Uterine glands: development, function and experimental model systems

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ABSTRACT: Development of uterine glands (adenogenesis) in mammals typically begins during the early post-natal period and involves budding of nascent glands from the luminal epithelium and extensive cell proliferation in these structures as they grow into the surrounding stroma, elongate and mature. Uterine glands are essential for pregnancy, as demonstrated by the infertility that results from inhibiting the development of these glands through gene mutation or epigenetic strategies. Several genes, including forkhead box A2, beta-catenin and members of the Wnt and Hox gene families, are implicated in uterine gland development. Progestins inhibit uterine epithelial proliferation, and this has been employed as a strategy to develop a model in which progestin treatment of ewes for 8 weeks from birth produces infertile adults lacking uterine glands. More recently, mouse models have been developed in which neonatal progestin treatment was used to permanently inhibit adenogenesis and adult fertility. These studies revealed a narrow and well-defined window in which progestin treatments induced permanent infertility by impairing neonatal gland development and establishing endometrial changes that result in implantation defects. These model systems are being utilized to better understand the molecular mechanisms underlying uterine adenogenesis and endometrial function. The ability of neonatal progestin treatment in sheep and mice to produce infertility suggests that an approach of this kind may provide a contraceptive strategy with application in other species. Recent studies have defined the temporal patterns of adenogenesis in uteri of neonatal and juvenile dogs and work is underway to determine whether neonatal progestin or other steroid hormone treatments might be a viable contraceptive approach in this species.

Key words: adenogenesis / contraception / endometrium / murine / ungulate

Introduction

The mammalian uterus differentiates from the fetal Müllarian ducts and typically consists of a central tubular epithelium surrounded by undifferentiated mesenchyme at birth. Uterine glands begin to develop as invaginations of luminal epithelium (LE) that progressively invade the mesenchyme, ultimately resulting in an extensive network of epithelial glands throughout the stroma. Here we provide an overview of how uterine glands develop and their role in implantation and early conceptus development. Recent experimental approaches that allow gland development to be modulated by genetic or other means are discussed, including how these model systems have helped to define the roles of uterine endometrial glands in uterine biology. Prospects for future use of these systems to advance understanding of adenogenesis and to provide a permanent contraceptive methodology in some species are discussed.

Uterine glands: historical perspective

While empirical evidence that uterine glands are required to support pregnancy was provided only recently (Gray et al., 2001b; Cooke et al., 2012a), the idea that uterine secretions are essential to the success of pregnancy is an ancient one. Hippocrates (460–370 BC) and his students argued that the fetus was nourished per os by sucking on maternal cotyledons or ‘uterine paps’ (DeWitt, 1959). 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 (DeWitt, 1959; Needham, 1959; Saunders and O’Malley, 1982).

William Harvey (1578–1657 AD) was among the first to recognize that the conceptus was nourished by substances within the uterus (‘vicar of the breasts’), much as the neonate is nourished by milk (Needham, 1959). 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’ (Needham, 1950, 1959; Amoroso, 1952). In the late nineteenth century, von Hoffman (1884) 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’. According to Amoroso (1952), the term embryotroph was coined in the late nineteenth century, was used to describe all available material supplied to the conceptus in utero. In the early twentieth century, the terms hemotroph and histotroph were coined to describe those substances essential for the support of conceptus and fetal development supplied via the uterus either: (i) directly from blood; or (ii) by biosynthetic activity of uterine endometrium. Direct evidence of the essential nature of uterine secretions came with the demonstration that endometrial glands are required for establishment and maintenance of pregnancy.

**Uterine gland development in various species**

The organogenetic development and differentiation of most reproductive tract organs is completed during the fetal period; however, the uterus is not fully developed or differentiated at birth (Fig. 1). Establishment of tissue-specific histoarchitecture is completed post-natally in laboratory rodents, domestic animals and presumably humans (Cunha, 1976c; Bartol et al., 1993; Bartol et al., 1999; Gray et al., 2001a; Spencer et al., 2005a, b; Kurita and Nakamura, 2008; Spencer et al., 2012). The process of post-natal radial patterning establishes the three histological elements of the uterine wall: (i) endometrium; (ii) myometrium, consisting of inner circular and outer longitudinal smooth muscle layers and (iii) perimetrium. Events common to post-natal uterine morphogenesis include: (i) organization and stratification of endometrial stroma; (ii) myometrial differentiation and growth and (iii) development of endometrial glands. The timing of these developmental events differs among species and reflects differences in uterine maturity at birth. Importantly, uterine gland morphogenesis is primarily, if not uniquely, a post-natal event (Fig. 1). Indeed, the functional capacity of the adult uterus is established by developmental events associated with ‘programming’ of uterine tissues during prenatal and post-natal life (Bartol et al., 1999, 2008; Sassoon, 1999; Kobayashi and Behringer, 2003; Mericskay et al., 2004; Crain et al., 2008; Masse et al., 2009; Walker, 2011).

