in length and weight of the trophectoderm 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 (Bartol *et al*., 1999, Gray *et al*., 2001a, Spencer *et al*., 2012). Work involving rodents (Bigsby and Cunha, 1985, Ogasawara *et al*., 1983) and sheep (Bartol *et al*., 1988a, Bartol *et al*., 1988b) established that onset of adenogenesis was independent of the ovary or steroid hormones and progestins could suppress uterine epithelial cell proliferation. Since uterine tissues are steroid responsive perinatally, it was hypothesized that birth provides an endocrine cue for onset of uterine gland genesis by delivering uterine tissues from an anti-proliferative fetal endocrine environment. This hypothesis was tested by Frank “Skip” Bartol who exposed ewe lambs to norgestomet, a non-metabolizable and potent synthetic progestin, from birth to postnatal day 13, which inhibited uterine adenogenesis (Bartol *et al*., 1988a). Removal of the progestin block to adenogenesis on postnatal day 13 permitted glands to develop by postnatal day 26; however, these glands were underdeveloped and histologically abnormal. This original observation served as the foundation for the idea that prolonged exposure of neonatal ewes to progestins during adenogenesis could permanently inhibit uterine gland differentiation, thereby producing a uterine gland knockout (UGKO) phenotype in adults (Bartol *et al*., 1999). Subsequently, exposure of neonatal ewes to norgestomet from birth to 8 weeks of life was used to permanently ablate the postnatal differentiation of uterine glands, resulting in a UGKO

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phenotype in the adult (Gray *et al*., 2000a, Gray *et al*., 2001b, Gray *et al*., 2002). 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 (Gray *et al*., 2001b, Gray *et al*., 2000b). 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 (Gray *et al*., 2001b).

The infertility of UGKO sheep revealed an essential role for uterine glands and their secretions in conceptus survival and development during early pregnancy. Indeed, adult UGKO ewes were completely infertile and exhibited recurrent pregnancy loss (Gray *et al*., 2001b, Gray *et al*., 2002, Gray *et al*., 2001c). Morphologically normal blastocysts were present in UGKO ewes after mating on day 9, but conceptuses were absent or severely growth-retarded by day 12 or 14. Of note, the majority of natural pregnancy losses in domestic ruminants occur during the first two weeks of pregnancy (Diskin and Morris, 2008).

Glandular secretions and uterine function in sheep

Although blastocysts can develop entirely *in vitro*, the overall success of this process and quality of the blastocysts is markedly lower than for those developed *in vivo*, and they must be transferred into a receptive uterus for growth and development into an elongated, filamentous conceptus. The lack of conceptus elongation in UGKO ewes is presumably due to the absence of specific secretions that emanate from the glands of the uterus (Gray *et al*., 2001a, Gray *et al*., 2002). Histotroph in the uterine luminal fluid (ULF) of sheep and other ruminants is a complex and rather undefined mixture of ions, AA, carbohydrates, proteins, lipids, and other substances that are selectively transported into the uterine lumen by the endometrial epithelia from serum transudate as well as specific secretory products encoded by genes expressed in the LE and GE (see (Bazer, 1975, Gray *et al*., 2001a, Spencer *et al*., 2008)). 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, prostaglandins and cortisol) as well as the endometrium itself (prostaglandins, cortisol) (see (Bazer *et al*., 2010, Dorniak *et al*., 2013) for review). The genes and functions regulated by these hormones and factors in the endometrial epithelia trigger specific changes in the intrauterine histotrophic milieu that are necessary for conceptus elongation.

Given the recurrent pregnancy loss phenotype of the UGKO ewe, the secretions produced by the uterine glands are of primary import for conceptus elongation; however, knowledge of the GE transcriptome and secrotome remains incomplete. A mass spectrometry experiment found that a large number of proteins are present in the ULF of day 16 cyclic and early pregnant sheep (Koch *et al*., 2010). The uterine glands of early pregnant sheep express genes that encode for secreted factors (CTGF, GRP, WNT11), AA 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

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protease inhibitors (CST3, CST6), and an immunomodulatory factor (SERPINA14; also known as uterine milk protein or uterine serpin). As in mice and humans, some of those genes are also expressed in the LE. Those genes alter the intrauterine milieu or histotroph, such as increasing select AA, glucose, cytokines and growth factors, to support blastocyst survival and growth into an ovoid conceptus and elongation to form a filamentous conceptus in sheep and cattle (see (Bazer *et al*., 2010, Dorniak *et al*., 2013, Forde and Lonergan, 2012, Spencer *et al*., 2008)).

