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Uterine Glands: Development Biology and Function During Pregnancy

Contents

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Abstract

This review focuses on the developmental biology and functional roles of endometrial glands in mammalian uteri with emphasis on domestic livestock. All uteri contain endometrial glands that synthesize or transport and secrete substances essential for survival and development of the conceptus (embryo/fetus and associated extraembryonic membranes). Analyses of the ovine uterine gland knockout (UGKO) model support a primary role for endometrial glands and, by default, their secretions in peri-implantation conceptus survival and development. Uterine adenogenesis is the process whereby endometrial glands develop and is primarily a postnatal event in domestic livestock, rodents and humans. Endometrial adenogenesis involves differentiation and budding of glandular epithelium (GE) from lumenal epithelium (LE), followed by invagination and extensive tubular coiling and branching morphogenesis throughout the stroma to the myometrium. In neonatal ungulates, studies implicate prolactin, estradiol-17β and their receptors in mechanisms regulating endometrial adenogenesis. In sheep and pigs, extensive endometrial gland hyperplasia and hypertrophy occurs during gestation, presumably to provide increasing histotrophic support for fetoplacental growth and development. A servomechanism is proposed to regulate endometrial gland morphogenesis and function during pregnancy that involves sequential actions of ovarian steroid hormones, pregnancy recognition signals, and lactogenic hormones from the pituitary or placenta. Disruption of uterine development during critical organizational periods can alter the functional capacity and embryotrophic potential of the adult uterus. These findings reinforce the importance of understanding the mechanisms regulating uterine gland morphogenesis in livestock and humans.

Keywords:uterus, endometrium, glands, development, pregnancy.

Introduction and Historical Background

All mammalian uteri contain endometrial glands that synthesize and secrete or transport a complex array of proteins and related substances termed histotroph. The idea that uterine secretions nourish the developing conceptus (embryo/fetus and associated extraembryonic placental membranes) was discussed by both Aristotle, in the third century BC, and William Harvey in the 17th century. In 1882, Bonnett concluded that secretions of uterine glands were important for fetal well-being in ruminants. Evidence accumulated from primate and subprimate species during the last century supports an unequivocal role for secretions of endometrial glands as primary regulators of conceptus survival, development, production of pregnancy recognition signals, and implantation/placentation (for reviews see Amoroso, 1952; Bazer, 1975; Bazer et al., 1979; Bell, 1988; Roberts & Bazer, 1988; Bell & Drife, 1989; Simmen & Simmen, 1990; Fazleabas et al., 1997; Kane et al., 1997;

Stewart & Cullinan, 1997; Armant et al., 2000; Carson et al., 2000). In marsupials, carnivores and roe deer, changes in endometrial gland secretory activity are proposed to regulate delayed implantation (Given & Enders, 1989; Renfree, 1993). In rodents, several factors, including leukemia inhibitory factor (LIF) and calcitonin, are expressed exclusively by endometrial glands and are essential for establishment of uterine receptivity and embryo implantation (for reviews see Stewart & Cullinan, 1997; Carson et al., 2000; Bagchi et al., 2001).

Uterine secretions, produced primarily by the endometrial glands, are hypothesized to be particularly important for conceptus survival and development in sheep, cattle, pigs, and horses, given the prolonged nature of the peri-implantation period that precedes superficial attachment and placentation (Bazer, 1975; Bazer et al., 1979). Adenogenesis is a term for the process of endometrial gland morphogenesis (Bartol et al., 1999; Gray et al. 2001[c]). The importance of endometrial adenogenesis in neonatal ungulates is highlighted by studies demonstrating that inappropriate exposure of the developing neonatal uterus to steroids permanently alters adult endometrial structure and function and compromises conceptus survival and development. Exposure of neonatal ewes to a progestin ablated endometrial gland differentiation and produced adult uterine gland knockout (UGKO) ewes that lacked endometrial glands (Spencer et al., 1999[a]; Gray et al., 2000[a]). Studies of conceptus survival and development in the ovine UGKO uterus showed that a normal glandular endometrium is essential for peri-implantation conceptus survival and growth (Gray et al., 2000[a], 2001[a,b]). Partial to complete UGKO phenotypes have also been produced in adult cows exposed from birth to a combination of progesterone plus estradiol benzoate (P+E) (Bartol et al., 1995, 1999). Consistently, pregnancy rates were reduced in neonatally P+E-exposed adult heifers with reduced endometrial gland numbers. In mares, infertility and sub-fertility are associated with fibrotic lesions in the endometrium that are thought to compromise the integrity and functionality of endometrial glands (Gerstenberg et al., 1999). In sheep (Wimsatt, 1950; Stewart et al., 2000[a]), cattle (King et al., 1981), and pigs (Perry & Crombie, 1982; Sinowatz & Friess, 1983), endometrial glands undergo extensive hyperplasia and hypertrophy during pregnancy, presumably in response to increasing demands of the developing conceptus for uterine histotroph (Bazer, 1975; Samuel et al., 1977; Stewart et al., 2000[a]). In the sheep, rabbit and pig, a servomechanism is proposed to regulate endometrial gland morphogenesis and function during pregnancy that involves sequential actions of ovarian steroid hormones, pregnancy recognition signals, and lactogenic hormones from the pituitary or placenta (Chilton et al. ,1988; Young et al., 1990; Spencer et al., 1999[c]; Gray et al., 2001[c]).

