Commentary

The Glands Have It¹

Ann E. Sutherland²

Department of Cell Biology, University of Virginia, Charlottesville, Virginia

For all mammals, the initial stages of development occur within the uterus, which is specialized in many ways to nourish and protect the embryo and fetus. At the heart of uterine function is the glandular epithelium (GE); it not only provides a nutrient-rich histotroph to support embryonic development until the placenta is functional [1], but it also regulates the complex coordination of activities between the embryo and uterus, thus resulting in successful implantation and placenta-tion [2]. Implantation is initiated in the progesterone-sensitized uterus by a nidatory estradiol-17 β (E₂) surge that triggers changes in the uterus, making it receptive to the embryo, and changes in the embryo making it capable of attaching and implanting [3]. In particular, substances secreted by the uterine GE, such as leukemia inhibitory factor (LIF) [4] and calcitonin [5], orchestrate changes in the luminal epithelium (LE) that permit attachment and subsequent implantation of the embryo, as well as decidualization of the underlying stromal cells (Fig. 1). LIF appears to be a primary effector of the E₂ signal, leading to activation of Stat3, changes in the expression of several genes—for example, heparin-binding EGF (*HB-EGF*), cochlin (*Coch*), insulin-like growth factor binding protein-3 (*IGFBP-3*), and immune response gene 1 (*IRG1*) (Fig. 1)—and changes in cell morphology in the LE [6, 7]. Induction of the decidual response requires not only LIF, but also a blastocyst-derived signal [7, 8], although the blastocyst signal can be replaced by a nonspecific stimulation of the LE by oil injection or mechanical scratching [9].

Given the key roles of uterine GE in the establishment of pregnancy and nourishment of the conceptus, it is important to understand both the mechanisms regulating gland formation (adenogenesis) and glandular functions at implantation. Work from many laboratories over the past two decades has provided important new insights into both aspects. In many species, including sheep and mice, the uterus of newborn females consists of a simple luminal epithelium surrounded by layers of stroma and muscle, and adenogenesis occurs after birth. Adenogenesis begins on Postnatal Day 5 (PND5) in the mouse, and progresses through PND60 [10], with distinct postnatal and pubertal periods. There is also regenerative adenogenesis in the postpartum uterus [11]. An important advance has been the use of conditional, uterine-specific gene knockouts to dissect the molecular pathways regulating gland formation in the mouse [12–14]. This approach has so far resulted in the identification of both Wnt signaling elements

(Wnt5a, Wnt7a, Ctnnb) and the transcription factor FoxA2 as critical elements in the formation, and potentially the maintenance, of the GE [12, 15, 16].

Adenogenesis in the early postnatal period is normally independent of steroid hormones, yet it is exquisitely sensitive to their presence, as exposure to either E₂ or progesterone (P₄) for a defined period of time during the postnatal period leads to failure of adenogenesis and infertility in both sheep and mice [11, 14, 17, 18]. Interestingly, in mice, the effects of P₄ treatment vary between strains, from complete inhibition of adenogenesis in the inbred C57Bl/6 strain [10, 18] to a delay and a decrease in the number of glands in the outbred Swiss Webster strain [11]. The period of sensitivity is quite specific (PND2–PND10); even one day of difference in timing of steroid treatment changes the overall severity of the effect in C57Bl/6 mice [10, 18]. Treatment of postnatal C57Bl/6 mice with P₄ between PND2 and PND10 causes a decrease in the expression of several genes, such as *FoxA2*, *Wnt* ligands, and *Hox* genes, known to be important for proliferation and differentiation of the GE [10, 11, 18].

In this month's issue of *Biology of Reproduction*, a new study by Filant and Spencer [19] builds on their previous demonstration of the uterine gland knockout (PUGKO) mouse model [18] to determine the functional effects on implantation and decidualization of an absence of uterine glands. They show that, in the absence of GE, embryos are unable to implant, and the uterine stroma does not decidualize. Ovulation, fertilization, and blastocyst formation are unaffected, and the levels of steroid hormone receptors and many other P₄- and E₂-regulated genes (e.g., *Hand2*, *Hoxa10*, *Il13ra2*, *Igf1*, *Muc1*) in the PUGKO uterus are equivalent to control uteri. These observations complement those of earlier studies in which uterine gland formation was inhibited to a greater or lesser extent, either by neonatal P₄ treatment or by uterine knockout of a specific protein [10–12, 14], and underscore the absolute importance of GE to implantation and decidualization. Taken together, all of the above studies also show a threshold effect of the GE on implantation and ultimately, fertility, likely due to the absolute amount of secretions produced by the variable number of uterine glands.