In sheep, the uterine glands undergo a program of hyperplasia followed by hypertrophy that appears to be dependent on temporal and spatial actions of hormones from the ovary (progesterone) and placenta (placental lactogen and growth hormone) (Bazer *et al*., 2010, Gray *et al*., 2001a). Uterine gland morphogenesis during pregnancy allows for the endometrium to increase output of secretory proteins that are transported to the fetus by specialized areas of the placenta termed areolae, which provide histotrophic nutrition to the fetus throughout pregnancy. Similarities exist among other domestic animals (cattle, goats, pigs) as well as humans during the first trimester of pregnancy (Burton *et al*., 2011).

Uterine glands and pregnancy in mice

Laboratory rodents (mouse and rat) have a long duplex uterus with a dual cervix (Spencer *et al*., 2012). 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 or branched as found in the uterus of humans and domestic animals.

Early pregnancy events in mice

In rodents, blastocyst implantation involves trophectoderm apposition, attachment, adhesion to the LE followed by penetration and growth of the trophectoderm into the decidualizing stroma (see (Cha *et al*., 2012, Ramathal *et al*., 2010, Wang and Dey, 2006, Wetendorf and DeMayo, 2012, Zhang *et al*., 2013)). This complex process requires dialogue between an implantation-competent blastocyst and a receptive uterus. Dynamic changes in ovarian estrogen and progesterone secretion regulate endometrial function and blastocyst implantation. 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 that occurs at midnight on day 3.5. Recent evidence suggests that there are two separate uterine signals regulating blastocyst 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 (Gonzalez *et al*., 2012). By day 5.5, the LE cells lining the implantation chamber and near the blastocyst undergo apoptosis allowing the motile trophectoderm to come into contact with stromal cells that are differentiating into decidual cells in a process that involves endoreduplication. Decidualization is required for successful pregnancy and important for implantation of the blastocyst and formation of a functional placenta.

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Mouse gene knockouts affecting uterine adenogenesis or gland function

Uterine glands have been known to be essential for blastocyst implantation in rodents for the past 20 years. In the early 1990s, the work of Colin Stewart established that leukemia inhibitory factor (LIF) is expressed specifically by the glands of the mouse uterus in response to the nidatory surge of estrogen from the ovary (Bhatt *et al*., 1991). Subsequently, LIF was found to be essential for blastocyst implantation in mice, as *Lif* null mice were infertile due to a failure of blastocyst implantation (Stewart *et al*., 1992). Recent studies found that 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, wingless-related MMTV integration site 4* or *Wnt4; and Wnt7a*] lack glands in the adult uterus (Dunlap *et al*., 2011, Franco *et al*., 2011, Franco *et al*., 2010, Jeong *et al*., 2010, Jeong *et al*., 2009, Shelton *et al*., 2012). Many of those mice exhibit defects in blastocyst implantation and uterine decidualization and thus fertility, which can be attributed to the absence of LIF and other uterine gland-derived factors.

Progesterone effects on uterine epithelial proliferation: a tool to regulate adenogenesis and create uterine gland knockout (PUGKO) mice

The uterine epithelium in mice, rats, dogs and other species proliferates rapidly during the neonatal period (Bigsby and Cunha, 1985; Cooke *et al*., 2012b). In mice, uterine epithelial proliferation in the neonate is almost completely blocked by progesterone (Bigsby and Cunha, 1985), just as progesterone abolishes epithelial proliferation induced by estrogen in ovariectomized mice (Martin and Finn, 1971). Proliferation of the uterine epithelium is important for gland development, such as budding differentiation of GE from the LE and growth into the stroma of nascent uterine glands. In the neonate, the onset of adenogenesis was found to be ovary-, adrenal-and steroid-independent, and both progestins and glucocorticoids could suppress uterine epithelial proliferation in rodents (Bigsby and Cunha, 1985, Ogasawara *et al*., 1983). Subsequently, Paul Cooke found that progesterone treatment could be used to inhibit uterine adenogenesis in neonatal mice, resulting in an UGKO phenotype in the adult (Cooke *et al*., 2012). Treatment of C57BL/6 mice with progesterone from postnatal days 2 to 10 altered expression of morphoregulatory genes, including upregulation of *indian hedgehog* (*Ihh*), and inhibited epithelial cell proliferation, leading to a disruption of uterine gland development in the neonate (Cooke *et al*., 2012, Filant *et al*., 2012). The resulting adult UGKO mice cycled normally but were infertile.

