COMMENTARY

Hunting for Fox(A2): Dual roles in female fertility

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Pregnancy encompasses a complex orchestration of several sequential steps across tissue and cell types in the mother and fetus for embryo development and delivery of offspring at an opportune time for survival. These steps include embryo implantation, stromal cell proliferation and differentiation (decidualization), placentation, and ultimately parturition with delivery of offspring (1, 2). The outcome of each stage depends on the success of the preceding stages. Most pregnancy events in mice and other mammals are primarily regulated by ovarian estrogen and progesterone (2), yet the molecular discourse between the reproductive system and the embryo that guides pregnancy events is a prevailing mystery. The uterus is primarily comprised of two compartments: the outermost muscle layer (myometrium) and the underlying endometrium. The endometrium is composed of the stroma, the epithelium, and glands. These glands have been seen throughout pregnancy and are acknowledged as essential for implantation and pregnancy success (3), yet their formation and particular genetic and molecular contributions during distinct stages of pregnancy have not been fully appreciated. In PNAS, Kelleher et al. (4) show that Forkhead box a2 (FOXA2), a transcription factor expressed in neonatal and adult mouse uteri, plays dual, stage-specific roles in maternal tissues during pregnancy (Fig. 1).

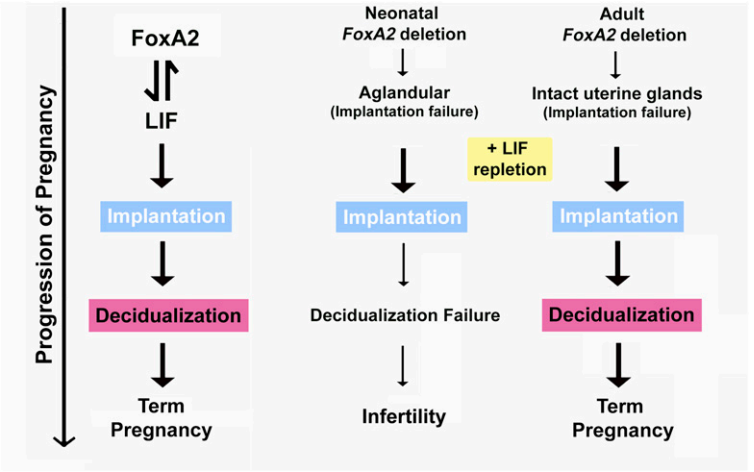

Fig. 1. A schematic of uterine FoxA2 function. (*Left*) In control females, FoxA2 regulates the secretion of glandular LIF, a requisite factor in implantation. With deletion of neonatal FoxA2 (*Center*), adenogenesis is impaired, with uteri becoming aglandular, reduced *Lif* expression, and infertility resulting from implantation failure. Deletion of adult FoxA2 (*Right*) does not perturb gland formation but disrupts gland differentiation and secretion of LIF, resulting in implantation failure and infertility. LIF replacement rescues implantation in both types of FoxA2 mutant mice but cannot sustain pregnancy in aglandular *FoxA2 Pgr-Cre* females. In contrast, LIF substitution in mice with uterine glands (*FoxA2 Ltf-Cre*) females can progress to term pregnancy.

The development of glands (adenogenesis) begins in the postnatal uterus, and their maturation and differentiation is under endocrine control during adulthood. Gland formation and secretions during pregnancy are essential for successful reproduction in mammals. Uterine gland knockout ewes do not form glands, nor do they cycle. These ewes are infertile (3, 5), suggesting that uterine glands are required for conceptus survival and development. Adenogenesis is associated with several signaling pathways and their development and maturation has been linked with growth factors, Wnt signaling, and homeobox signaling pathways (6, 7).

FoxA2 is a homeobox transcription factor required during embryogenesis and plays multiple critical roles in gastrulation, neural tube patterning, and gastrointestinal development (8, 9). FoxA2 is also expressed in glands in the pregnant mouse uterus, suggesting its role in pregnancy (10). Because systemic deletion of *FoxA2* results in embryonic lethality, its role was evaluated during pregnancy by transgenic mice with *FoxA2* conditionally ablated in the mouse uterus (10, 11). These mice had significantly reduced fertility with smaller litter sizes as a result of disrupted embryo implantation. Blastocysts in mutant females could attach to the uterine luminal epithelium (LE) with appropriate expression of uterine receptivity marker genes. However, the embryo could not penetrate the LE for implantation or initiate stromal cell decidual responses in most of the females studied. The number of endometrial glands was severely reduced in *FoxA2* mutant mice, precluding the secretion of leukemia inhibitory factor (*Lif*) expression, a critical factor in implantation success. *Lif*-null females and mice with impaired secretion or expression of *Lif* have been shown to have implantation defects (12, 13).