**Comparative development of the uterus**

Knowledge of prenatal uterine development is more extensive in rodents than in other mammals and this process is assumed to be similar among other species (Kobayashi and Behringer, 2003; Spencer et al., 2005b; Kurita, 2011). In contrast, post-natal uterine morphogenesis depends on the state of development of the uterus at birth, which can be a function of gestation length and, perhaps, the interval between birth and puberty (Gray et al., 2001a). For instance, initially, development of the rodent uterus after birth involves the differentiation of mesenchyme into stroma and myometrium, whereas uterine mesenchyme is already patterned radially into stroma and myometrium at birth in domestic animals and humans. Post-natal development of the uterus is summarized for several species (rodents, pig and sheep) in Fig. 1 and is discussed in detail below.

**Laboratory rodents**

Laboratory rodents (mouse and rat) have a long duplex uterus with a dual cervix. The adult rodent endometrium consists histologically of simple columnar LE surrounded by stromal cells containing endometrial glands lined by simple cuboidal glandular epithelium (GE). The endometrium contains glands but does not contain the tightly coiled, branched glands characteristic of humans and domestic animals (Branhman et al., 1985). Surrounding the endometrium are inner circular and outer longitudinal layers of smooth muscle comprising the myometrium (Cunha, 1976a; Cunha et al., 1985; Brody and Cunha, 1989).

Gestation length in mice and rats is 20 and 21 days, respectively. Post-natal uterine development is similar in rats and mice (Brody and Cunha, 1989). At birth, uteri of mice and rats lack endometrial glands and consist of a simple epithelium supported by undifferentiated mesenchyme (Fig. 1). Between birth and post-natal Day 5 (P5) in mice, epithelial invaginations or buds appear, indicating the formation of GE (Kurita et al., 2001; Cooke et al., 2012a). Also, during this period, three layers of mesenchyme become segregated distinctly into radially oriented endometrial stroma and inner circular myometrium (Brody and Cunha, 1989).

Histologically, distinct uterine glands do not appear in the endometrium until P7 and P9 in mice and rats, respectively (Branham et al., 1985). By P10 in mice, nascent uterine glands extend from the LE into surrounding endometrial stroma, and the outer longitudinal layer of the myometrium becomes organized into bundles (Brody and Cunha, 1989). The basic adult configuration of the murine uterus is established by P15 (Hu et al., 2004). In the rat, uterine gland morphogenesis or adenogenesis proceeds from P9 through P15 (Branham et al., 1985) and is characterized by formation of simple, tubular glands that, unlike endometrial glands in humans and domestic animals, are neither tightly coiled nor extensively branched (Hu et al., 2004).

**Pigs**

The mature uterine wall in adult gilts or sows has a similar architecture to that of humans and other domestic animals in that the endometrium contains hundreds of glands in a uterine cross section. Gestation in the pig is ~ 114 days. Transformation of the neonatal porcine uterine wall to maturity occurs within 120 days of birth (Bal and Getty, 1970; Bartol et al., 1993; Spencer et al., 1993; Tarleton et al., 1998). Uterine development in neonatal pigs includes appearance and proliferation of endometrial glands, stromal organization, development of endometrial folds and growth of the myometrium (Fig. 1). At birth (P0), the porcine uterus consists of a simple columnar LE that is supported by undifferentiated mesenchyme and encircled by a rudimentary myometrium (Bartol et al., 1993, 1999; Spencer et al., 1993). Endometrial adenogenesis is initiated after birth when GE develops into simple epithelial tubes that extend radially from the LE into the stroma. By P7, stromal zones, including a
shallow stratum compactum and a deep stratum spongiosum, are evident and distinct, simple, coiled, tubular glands are present throughout the shallow stroma. Eventually, tubular glands undergo coiling and some branching within the stroma until they reach the myometrium. By P14, coiled tubular glands extend approximately one-third of the distance from the LE to the myometrium, which has differentiated into inner circular and outer longitudinal layers. On P28, many coiled glands have obvious branches and GE is present throughout the stroma. Well-developed endometrial folds are apparent by P28 and endometrial glands are numerous and extensive by P56. The pig uterus is capable