Next, progesterone-induced UGKO (PUGKO) mice were used to investigate the biological role of uterine glands in blastocyst implantation and stromal cell decidualization (Filant and Spencer, 2013b). 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 expression of steroid hormone receptors and their regulated genes were not different. The defect in blastocyst implantation in PUGKO mice is likely due to inadequate uterine receptivity and/or blastocyst activation (trophectoderm attachment and motility).

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Uterine gland secretions in mice

Available evidence supports the hypothesis that uterine glands and, by inference, their paracrine-acting secretions have important biological roles in blastocyst implantation, establishment of uterine receptivity, and stromal cell decidualization. Available data indicate that the ULF contains AA, ions, carbohydrates (glucose, lactate, pyruvate), lipids, and proteins (cytokines, enzymes, hormones, growth factors, proteases and their inhibitors, transporters, etc.). Carbohydrates and 19 AA were measured in ULF of mice at estrus (Harris *et al*., 2005), but no data is available for nutrients in the ULF of the peri-implantation mouse uterus. In fact, LIF has never been documented as present in the ULF of 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 that they act differentially and synergistically to establish uterine receptivity and govern trophectoderm attachment and motility for blastocyst implantation and influence stromal cell decidualization (Cha *et al*., 2012, Evans *et al*., 2012, Filant *et al*., 2013, Filant and Spencer, 2013a, Niklaus and Pollard, 2006, Wang and Dey, 2006, Wetendorf and DeMayo, 2012, Zhang *et al*., 2013). The LE has genes that are solely expressed at peri-implantation stages (*Areg, Calb1, Hdc, Hegf1, Irg1, Ptgs2*) as well as the GE (*Calca, Lif, Il6st*) (Tables 1 and 2). Both epithelial cell types can uniquely express certain genes, while other genes appear to be coordinately expressed (*Cdh1, Ihh, Klf5, Msx1/Msx2, Ptgs1*) in both LE and GE (Table 2). Further, genes are also down regulated in either or both epithelial cell types during the receptive window such as *Muc1* and *Pgr*. In adult rodents, blastocyst implantation defects arise from the loss of genes expressed specifically in the GE [LIF and calcitonin/calcitonin-related polypeptide, alpha (CALCA) (Stewart *et al*., 1992, Zhu *et al*., 1998)] as well as those in the LE and GE (i.e., *Ihh, Klf5, Msx1/Msx2*) (Daikoku *et al*., 2011, Lee *et al*., 2006, Wang and Dey, 2006) and LE and stroma (i.e., *Ptgs2*) (Lim *et al*., 1999, Lim *et al*., 1997). Of particular note, very few studies have evaluated the substances present in the ULF of mice (Daikoku *et al*., 2005, Gonzalez *et al*., 2012, Harris *et al*., 2005). Given the cellular complexity of the uterus, analysis of the entire uterine transcriptome is not entirely advantageous given the preponderance of stroma and myometrium relative to the endometrial epithelia. Niklaus and Pollard (Niklaus and Pollard, 2006) and Filant and coworkers (Filant *et al*., 2013, Filant and Spencer, 2013a) have used laser-capture microdissection (LCM) and microarray technology to define the epithelial transcriptome of the peri-implantation mouse uterus. In those studies, LE-expressed genes were enriched for metabolic processes and steroid biosynthesis, whereas retinoid signaling, tight junction, extracellular matrix, and regulation of kinase activity were enriched in the GE. The transcriptome differences in the epithelia support the idea that each cell type has unique genetic signatures that dictate their differential and synergistic function in the uterus. The candidate genes and regulatory networks identified in those studies provide a framework to discover new mechanisms regulating epithelial function, uterine receptivity and blastocyst implantation in early pregnancy (Filant *et al*., 2013, Filant and Spencer, 2013a, Niklaus and Pollard, 2006).