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Although implantation could be rescued in *FoxA2* mutant mice by intrauterine injection of LIF, decidualization was only partially rescued, suggesting that mice with a reduced number of endometrial glands cannot sustain an adequate decidualization response. Mutant females that could mount decidualization showed blunted, partial responses, and abnormal stromal morphology. Additional studies showed that mutant mice were unable to form a deciduoma with artificial stimulation and reduced decidual expression of prostaglandin synthase 2 (*Ptgs2*; encoding Cox2), a rate-limiting enzyme in prostaglandin synthesis, required for implantation and placentation (14, 15). These findings implied a role for FOXA2 in glandular function and differentiation at later stages of pregnancy. However, because *FoxA2* mutant uteri generated by the *PR-Cre* driver show implantation failure, stage-specific functions of FOXA2 in later stages of pregnancy could not be addressed.

In the current study, Kelleher et al. (4) used and compared stage- and tissue-specific deletion of *FoxA2* using conditional mouse lines developed with different *Cre* drivers. *Cre* expression under the *Pgr-Cre* becomes operative in mouse uteri around day 10 of postnatal life, and *FoxA2* deleted in neonatal uteri (*FoxA2 Pgr-Cre*) resulted in adult females lacking uterine glands. In contrast, *Cre* expression under the lactoferrin promoter appears at later stages around the time of puberty and thereafter, and *FoxA2* is deleted in uteri of sexually mature mice (*Ltf-Cre*) with intact uterine gland formation, without disruption of oviductal or cervical *FoxA2* expression (11, 16).

The Kelleher et al. study (4) identifies implantation failure because of *Lif* deficiency in *FoxA2 Pgr-Cre* and *FoxA2 Ltf-Cre* females, but for different reasons: deletion of *FoxA2* in the uterus by *Pgr-Cre* prevented gland formation as seen previously, whereas deletion of *FoxA2* by *Ltf-Cre* dysregulated glandular differentiation. Using this strategy, the authors identified FOXA2-related pleiotropic effects in the LE and stroma, which influence implantation and decidualization. Intraperitoneal injections of LIF in FOXA2-deficient mice with (*Ltf-Cre*) or without (*Pgr-Cre*) uterine glands before implantation elicited blastocyst implantation. In fact, LIF injections during the uterine receptive phase rescued pregnancy in gland-containing *FoxA2 Ltf-Cre* mice with a full complement of pups comparable to control females. However, resorption was predominant on gestational days 5.5 through 9.5 in *FoxA2 Pgr-Cre* females without glands, and these mice remained infertile, suggesting that the presence of functional glands is critical for pregnancy maintenance. Previously, Jeong et al. showed that administering LIF only partially rescued deciduoma formation in glandless *FoxA2 Pgr-Cre* mice and had no effect on progesterone-induced uterine gland knockout mice (10).

Of further interest, *FoxA2 Ltf-Cre* females showed somewhat aberrant gene expression despite rescue of pregnancy. The decidual cells in the LIF-replaced glandless *FoxA2 Pgr-Cre* mice on gestational day 5.5 were clearly abnormal based on decidual marker gene analysis: *Alpl*, *Bmp2*, *Bmp8a*, *Prl8a2*, and *Wnt4* were substantially lower in the implantation site relative to control and LIF-replaced *FoxA2 Ltf-Cre* mice. *Ptgs2* was induced in the LE adjacent to the blastocyst in adult LIF-replaced FOXA2-deficient mice, but not in decidualizing stromal cells at the implantation site, similar to the phenotype of *Lif*-null mice (17). Additionally, abnormal expression of placental marker genes was reflected in aberrant placental morphology. These findings suggest that the presence and function of uterine glands during decidualization is critical for pregnancy maintenance. This finding begs the question of compensatory mechanisms by the uterine glands to allow pregnancy to proceed, because the *FoxA2* mutant mouse lines differed by the presence or absence of functional glands. Kelleher et al. (4) identify specific glandular secreted factors that were intact in LIF-treated *FoxA2 Ltf-Cre* mice, including PRSS28, PRSS29, SPINK3, and WFDC3. Are these factors critical for pregnancy maintenance? The biological roles for LIF and other secreted molecules in the uterus will require further investigation. The Kelleher et al. (4) study supports the utility of using different FOXA2-deficient mouse models with and without uterine glands to identify the gland-derived factors necessary for decidualization.

In PNAS, Kelleher et al. show that Forkhead box a2 (FOXA2), a transcription factor expressed in neonatal and adult mouse uteri, plays dual, stage-specific roles in maternal tissues during pregnancy.