Figure 1  Uterine morphology, radial patterning and post-natal development in rodents, sheep and pigs. (A) Diagrams of ideal frontal sections of uterine types. The drawings cut the oviducts off near the uterotubal junctions, and the vaginas just caudal to the cervixes. Rodents (rats and mice) have a long duplex type of uterus with dual cervixes. Pigs have a long bicornuate uterus with a short uterine body and a single cervix. Sheep have a medium-length bicornuate type of uterus and a short uterine body and a single cervix. (B) Diagrams of ideal radial patterns of the uterine wall. Curved lines in the endometrium denote the tubular, coiled and branched glands that extend from the uterine lumen to the inner myometrium. The rodent uterus contains only a few endometrial glands. The sheep uterus contains large numbers of glands in the intercaruncular areas of the endometrium, whereas the caruncles are glandless. The pig uterus contains large numbers of glands throughout the endometrium. (C) Histoarchitectural development of the uterine wall in the neonatal mouse, sheep and pig. The post-natal (P) age in days is shown in the bottom left of the images. Car, caruncle; LE, luminal epithelium; GE, glandular epithelium; S, stroma; M, myometrium. Reprinted from Spencer et al. (2012) with permission.
of supporting pregnancy by P120, indicating functional maturity (Bartol et al., 1999).

**Sheep**

Ruminants (cattle, goats and sheep) have a bicornuate uterus with a small common corpus and a single cervix (Fig. 1). The endometrium in adult sheep and cattle contains large numbers of raised aglandular caruncles, dense stromal protuberances covered by LE, and glandular intercaruncular areas (Wimsatt, 1950; Atkinson et al., 1984). As in humans and pigs, intercaruncular endometrial areas contain hundreds of glands in a cross section of the uterus. Caruncles are sites of superficial implantation and placentation (Wimsatt, 1950; Mossman, 1987). In synepitheliochorial placentation found in ruminants, interdigitation of placental cotyledons with endometrial caruncles forms placentomes, which serve in fetal–maternal gas exchange and derivation of micronutrients by the placenta (Wimsatt, 1950; Amoroso, 1952; Tomes, 1968). In adult sheep and cattle contains large numbers of raised aglandular caruncles, dense stromal protuberances covered by LE, and glandular intercaruncular areas (Wimsatt, 1950; Amoroso, 1952; Wooding, 1992).

Gestation length in sheep is ~147 days. The vagina, cervix and oviduct, but not the uterus, are fully developed histologically at birth in sheep (Gray et al., 2000; Gray et al., 2001b; Carpenter et al., 2003; Fig. 2). Post-natal uterine morphogenesis involves emergence and proliferation of endometrial glands, development of endometrial folds and, to a lesser extent, growth of endometrial caruncular areas and myometrium (Wiley et al., 1987; Bartol et al., 1988a, b; Taylor et al., 2000). Endometrial gland genesis is initiated between P0 and P7, when shallow epithelial invaginations appear along the LE in presumptive intercaruncular areas (Bartol et al., 1988b; Taylor et al., 2000). Between P7 and P14, nascent GE buds proliferate and invaginate into the stroma, forming tubular structures that coil and branch slightly by P21. After P21, the majority of glandular morphogenetic activity involves coiling and branching of tubular endometrial glands as they develop into the deeper stratum spongiosum of the stroma adjacent to the inner myometrium. By P56, caruncular and intercaruncular endometrial areas are histoarchitecturally similar to those of the adult uterus.

**Humans**

Humans have a simplex uterus consisting of a single uterine body. 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 (Padykula, 1991; Brenner and Slayden, 1994; Okulicz et al., 1997). 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 (Padykula, 1991; Okulicz, 2002; Chan et al., 2004; Gargett et al., 2008).

Knowledge of prenatal and post-natal uterine development is limited in humans (Gell, 2003; Kurita and Nakamura, 2008). Prior to E49, the embryo is ambisexual (Cunha, 1989). The uterine corpus and cervix differentiate by Week 12. As in rodents and ungulates, simple columnar LE gives rise to invaginations representing primordial GE buds. By 20–22 weeks of gestation, myometrium is well defined, but adenogenesis is superficial (Song, 1964). Uterine histoarchitecture at birth resembles that of the adult, but is less well developed. Neonatal endometrial LE is low columnar or cuboidal and GE is sparse and limited to the adluminal stroma (Valdes-Dapena, 1973). From birth to puberty, endometrial glands develop slowly. By 6 years of age, glands extend through one-third to one-half of the endometrium. Mature uterine histoarchitecture is observed at puberty, with endometrial glands extending to the myometrium (Valdes-Dapena, 1973). Although initiated fatally, human endometrial gland proliferation is completed post-natally, similar to domestic ungulates, and involves differentiation of GE from LE followed by the 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 (Okulicz et al., 1997; Huang et al., 2012).