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Biological role of uterine glands and their secretions in implantation and decidualization in mice

Blastocyst activation

While progress has been made in understanding the regulation of uterine receptivity, the process of blastocyst activation itself remains poorly understood (Gonzalez *et al*., 2012). Previous work indicates that AA play an important role in blastocyst activation (see (Martin *et al*., 2003) for review). 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 trophectoderm motility through activation of mammalian target of rapamycin (mTOR)-dependent signal transduction cascades only after the embryo has reached the early blastocyst stage (Martin *et al*., 2003). A short pulse (4–8 h) of AA exposure is sufficient to induce motility in the trophectoderm of the early blastocyst, leading to trophoblast outgrowth in an *in vitro* model of *in vivo* implantation behavior. The requirement for AA at this stage acts as a developmental checkpoint; when cultured *in vitro* without AA supplementation, blastocysts remain in a quiescent state, from which trophoblast motility can be induced at any time by adding AA to the culture medium. Once induced, the effect of AA is not reversible, as removing AA will not then switch embryos back to a quiescent state. A recent study found that leucine and arginine, and in particular, uptake of leucine through the SLC6A14 AA transporter, are each required individually and together are sufficient to induce blastocyst activation (Gonzalez *et al*., 2012). Blastocyst activation *in vivo* is accompanied by changes in mTOR localization, and these changes occur on day 4.5 of gestation, after the onset of embryo attachment, suggesting that there are two separate signals from the uterus regulating the phases of implantation. Although little is known about the actual composition of mouse ULF in terms of nutrients, recent transcriptional profiling experiments indicate that the uterine LE and GE of the peri-implantation mouse uterus differentially express a number of transporters for AA and glucose (Filant *et al*., 2013, Filant and Spencer, 2013a).

Blastocyst implantation and stromal cell decidualization

In both humans and rodents, uterine glands and their secretions have been hypothesized to play important roles in blastocyst implantation and uterine stromal cell decidualization in mice and humans (Burton *et al*., 2007, Burton *et al*., 2011, Cha *et al*., 2012, Hannan *et al*., 2010, Salamonsen *et al*., 2007, Zhang *et al*., 2013). Results with the aglandular PUGKO mouse model, along with those from conditional *Foxa2* mutant mice that have much reduced uterine glands (Jeong *et al*., 2010) and *Lif* null mice (Stewart *et al*., 1992), strongly support that hypothesis.

Blastocyst implantation—Many factors and pathways necessary for implantation have been identified in mice (see (Cha *et al*., 2012, Ramathal *et al*., 2010, Wang and Dey, 2006, Wetendorf and DeMayo, 2012, Zhang *et al*., 2013)). Of particular note, *Lif* null mice exhibit defects in blastocyst implantation (Stewart *et al*., 1992). In mice, LIF is induced in the uterine glands in response to the surge of nidatory estrogen on the morning of implantation (Bhatt *et al*., 1991, Chen *et al*., 2000) and is also expressed in the subluminal stroma at the

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implantation site (Song *et al*., 2000). It is critical for blastocyst implantation (Bhatt *et al*., 1991, Stewart *et al*., 1992) and has pleiotropic effects on both uterine epithelium and stroma (Chen *et al*., 2000). The absence of uterine glands and their essential secreted factors, including LIF and other factors, is hypothesized to underlie the blastocyst implantation failure observed in the uteri of PUGKO mice. Although LIF has a well-recognized role to establish receptivity of the LE for implantation, the role of many other genes expressed in the uterine glands has not been established (Tables 1 and 2). Recent transcriptional profiling experiments indicate that the glands of the day 2.5 and 3.5 pseudopregnant mouse uterus express many genes that encode for enzymes, transporters or secreted proteins that could regulate blastocyst implantation by modifying the ULF (Filant *et al*., 2013, Filant and Spencer, 2013a).