A critical observation in this new study, however, is that injection of LIF into primed, pseudopregnant PUGKO females does not rescue artificial decidualization induced by oil injection. This result considerably refines our understanding of the mechanism of action of LIF in inducing uterine receptivity. Previous studies have focused on the LE as the primary cellular target of LIF, have documented changes in gene expression and morphology, and have examined the importance of the LIF-induced genes (*HB-EGF*, *Coch*, *IGFBP-3*, *IRG1*) to uterine receptivity [7]. However, the present study shows that LIF effects on the LE cannot be the entire story.

¹Supported by National Institutes of Health grant 5RO3HD065925.

²Correspondence: E-mail: as9n@virginia.edu

FIG. 1. The function of uterine glands at the time of implantation. In response to a surge of estrogen, the glandular epithelium (GE) secretes specific factors into the uterine lumen, including LIF and calcitonin. LIF directly targets the luminal epithelium (LE), promoting phosphorylation of Stat3 in concert with signals from the blastocyst and triggering increased expression of many proteins, including HB-EGF, cochlin, IGFBP-3, and IRG1 by the LE. Secretions from the GE, together with signals from the blastocyst, are required for initiation of decidualization, and the study by Filant and Spencer [19] shows that LIF is necessary but insufficient by itself for the initiation of decidualization, implicating other, as yet unidentified, factors in this process.

While LIF injection, acting through phosphorylated Stat-3 can rescue implantation and decidualization in LIF-null uteri containing GE [6], it is ineffective in uteri lacking GE [19]. Thus, there must be other GE-expressed factors (either induced by LIF or induced by E_2 in parallel to LIF) that are essential to inducing decidualization (Fig. 1). Calcitonin is one such candidate GE-expressed factor because decreased expression in the rat uterus is associated with infertility regardless of the presence of LIF [5]. It is also clear that factors present in uterine fluid, and presumably derived from GE secretion, regulate blastocyst attachment and invasion [20, 21]. Such factors are unlikely to include LIF because embryos lacking receptors for LIF are still able to implant [7, 22, 23]. Future work to identify these regulatory factors and their functions in uterine receptivity and embryo activation will be important, and the PUGKO model should play a prominent role in these studies.