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**Figure 2** Hypothesized role of Wnt and Hox genes in radial patterning and gland formation in the post-natal uterus. Wnt7a and canonical WNT signaling via beta-catenin is required for correct epithelial organization, the radial growth and patterning of the adjacent mesenchymal cells, and the organization of the smooth muscle layers. Wnt7a is required for maintenance (dotted arrows) of Wnt5a, Wnt4, Hoxa10 and Hoxa11 gene expression. Wnt5a signals cooperate with an unknown factor to allow Wnt7a repression that may be requisite for gland formation. Foxa2 governs GE differentiation from the LE and development. Adapted from Mericskay et al. (2004).
Progestosterone effects on uterine epithelial proliferation: a tool to regulate adenogenesis

Estrogens regulate a wide variety of maturational and functional uterine processes including cell proliferation, growth, decidualization and embryo implantation. Estrogens are a primary mitogen for uterine epithelium and the rise in estrogen concentrations around estrous in mice induces increases in proliferation of both uterine LE and GE (Finn and Martin, 1969). Mitogenic responses to estrogens are frequently studied in ovariectomized mice, in which the endocrine environment can be controlled and manipulated, and administration of 17β-estradiol (E2) or other xenoestrogens (Finn and Martin, 1969; Selvaraj et al., 2004) to ovariectomized mice produces a wave of epithelial proliferation mimicking that observed during the estrous cycle.

Progestins inhibit many estrogen actions in the uterus, including stimulation of epithelial proliferation. This occurs naturally during the estrous cycle when rising progesterone (P4) following ovulation inhibits E2-induced epithelial proliferation and has been demonstrated in ovariec- tomized animals, in which pretreatment with P4 essentially abolishes epithelial proliferation typically seen in response to estrogens (Finn and Martin, 1971).

Uterine epithelial proliferation in response to estrogen is regulated by estrogen receptor 1 (ESR1, previously known as estrogen receptor α), as demonstrated by the lack of proliferative responses to estrogen in ESR1 null mice (Lubahn et al., 1993). These epithelial estrogen effects are mediated by ESR1 in stromal cells (Cooke et al., 1997; Winuthayanon et al., 2010). Inhibitory effects of P4 on such estrogen-induced proliferation were reported to involve stromal progesterone receptors (PGR; Kurita et al., 1998), although other results suggest that this effect may be mediated through epithelial PGR (Franco et al., 2011). Recently, Li et al. (2011) found that inhibitory effects of P4 on estrogen-induced epithelial proliferation are mediated through the transcription factor Hand2, which is expressed only in the stroma. Hand2 appears to act by suppressing the production of several fibroblast growth factors (FGFs) that are normally produced in the stroma in response to estrogen and act through receptors in uterine epithelium to stimulate epithelial mitogenesis. These findings provide a partial mechanistic explanation for the proliferative epithelial response to estrogen and how this process is blocked by P4.

Uterine epithelium in mice, rats, dogs and other species proliferates rapidly during the neonatal period (Bigsb y and Cunha, 1985; Cooke et al., 2012b). In mice, neonatal uterine epithelial proliferation is almost completely blocked by P4 (Bigsb y and Cunha, 1985), just as P4 abolishes epithelial proliferation induced by estrogen in ovariectomized mice. Neonatal uterine epithelial proliferation is permissiv e for critical aspects of uterine adenogenesis such as budding of GE and stromal invasion by nascent uterine glands. Evidence that P4 could be used to inhibit uterine epithelial proliferation provided an essential tool with which to develop experimental systems designed to prevent neonatal uterine adenogenesis and establish model systems for assessment of the functional role of uterine glands in processes such as embryo implantation and fertility.

Effects of neonatal steroids in sheep and pigs

The endometrium contains extensive uterine glands that synthesize and secrete substances into the uterine lumen, including a complex array of enzymes, growth factors, cytokines, lymphokines, hormones, transport proteins and other substances, collectively termed histotroph (Bazer, 1975; Kane et al., 1997). These secretions influence conceptus survival and development, transduction of pregnancy recognition signals, and placental and fetal growth in humans and other species (Bazer, 1975; Burton et al., 2002, 2007; Hempstock et al., 2004; Spencer et al., 2008; Bazer et al., 2010).