Uterine decidualization—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 (Filant and Spencer, 2013b). Thus, an artificial model of decidualization was used that involves ovariectomy, hormone replacement and intrauterine administration of oil as a deciduogenic stimulus (Filant and Spencer, 2013b). Using that artificial model, the PUGKO mice exhibited a distinct lack of uterine stromal cell decidualization. Interestingly, LIF can substitute for nidatory estrogen at inducing both implantation and decidualization in hormonally prepared, ovariectomized bred mice that that have glands in their uterus (Chen *et al*., 2000). However, intrauterine LIF failed to rescue decidualization in ovariectomized PUGKO mice that lack uterine glands (Filant and Spencer, 2013b). Similarly, mice that have a conditional ablation of *Foxa2* have a uterus with much reduced numbers of glands and exhibit severe defects in blastocyst implantation as well as stromal cell decidualization using an artificial model (Jeong *et al*., 2010). Similarly, the uterus of conditional *Wnt4* ablated mice also lack uterine glands and have a severely impaired artificial decidual response (Franco *et al*., 2011). However, 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 expressed only in the GE of the mouse uterus, SPINK3 protein was detected in the LE and decidual cells as well as uterine glands (Chen *et al*., 2010). 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 (Lejeune *et al*., 1981). 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 optimal uterine decidualization.

In women, the LE and GE maximally express LIF during the mid-secretory phase of the menstrual cycle, the period during which the uterus is receptive to an implanting blastocyst (Paiva *et al*., 2009). A number of studies have found that locally and temporally produced products of the endometrium, such as cytokines and growth factors [interleukin-11 (IL11), relaxin, prostaglandin E2 (PGE2), activin A, corticotrophin-releasing hormone (CRH), and

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LIF], enhance *in vitro* progesterone-induced decidualization of endometrial stromal cells sourced from humans and/or mice (Dimitriadis *et al*., 2005, Shuya *et al*., 2011). Thus, paracrine factors from the endometrium and/or embryo enhance decidualization induced in endometrial stromal cells by ovarian progesterone (Bany and Cross, 2006, Cha *et al*., 2012, Dimitriadis *et al*., 2005, Kashiwagi *et al*., 2007). Indeed, recent transcriptional profiling experiments indicate that the glands of the day 2.5 and 3.5 pseudopregnant mouse uterus express many genes that encode for enzymes, transporters or secreted proteins that could regulate stromal cell decidualization in a paracrine manner (Filant *et al*., 2013, Filant and Spencer, 2013a).

Uterine glands and pregnancy in humans

Humans have a simplex uterus consisting of a single uterine body (Spencer *et al*., 2012). The endometrium is lined by a simple LE and contains 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 glands surrounded by loose stroma, and the lower stratum basalis consisting of branched coiled glands and dense stroma. The endometrial functionalis is lost during menses. Histologically, the basalis includes a zone that contains loose stroma and bodies of endometrial glands, and another zone where endometrial glands terminate and endometrial progenitor and stem cells reside. The endometrial 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 menstruating primates including women, providing stem cells from which the functionalis regenerates after each cycle or following gestation. Although initiated fetally, human uterine gland proliferation is completed postnatally, similar to domestic ungulates, and involves differentiation of GE from LE followed by radial development of coiled, tubular glands through endometrial stroma. This pattern of endometrial development is distinct from gland genesis in uteri of adult women and primates, where endometrial glands develop adluminally from the basalis during the proliferative phase after menses and possibly from the stroma.

The human conceptus undergoes interstitial implantation, beginning around day 7 post-fertilization and being complete by days 10 to 12 (see (Burton *et al*., 2011, Red-Horse *et al*., 2004, Wooding and Burton, 2008)). When the trophectoderm adheres to the uterine surface it undergoes transformation into syncytiotrophoblast, projections of which penetrate between the uterine epithelial cells into the underlying stroma. By a combination of invasion and most likely proliferation of the surrounding stromal cells, the conceptus becomes encapsulated within the superficial endometrium, with the uterine epithelium regrowing over the implantation site. The endometrium has a high density of uterine glands with approximately 15 openings per mm^2 of uterine surface in the non-pregnant state. As the mantle of syncytiotrophoblast enlarges, it encircles and erodes into the necks of the glands. Consequently, connections between the lumens of glands and the developing intervillous space of the placenta can be observed as early as day 17 post-fertilization (Hamilton and Gladstone, 1942), and those connections persist throughout the first trimester (Burton *et al*., 2002).