REFERENCES

- Burton GJ, Watson AL, Hempstock J, Skepper JN, Jauniaux E. Uterine glands provide histiotrophic nutrition for the human fetus during the first trimester of pregnancy. *J Clin Endocrinol Metab* 2002; 87:2954–2959.
- Gray CA, Bartol FF, Tarleton BJ, Wiley AA, Johnson GA, Bazer FW, Spencer TE. Developmental biology of uterine glands. *Biol Reprod* 2001; 65:1311–1323.
- Finn CA, Martin L. The role of the oestrogen secreted before oestrus in the preparation of the uterus for implantation in the mouse. *J Endocrinol* 1970; 47:431–438.
- Stewart CL, Kaspar P, Brunet LJ, Bhatt H, Gadi I, Kontgen F, Abbondanzo SJ. Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. *Nature* 1992; 359:76–79.
- Zhu LJ, Bagchi MK, Bagchi IC. Attenuation of calcitonin gene expression in pregnant rat uterus leads to a block in embryonic implantation. *Endocrinology* 1998; 139:330–339.
- Chen JR, Cheng JG, Shatzer T, Sewell L, Hernandez L, Stewart CL. Leukemia inhibitory factor can substitute for nidatory estrogen and is essential to inducing a receptive uterus for implantation but is not essential for subsequent embryogenesis. *Endocrinology* 2000; 141:4365–4372.
- Kimber SJ. Leukaemia inhibitory factor in implantation and uterine biology. *Reproduction* 2005; 130:131–145.
- Teng CB, Diao HL, Ma XH, Xu LB, Yang ZM. Differential expression and activation of Stat3 during mouse embryo implantation and decidualization. *Mol Reprod Dev* 2004; 69:1–10.
- Finn CA, Hinchliffe JR. Reaction of the mouse uterus during implantation and decidualization as demonstrated by changes in the distribution of alkaline phosphatase. *J Reprod Fertil* 1964; 8:331–338.
- Cooke PS, Ekman GC, Kaur J, Davila J, Bagchi IC, Clark SG, Dziuk PJ, Hayashi K, Bartol FF. Brief exposure to progesterone during a critical neonatal window prevents uterine gland formation in mice. *Biol Reprod* 2012; 86:63.
- Stewart CA, Fisher SJ, Wang Y, Stewart MD, Hewitt SC, Rodriguez KF, Korach KS, Behringer RR. Uterine gland formation in mice is a continuous process, requiring the ovary after puberty, but not after parturition. *Biol Reprod* 2011; 85:954–964.
- Dunlap KA, Filant J, Hayashi K, Rucker EB III, Song G, Deng JM, Behringer RR, DeMayo FJ, Lydon J, Jeong JW, Spencer TE. Postnatal deletion of *wnt7a* inhibits uterine gland morphogenesis and compromises adult fertility in mice. *Biol Reprod* 2011; 85:386–396.
- Franco HL, Dai D, Lee KY, Rubel CA, Roop D, Boerboom D, Jeong JW, Lydon JP, Bagchi IC, Bagchi MK, DeMayo FJ. WNT4 is a key regulator of normal postnatal uterine development and progesterone signaling during embryo implantation and decidualization in the mouse. *FASEB J* 2011; 25:1176–1187.
- Jeong JW, Kwak I, Lee KY, Kim TH, Large MJ, Stewart CL, Kaestner KH, Lydon JP, DeMayo FJ. Foxa2 is essential for mouse endometrial gland development and fertility. *Biol Reprod* 2010; 83:396–403.
- Mericskay M, Kitajewski J, Sassoon D. Wnt5a is required for proper epithelial-mesenchymal interactions in the uterus. *Development* 2004; 131:2061–2072.
- Jeong JW, Lee HS, Franco HL, Broadus RR, Taketo MM, Tsai SY, Lydon JP, DeMayo FJ. Beta-catenin mediates glandular formation and dysregulation of beta-catenin induces hyperplasia formation in the murine uterus. *Oncogene* 2009; 28:31–40.
- Allison Gray C, Bartol FF, Taylor KM, Wiley AA, Ramsey WS, Ott TL, Bazer FW, Spencer TE. Ovine uterine gland knock-out model: effects of gland ablation on the estrous cycle. *Biol Reprod* 2000; 62:448–456.
- Filant J, Zhou H, Spencer TE. Progesterone inhibits uterine gland development in the neonatal mouse uterus. *Biol Reprod* 2012; 86(146): 141–149.
- Filant J, Spencer TE. Endometrial glands are essential for blastocyst implantation and decidualization in the mouse uterus. *Biol Reprod* 2013; 88:93,1–9.
- Paria BC, Song H, Dey SK. Implantation: molecular basis of embryo-uterine dialogue. *Int J Dev Biol* 2001; 45:597–605.
- Gonzalez IM, Martin PM, Burdsal C, Sloan JL, Mager S, Harris T, Sutherland AE. Leucine and arginine regulate trophoblast motility through mTOR-dependent and independent pathways in the preimplantation mouse embryo. *Dev Biol* 2012; 361:286–300.
- Yoshida K, Taga T, Saito M, Suematsu S, Kumanogoh A, Tanaka T, Fujiwara H, Hirata M, Yamagami T, Nakahata T, Hirabayashi T, Yoneda Y, et al. Targeted disruption of gp130, a common signal transducer for the interleukin 6 family of cytokines, leads to myocardial and hematological disorders. *Proc Natl Acad Sci U S A* 1996; 93:407–411.
- Ware CB, Horowitz MC, Renshaw BR, Hunt JS, Liggitt D, Koblar SA, Gliniak BC, McKenna HJ, Papayannopoulou T, Thoma B, Cheng L, Donovan PJ, et al. Targeted disruption of the low-affinity leukemia inhibitory factor receptor gene causes placental, skeletal, neural and metabolic defects and results in perinatal death. *Development* 1995; 121: 1283–1299.