Adenogenesis in domestic and laboratory animals occurs rapidly after birth (Bartol et al., 1999; Gray et al., 2001a; Spencer et al., 2012). Anti-proliferative effects of both progestins (Rider, 2002; Vicent et al., 2006; Dressing and Lange, 2009) and glucocorticoids (Dickmeis and Foulke s, 2011) are well known. Work involving rodents (Ogasawara et al., 1983; Bigsb y and Cunha, 1985) established that the onset of adenogenesis was ovary-, adrenal- and steroid independent and that both progestins and glucocorticoids could suppress uterine epithelial proliferation. Since uterine tissues are steroid responsive perinatally, it has been hypothesized that perinatal endocrine conditions, characterized by progest erone dominance gestationally and involving increased presence of glucocorticoids during parturition, could create a transient anti-proliferative state in the endometrium. Thus, birth would provide the cue for onset of endometrial gland genesis by delivering uterine tissues from an anti-proliferative fetal endocline environment (Wiley et al., 1987; Bartol et al., 1988a).

This hypothesis was tested by exposing ewe lambs to the synthetic progestin norgestomet (NOR) from birth to P13, which inhibited endometrial adenogenesis (Bartol et al., 1988a). Removal of the progestin block to adenogenesis on P13 permitted glands to develop by P26; however, these glands were underdeveloped and histologically abnormal. This original observation served as the foundation for the hypothesis that prolonged exposure of neonatal ewes to progestins during adenogenesis could permanently inhibit endometrial gland differentiation, thereby producing a uterine gland knockout (UGKO) phenotype in adults (Bartol et al., 1999; Gray et al., 2001a). The hypothesis that neonatal progesterin withdrawal was a factor in the induction of endometrial adenogenesis also predicted that local paracrine and autocrine conditions within developing tissues would change following removal of the developmentally inhibitory cue. Indeed, NOR-induced inhibition of ovine endometrial adenogenesis was reported to involve the down-regulation of epithelial ESR1 and PGR expression, as well as reduced FGF receptor 2IIIb (FGFR2IIIb) and uterine hepatocyte growth factor expression, both of which are involved in epithelial–mesenchymal interactions (Gray et al., 2000) and important for morphogenesis of Müllerian duct-derived tissues including uterus (Cunha, 1976c).

In sheep, the exposure of neonatal ewes to NOR did not affect the development of the ovary, hypothalamic–pituitary–ovarian axis, ovary or other Müllerian duct derivatives, including oviduct, cervix and vagina (Gray et al., 2000, 2001b). Uteri of UGKO ewes weighed less, were essentially devoid of endometrial glands and lacked intercaruncular endometrial areas characterized of normal ewes (Gray et al., 2001b). Consistent with reduced uterine size and weight, area and LE length were decreased, whereas myometrial width and morphology were
similar to normal ewes. Specific targeting of only the endometrium makes the UGKO sheep an attractive model with which to study mechanisms regulating neonatal endometrial organization and adenogenesis, as well as the functional roles of endometrial glands in adults (Spencer and Gray, 2006).

The UGKO model revealed an essential role for endometrial glands and their secretions in normal estrous cycles and peri-implantation conceptus survival and growth. Mature UGKO ewes lacked normal estrous cycles as a result of insufficient uterine production of luteolytic pulses of prostaglandin F2 alpha (PGF2α; Gray et al., 2001c). However, exogenous PGF2α induced luteolysis in UGKO ewes, and they displayed normal estrous mating behavior (Gray et al., 2001c). Adult UGKO ewes are unable to establish pregnancy (Gray et al., 2001b, 2001c, 2002); morphologically, normal blastocysts were present in bred UGKO ewes after mating; however, by Day 14, conceptuses were absent or severely growth retarded. This was suggested to reflect the absence of specific endometrial gland secretions (Gray et al., 2001a, 2002). Interestingly, the majority of pregnancy losses in domestic ruminants and humans occur during the first 2 weeks of pregnancy (Diskin and Sreenan, 1980; Koot et al., 2012).