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Uterine gland secretions in humans

Uterine gland secretions include AA, ions, carbohydrates (glucose), lipids, proteins (cytokines, enzymes, hormones, growth factors, proteases and their inhibitors, transporters, etc.) and likely other substances in the human uterus (Hannan *et al*., 2010, Hempstock *et al*., 2004, Kane *et al*., 1997, Salamonsen *et al*., 2007, Vilella *et al*., 2013). They are presumed to be important mediators of uterine receptivity, blastocyst implantation (trophoblast attachment, growth, and invasion), stromal cell decidualization and conceptus growth in humans (Burton *et al*., 2007, Burton *et al*., 2011, Cha *et al*., 2012, Salamonsen *et al*., 2007). Deficient glandular activity, usually described as a “secretory phase defect”, is hypothesized to be an underlying cause of early pregnancy failure in humans (Burton *et al*., 2007, Dimitriadis *et al*., 2006), and recent proteomic studies using mass spectrometry found that proteins in the ULF of fertile women change during the menstrual cycle and are altered in ULF from fertile as compared to infertile women (Boomsma *et al*., 2009, Hannan *et al*., 2011, Hannan *et al*., 2010). In women, decidualization is initiated during the mid-late secretory phase by progesterone, independent of the presence of an implanting blastocyst, however the decidua of pregnancy is only formed following blastocyst implantation. Thus, paracrine crosstalk between the GE and stroma may be needed to establish a receptive endometrium and stromal cell decidualization that is important for blastocyst implantation in humans (Koot *et al*., 2012).

Uterine gland secretions and conceptus nutrition during the first trimester

In addition to a primary role in blastocyst implantation and perhaps stromal cell decidualization, evidence over the past 10 years provides support for the idea that uterine glands represent an important source of nutrients for the conceptus during the first trimester (Burton *et al*., 2007, Burton *et al*., 2011, Hempstock *et al*., 2004). 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 in all species, prior to the establishment of the placenta (Wooding and Burton, 2008). 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 (Burton *et al*., 2002). These accumulations are greatest at the materno-fetal interface, suggesting a concentration dependent uptake by the trophoblast. In addition, during the first trimester the syncytiotrophoblast was shown to phagocytize maternal glycoproteins, including glycodelin A (Burton *et al*., 2002). Since the glycodelin A mRNA is not detected in placental tissues (Seppala *et al*., 1992), the presence of vesicles immunoreactive for the glycoprotein within the syncytiotrophoblast confirms uptake.

The importance of the glands during the first trimester has only really been appreciated since it has been recognized that the maternal arterial circulation to the placenta is not fully established until 10 to 12 weeks of pregnancy (Burton *et al*., 2011, Jauniaux *et al*., 2003). Although the invading syncytiotrophoblast erodes into the sub-mucosal capillary network, the spiral arteries do not penetrate into the superficial third of the endometrium. Thus, the conceptus has to enlarge considerably before it makes contact with their tips. When the conceptus does break into the arteries, large quantities of endovascular trophoblast migrate down the lumens of the vessels and act as plugs and prevent any blood flow (Burton *et al*.,

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1999). Normally, the plugs dissipate towards the end of the first trimester, and it is only then that moving echoes indicative of significant flow can be detected within the intervillous space on ultrasound (Jauniaux *et al*., 2000). Prior to this time, the intervillous space is filled with a clear fluid that is most likely derived from secretions of the uterine glands. Consequently, the relationship is equivalent to that in the horse or the sheep, except that uterine gland secretions are intraplacental rather than into the uterine luminal fluid (Burton *et al*., 2011).

Conclusion

Uterine glands and their secretions have important biological roles in mammalian pregnancy. Some of the cellular events and molecular pathways involved in uterine gland development and function have been identified through gene expression studies and genetically engineered mouse models. However, a significant gap in our knowledge of how the glands function to support early pregnancy events remains. A comprehensive understanding of their biological roles in blastocyst/conceptus growth and implantation, uterine receptivity, and uterine decidualization is needed to generate new knowledge essential for understanding implantation failure and recurrent pregnancy loss and to improve pregnancy rates in women and domestic animals.