ESR1 (encoding estrogen receptor α, ERα) expression is postulated to involve FOXA2 transcription activity (18). Indeed, target gene expression downstream of ERα and FoxA2 significantly overlaps (nearly 82%) in a model of hepatocellular carcinoma; this helps to explain the influence of FoxA1/A2 in sexual dimorphism seen in this neoplasm (19). Intriguingly, single nucleotide polymorphisms at FOXA2 binding sites were shown to reduce binding of both FOXA2 and ERα to their targets in the human liver and correlated with hepatocellular carcinoma development in women. Future studies on FOXA2 programming of the glandular epithelial transcriptome and mediating steroid hormone responsiveness of uterine glands for maturation and differentiation are warranted. In line with this thought process, additional questions arise: What determines the number of glands formed in the uterus? Are they made on demand or is the number predetermined? Future research will help to garner not only new knowledge in glandular epithelial biology but also identify new gaps in knowledge that were not previously appreciated.

Overall, the Kelleher et al. (4) study successfully compares stage and cell-type–specific transgenic models of *FoxA2* deficiency and finds that: (*i*) FOXA2 has a role in regulating *Lif* expression in the uterine glands for implantation and partial maintenance of decidualization, and (*ii*) LIF-initiated FOXA2-independent genes in the glands can influence stromal cell decidualization and placental growth and development for pregnancy success. These findings highlight the presence and function of uterine glands in successful pregnancy and identify pleiotropic roles of FOXA2 to support glandular function in distinct stages of pregnancy. Because uterine gland dysfunction is linked to pregnancy loss and complications, such as miscarriage, preeclampsia, and fetal growth retardation (20), further research in uterine gland biology is necessary to improve pregnancy success in humans.

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1 Wang H, Dey SK (2006) Roadmap to embryo implantation: Clues from mouse models. *Nat Rev Genet* 7(3):185–199.
2 Cha J, Sun X, Dey SK (2012) Mechanisms of implantation: Strategies for successful pregnancy. *Nat Med* 18(12):1754–1767.

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3 Gray CA, et al. (2001) Developmental biology of uterine glands. *Biol Reprod* 65(5):1311–1323.
4 Kelleher AM, et al. (2017) Forkhead box a2 (FOXA2) is essential for uterine function and fertility. *Proc Natl Acad Sci USA* 114:E1018–E1026.
5 Gray CA, Burghardt RC, Johnson GA, Bazer FW, Spencer TE (2002) Evidence that absence of endometrial gland secretions in uterine gland knockout ewes compromises conceptus survival and elongation. *Reproduction* 124(2):289–300.
6 Shelton DN, et al. (2012) The role of LEF1 in endometrial gland formation and carcinogenesis. *PLoS One* 7(7):e40312.
7 Guimarães-Young A, Neff T, Dupuy AJ, Goodheart MJ (2016) Conditional deletion of Sox17 reveals complex effects on uterine adenogenesis and function. *Dev Biol* 414(2):219–227.
8 Friedman JR, Kaestner KH (2006) The Foxa family of transcription factors in development and metabolism. *Cell Mol Life Sci* 63(19-20):2317–2328.
9 Kaestner KH (2010) The FoxA factors in organogenesis and differentiation. *Curr Opin Genet Dev* 20(5):527–532.
10 Jeong JW, et al. (2010) Foxa2 is essential for mouse endometrial gland development and fertility. *Biol Reprod* 83(3):396–403.
11 Soyal SM, et al. (2005) Cre-mediated recombination in cell lineages that express the progesterone receptor. *Genesis* 41(2):58–66.
12 Bhatt H, Brunet LJ, Stewart CL (1991) Uterine expression of leukemia inhibitory factor coincides with the onset of blastocyst implantation. *Proc Natl Acad Sci USA* 88(24):11408–11412.
13 Stewart CL, et al. (1992) Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. *Nature* 359(6390):76–79.
14 Lim H, et al. (1997) Multiple female reproductive failures in cyclooxygenase 2-deficient mice. *Cell* 91(2):197–208.
15 Lim HJ, Wang H (2010) Uterine disorders and pregnancy complications: Insights from mouse models. *J Clin Invest* 120(4):1004–1015.
16 Daikoku T, et al. (2014) Lactoferrin-iCre: A new mouse line to study uterine epithelial gene function. *Endocrinology* 155(7):2718–2724.
17 Song H, Lim H, Das SK, Paria BC, Dey SK (2000) Dysregulation of EGF family of growth factors and COX-2 in the uterus during the preattachment and attachment reactions of the blastocyst with the luminal epithelium correlates with implantation failure in LIF-deficient mice. *Mol Endocrinol* 14(8):1147–1161.
18 Hurtado A, Holmes KA, Ross-Innes CS, Schmidt D, Carroll JS (2011) FOXA1 is a key determinant of estrogen receptor function and endocrine response. *Nat Genet* 43(1):27–33.
19 Li Z, Tuteja G, Schug J, Kaestner KH (2012) Foxa1 and Foxa2 are essential for sexual dimorphism in liver cancer. *Cell* 148(1-2):72–83.
20 Burton GJ, Jauniaux E, Charnock-Jones DS (2010) The influence of the intrauterine environment on human placental development. *Int J Dev Biol* 54(2-3):303–312.

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