The neonatal porcine endometrium presents a different model with respect to mechanisms regulating the onset of uterine adenogenesis. In this species, endometrial glands begin to develop by P2 (Bartol and Bagnell, 2012; Bartol et al., 2013) and proliferate rapidly and in an ovary-independent manner through, at least, the first 15–30 days postnatailly (Bartol et al., 1993). An image depicting the typical pattern of ESR1 expression in a nascent porcine endometrial gland on P5 is shown in Fig. 3. Uterine adenogenesis in the neonatal pig is both ESR1-dependent and estrogen sensitive (Tarleton et al., 1998; Tarleton et al., 1999, 2001). Anti-estrogen treatment of gilt from birth inhibited neonatal adenogenesis (Tarleton et al., 1999), whereas progesterins were ineffective (unpublished). Nevertheless, disruption of ESR1-dependent, estrogen-sensitive development by estrogen exposure of gilts for 2 weeks from birth reduced adult uterine capacity to support pregnancy and altered biochemical characteristics of adult endometrium (Bartol et al., 1993; Tarleton et al., 2003; Chen et al., 2010). Consistent features here include the facts that uterine adenogenesis: (i) occurs neonatally; (ii) is steroid hormone (estrogen) sensitive and that (iii) if disrupted, alters adult uterine function permanently.

Based on porcine data (Yan et al., 2006; Bartol et al., 2009; Bartol and Bagnell, 2012), the lactocrine hypothesis was proposed to explain how milk-borne bioactive factors, transmitted to the offspring in colostrum (first milk), could regulate the development of neonatal uterine and other female reproductive tissues (Chen et al., 2011; Frankshun et al., 2012). Recent data (Miller et al., 2013) indicate that lactocrine signaling is required to support normal ESR1 expression and adenogenesis in neonatal porcine endometrium. These results, together with the observations indicating that patterns of neonatal uterine growth are comparable in ovary-intact gilts and gilts ovariectomized at birth (Bartol et al., 1993), suggest that lactocrine signaling is an important mechanistic element supporting uterine development in neonatal pigs. Given that all mammals nurse their young, lactocrine regulation of neonatal development may have broad implications (Bartol and Bagnell, 2012; Bartol et al., 2013).

Neonatal progestin treatment of mice as a tool for obtaining mechanistic insights into uterine gland development

Endometrial adenogenesis is critical for uterine development and determines, in part, the embryotrophic potential of the adult uterus (Bartol et al., 1999; Gray et al., 2001a; Spencer et al., 2011). Endometrial glands produce substances needed for conceptus (embryo/fetus and associated placenta) survival, development and implantation (Burton et al., 2002; Dey et al., 2004; Spencer et al., 2011). For example, leukemia inhibitory factor (LIF) is produced and secreted by endometrial glands in mouse uterus (Stewart et al., 1992). The LIF null mouse is infertile due to a failure of blastocyst implantation (Chen et al., 2000) due to potentially impaired uterine gland function, although more recent work has indicated that LIF is also produced in the LE and stroma. Thus, several cell types may be affected in the uterus of the LIF null mouse. Several mouse model systems were recently developed for studying neonatal uterine development and how it can be manipulated in order to understand both developmental and functional consequences of the inhibition of adenogenesis. These systems involve neonatal administration of P4 (Stewart et al., 2011; Cooke et al., 2012a; Filant et al., 2012) and other steroids such as E2 (Stewart et al., 2011).

Treatment of neonatal C57BL/6 mice with P4 altered expression of morphoregulatory genes and inhibited epithelial cell proliferation, leading to a disruption of adenogenesis in the developing uterus (Filant et al., 2012; Cooke et al., 2012a). Treatment from P3 to P10 permanently blocked endometrial adenogenesis (Filant et al., 2012; Cooke et al., 2012a).
involved in adenogenesis in both epithelial and stromal cell compartments (Cunha, 1976b; Sharpe and Ferguson, 1988).

 Knocking out Ctnnb1 (a critical intracellular mediator of Wnt signaling) (Jeong et al., 2009) or its downstream target gene, the transcription factor Left1 (Shelton et al., 2012), perturbs gland formation in neonatal uteri (Table I). In addition, forkhead box A2 (FOXA2) is an essential transcriptional factor for adenogenesis (Jeong et al., 2010). FOXA2 is detected specifically in mouse uterine GE and ablation of FOXA2 severely reduces endometrial gland numbers (Jeong et al., 2010). Similarly, the loss of Cdh1 in the uterus also results in the loss of endometrial glands (Reardon et al., 2012). Both Foxa2 and Cdh1 are thought to be involved in the WNT signaling pathway, further emphasizing the key role of Wnts in adenogenesis (Table I).