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Abbreviations used in this paper

AA	amino acids
GE	glandular epithelium
LE	luminal epithelium
UGKO	uterine gland knockout
ULF	uterine luminal fluid

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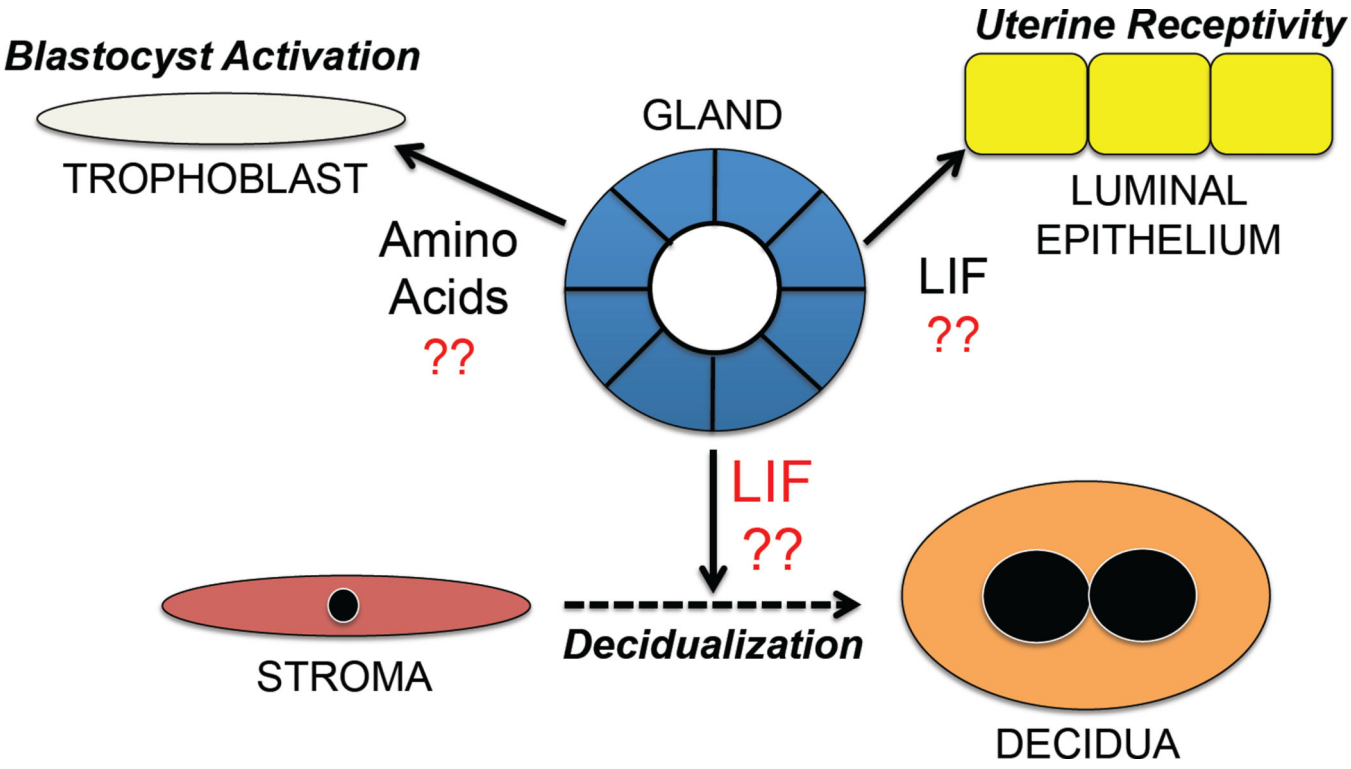

Fig. 1. Hypothesis on the biological roles of uterine glands in blastocyst activation, uterine receptivity to blastocyst implantation, and stromal cell decidualization during pregnancy in mice

See text for detailed description of hypotheses and supporting data.

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Table 1

Genes expressed in the glandular epithelia of the mouse uterus

Symbol	Name	Expression[1]	Null/Conditional Phenotype[2]	Reference
Cxcl15	chemokine (C-X-C motif) ligand 15	GE, LE	Viable and fertile	(Chen *et al*., 2001, Schmitz *et al*., 2007)
Fnbp1	formin binding protein 1	GE (GD4-5)	N/A	(Niklaus and Pollard, 2006)
Foxa2	forkhead box A2	GE (neonatal and adult)	Embryonic lethal, Implantation defect (conditional)	(Ang and Rossant, 1994, Jeong *et al*., 2010)
Gulo	gulonolactone (L-) oxidase	GE>>LE (GD4-5)	Viable and fertile	(Maeda *et al*., 2000, Niklaus and Pollard, 2006)
Il6st	interleukin 6 signal transducer	GE (GD3-5) Decidua (GD7)	Viable and fertile	(Ni *et al*., 2002, Yoshida *et al*., 1996)
Lif	leukemia inhibitory factor	GE>>Stroma (GD4)	Implantation defect	(Stewart *et al*., 1992)
Lyz2	lysozyme 2	Stroma, GE (GD3-5)	Viable and fertile (knock-in)	(Clausen *et al*., 1999, Niklaus and Pollard, 2006)
Prss28	protease, serine, 28	GE (GD5-8)	N/A	(O'Sullivan *et al*., 2002)
Prss29	protease, serine, 29	GE (GD5-9)	N/A	(O'Sullivan *et al*., 2001)
Sh3tc2	SH3 domain and tetratricopeptide repeats 2	GE (GD4-5)	Viable and fertile	(Arnaud *et al*., 2009, Niklaus and Pollard, 2006)
Slc23a2	solute carrier family 23 (nucleobase transporters), member 2	GE	Postnatal lethal	(Niklaus and Pollard, 2006, Sotiriou *et al*., 2002)
Spink3	serine peptidase inhibitor, Kazal type 3	GE (onset GD4)	Postnatal lethal	(Chen *et al*., 2010, Ohmuraya *et al*., 2005)
Sult1d1	sulfotransferase family 1D, member 1	GE>>LE (GD3-4)	N/A	(Niklaus and Pollard, 2006)
Ttr	transthyretin	GE only (peaks on GD4)	Viable and fertile	(Diao *et al*., 2010, Episkopou *et al*., 1993)

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

[2] N/A, not available

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Table 2

Genes expressed in the luminal (LE) and glandular epithelia (GE) of the mouse uterus

Symbol	Name	Expression[1]	Null/Conditional Phenotype	Reference
Cdh1	E-cadherin	LE and GE (GD1-4), stroma (GD5-8)	Embryonic lethal, Implantation defect (conditional)	(Paria *et al*., 1999, Reardon *et al*., 2012)
Clca3	chloride channel calcium activated 3	LE and GE (peaks on GD1)	Viable and fertile	(Jeong *et al*., 2006, Robichaud *et al*., 2005)
Ihh	Indian Hedgehog	LE and GE (peaks on GD3-4)	Embryonic lethal, Implantation defect (conditional)	(Lee *et al*., 2006)
Klf5	Kruppel-like factor 5	LE and GE (GD1-5) Decidua (GD5-8)	Embryonic lethal, Implantation defect (conditional)	(Sun *et al*., 2012)
Ltf	lactotransferrin	LE and GE (GD1-2)	Viable and fertile	(McMaster *et al*., 1992, Ward *et al*., 2003)
Msx1	homeobox, msh-like 1	LE and GE (peaks on GD4 and declines)	Embryonic lethal, Subfertile (conditional)	Daikoku 2011
Msx2	homeobox, msh-like 2	LE and GE (peaks on GD4)	Viable and fertile, Infertile (double conditional)	(Daikoku *et al*., 2011, Satokata *et al*., 2000)
Ptgs1	Prostaglandin-endoperoxide synthase 1	LE and GE (peaks on GD4)	Viable, delayed parturition	(Chakraborty *et al*., 1996, Gross *et al*., 1998)
Tro	Trophinin	LE and GE (peaks between GD4 and 6)	Viable and fertile	(Suzuki *et al*., 2000)

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