As discussed above, neonatal P4 treatment inhibits uterine epithelial proliferation and adenogenesis (Cooke et al., 2012a). Although conditional ablation of Wnt7a in neonatal uterus does not affect epithelial proliferation (Dunlap et al., 2011). Wnt7a is completely suppressed in the epithelium by P4 treatment (Cooke et al., 2012a). Thus, P4 can perturb cell proliferation as well as the expression of adenogenic factors. In fact, neonatal P4 treatment alters the expression of other Wnt genes and their Fzd receptors during neonatal uterine development (Cooke et al., 2012a). Recent work with neonatal administration of the synthetic estrogen diethylstilbestrol (DES), which also inhibits adenogenesis, revealed changes in Wnt4, Wnt7a and Wnt11 expression following exposure from birth to Day 5 (Hayashi et al., 2011). These genes show similar patterns of change in expression following the neonatal P4 administration, suggesting that genes altered similarly in both models may be involved in inhibition of adenogenesis induced by either hormone. In contrast, some changes in gene expression are seen with one treatment, but not the other. For example, in the neonatal uterus, P4 suppresses Fzd6 expression, while DES does not, and DES decreases Wnt5a and Wnt11 expression, while P4 stimulates Wnt11 expression. Effects seen with one hormone but not the other may reflect unique uterine effects of estrogen or progesterone. Expression of Hoxa10 and Hoxa11 is decreased in the neonatal uterus following P4 treatment, although opposite effects of P4 have been reported in the adult uterus (Daftary and Taylor, 2006). Wnt7a maintains uterine Hoxa10 and Hoxa11 expression (Miller and Sassoon, 1998; Mericskay et al., 2004; Spencer et al., 2005a,b, 2012). Thus, reduced Hoxa10 and Hoxa11 following neonatal P4 treatment may reflect downstream events resulting from decreased Wnt7a. Figure 2 illustrates the spatial expression patterns of FOXA2 and the major Wnts and Hox genes, and how these factors may interact to regulate adenogenesis.

### Inhibition of adenogenesis as a contraceptive strategy in companion animals

Experimental model systems in mice showing infertility following neonatal P4 treatment (Cooke et al., 2012a; Filant et al., 2012) corroborated observations in the original UGKO ovine model (Bartol et al., 1988a, 1999; Spencer et al., 1999; Spencer and Gray, 2006). These findings suggested that early progesterin treatment could permanently inhibit adenogenesis and fertility in many species.

Ovariohysterectomy (spaying) is the primary method used for canine sterilization. It is effective and permanent, but involves anesthesia and...
surgery with some attendant morbidity and mortality. Critically, both the surgical costs as well as time required to perform these procedures make them difficult to apply to large feral animal populations. Moreover, this methodology is logistically challenging for large-scale sterilizations or for the control of zoonotic disease transmission to humans. Development of non-surgical techniques based on inhibition of adenogenesis could be valuable in dogs and other companion animal species for addressing overpopulation problems, the accompanying euthanasia of

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<td>Stroma</td>
<td>None</td>
<td>Pgr&lt;sup&gt;Cre/+&lt;/sup&gt;</td>
<td>Hayashi, unpublished data</td>
</tr>
<tr>
<td>Fzd6</td>
<td>LE, GE</td>
<td>Not known</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Fzd10</td>
<td>LE, GE</td>
<td>Not known</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ctnnb1</td>
<td>All</td>
<td>Myogenesis switch to adipogenesis (&lt;em&gt;Amhr2&lt;sup&gt;Cre/+&lt;/sup&gt;&lt;/em&gt;), reduced uterine glands and squamous cell metaplasia (&lt;em&gt;Pgr&lt;sup&gt;Cre/+&lt;/sup&gt;&lt;/em&gt;)</td>
<td>—</td>
<td>Arango et al. (2005), Jeong et al. (2009)</td>
</tr>
<tr>
<td>Lef1</td>
<td>GE, stroma</td>
<td>No uterine glands</td>
<td>Null</td>
<td>Shelton et al. (2012)</td>
</tr>
<tr>
<td>Foxa2</td>
<td>GE</td>
<td>No uterine glands</td>
<td>Pgr&lt;sup&gt;Cre/+&lt;/sup&gt;</td>
<td>Jeong et al. (2010)</td>
</tr>
<tr>
<td>Hoxa10</td>
<td>Stroma</td>
<td>Homeotic transformation of the anterior part of uterus to an oviductal morphology</td>
<td>Null</td>
<td>Benson et al. (1996)</td>
</tr>
<tr>
<td>Hoxa11</td>
<td>Stroma</td>
<td>Partial anteriorization of uterus to oviduct, hypoplastic uterus and decreased uterine glands</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Vangll2</td>
<td>LE, GE</td>
<td>Reduced uterine glands</td>
<td>Long-tail Vangll2 mutant mice (xenograft)</td>
<td>Vandenberg and Sassoon (2009)</td>
</tr>
<tr>
<td>Cdh1</td>
<td>LE, GE</td>
<td>Epithelial degeneration, no uterine glands</td>
<td>Pgr&lt;sup&gt;Cre/+&lt;/sup&gt;</td>
<td>Reardon et al. (2012)</td>
</tr>
</tbody>
</table>

LE, luminal epithelium; GE, glandular epithelium.

*For the conditional mutants, mice in which Cre recombinase is knocked into the progesterone receptor locus (<em>Pgr<sup>Cre/+</sup></em>) have deletions of the target gene in both the uterine epithelium and stroma, while the use of mice with Cre recombinase knocked into the anti-Mullerian hormone type II receptor locus (<em>Amhr2<sup>Cre/+</sup></em>) results in target gene deletion only in the mesenchyme of the uterus.

Figure 4  Cell proliferation and adenogenesis in the neonatal canine uterus visualized by immunostaining for MKI67, a marker of cell proliferation (A) Cell proliferation was robust in both luminal epithelium (LE) and stroma (S) of the 1-week-old canine uterus. Adenogenesis was just beginning at this time, as evidenced by budding of epithelium into the underlying stroma. (B) In the 4-week-old canine uterus, proliferation of glandular (GE) and LE epithelium was reduced compared with the epithelial proliferation seen in the 1-week-old animal. Stromal proliferation was similarly reduced. At this age, glands are clearly seen, and they have extended some distance through the stroma. Scale bar = 50 μm for both photos.
millions of animals annually (Bartlett et al., 2005) and global public health issues exemplified by the continued widespread transmission of rabies to humans from feral dog bites (Panda et al., 2008).

Both murine and ovine model systems (Stewart et al., 2011; Cooke et al., 2012a; Filant et al., 2012) have been used to show that permanent inhibition of adenogenesis and fertility requires that progesterin treatment begins before the initiation of uterine gland development. Previous work on canine uterine development and gland genesis has focused on epithelial proliferation and functional activity during the estrous cycle in adults. Until recently, patterns of canine uterine adenogenesis in the neonate were unknown. Development of a canine contraceptive strategy based on the inhibition of uterine development and adenogenesis requires preliminary work to understand normal developmental biology of the canine uterus.

A recent study provided a first step toward this goal by defining the time course of post-natal uterine gland development in the dog (Cooke et al., 2012b). Canine adenogenesis begins during the first post-natal week. Proliferation of LE and nascent GE is initially robust (Fig. 4). Proliferation of LE, GE and stroma steadily declines during the neonatal period (Fig. 4 and Cooke et al., 2012b), becoming negligible by Week 8 post-natal. For example, 45% of glandular epithelial cells are proliferating at Week 2 post-natal, but this decreases to 15% by Week 4 (Fig. 3) and 2% at Week 6 before becoming almost undetectable by Week 8.

Understanding canine adenogenesis will facilitate the development of a rational strategy to test whether neonatal administration of a progestin or other steroids can be employed to inhibit uterine adenogenesis permanently and produce sterility in adults. However, numerous critical questions remain to be answered before a contraceptive approach of this kind can be validated, including identification of (i) the critical period for disruption of adenogenesis; (ii) the optimal progestin or other steroid compound(s) required to induce the desired effect and (iii) the optimal mode of delivery of the anti-adenogenic compound.

Conclusions

Studies in both sheep and mice illustrate the utility of P4 as a tool with which to alter or inhibit adenogenesis and discover mechanisms regulating post-natal uterine development. Overall, these studies indicated a narrow, well-defined window during which progesterin treatment (steroid hormone-induced disruption of development) may provide a potential tool with which to produce adult infertility through the induction of irreversible changes in uterine development. Available results support the hypothesis that P4 inhibits endometrial adenogenesis in the developing neonatal uterus by altering the expression of morphoregulatory genes and, consequently, disrupting normal patterns of cell proliferation and communication. Future studies are needed both to determine whether inhibiting adenogenesis can be used for contraception in dogs or other species. Additional work is also needed to identify and understand the novel factors and pathways involved in neonatal endometrial adenogenesis.

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Authors’ roles

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