Although a functional role for endometrial glands has been established in nearly all mammalian species, the developmental mechanisms regulating endometrial gland morphogenesis are not well understood. Genetic potential for

uterine function during pregnancy is defined at conception, but the success of developmental events regulating endometrial gland morphogenesis ultimately determines the functional capacity and embryotrophic potential of the adult uterus (Bartol et al., 1993, 1999; Gray et al., 2001c). Therefore, high and unexplained rates of peri-implantation embryonic losses in humans and livestock may reflect, in part, unrecognized defects in endometrial adenogenesis or function induced during critical organizational periods. In women and menstruating primates, the long pre- and peri-pubertal period during which endometrial adenogenesis occurs coupled with the cyclical nature of adult endometrial regeneration, provide significant and repeated opportunities for endometrial dysgenesis and development of pathological lesions that may contribute to infertility. Knowledge of endometrial gland developmental biology may also be useful to increase uterine capacity in livestock and humans. Therefore, the purpose of this review is to summarize current understanding of the comparative developmental biology and function of uterine glands in mammals with emphasis on domestic livestock and humans.

Comparative Developmental Biology of Uterine Gland Morphogenesis

In all mammals, the uterus develops as a specialization of the paramesonephric or Müllerian ducts, which gives rise to infundibula, oviducts, uterus, cervix and anterior vagina (Mossman, 1987). The mature uterine wall is comprised of two functional compartments, the endometrium and myometrium. The endometrium is the inner mucosal lining of the uterus, derived from the inner layer of ductal mesenchyme. Histologically, the endometrium consists of two epithelial cell types, luminal epithelium (LE) and glandular epithelium (GE), two stratified stromal compartments including a densely organized stromal zone (stratum compactum), and a more loosely organized stromal zone (stratum spongiosum), blood vessels and immune cells. The myometrium is the smooth muscle component of the uterine wall that includes an inner circular layer, derived from the intermediate layer of ductal mesenchymal cells, and an outer longitudinal layer, derived from subperimetrial mesenchyme.

Ontogenesis of Endometrial Glands in Livestock, Rodents, and Humans

Morphogenetic events common to development of all mammalian uteri include: (1) organization and stratification of endometrial stroma; (2) differentiation and growth of the myometrium; and (3) differentiation and morphogenesis of the endometrial glands (Bartol et al., 1993, 1999; Gray et al., 2001c). As illustrated schematically in Fig. 1, genesis of uterine glands involves differentiation and budding of GE from LE into the stroma which is followed by GE tubulogenesis and extensive

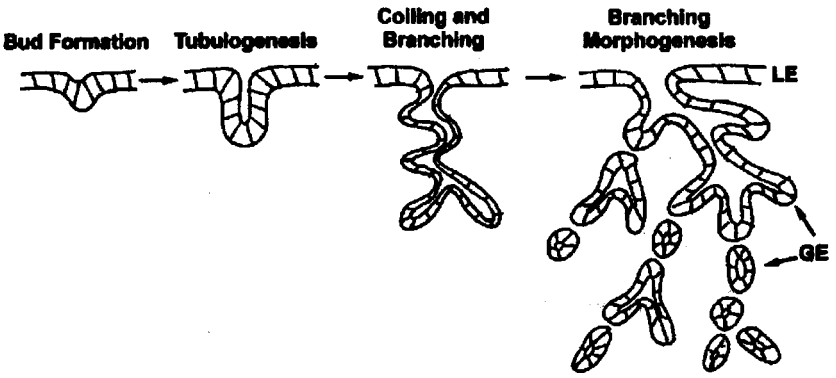

Figure 1. Diagrammatic representation of endometrial adenogenesis in the uterine wall of most species. In many cases where invagination of an epithelium is required for a developmental process, the first step is bud formation that involves conversion of a cuboidal cell to the geometry of a frustrum, a three-dimensional cone with the top removed parallel to the base. This creates a bulge in a basal portion of the epithelial cells at the point where the invagination is to occur. The next step is tubulogenesis, which involves subsequent cell divisions to convert the frustrum into a tube. The simple, tubular glands then begin to coil as they proliferate in the stroma towards the myometrium. The final stage of adenogenesis is the branching morphogenesis of the tubular, coiled glands throughout the stroma until the tips reach the inner circular layer of the myometrium. The overall process of endometrial adenogenesis is similar to adenogenesis that occurs during development of other epitheliomesenchymal organs, such as the mammary gland, salivary gland, and lung. Reprinted with permission from Gray et al. (2001[c]).

coiling and branching morphogenesis. In humans and livestock species, endometrial adenogenesis is completed postnatally and involves extensive coiling and branching morphogenesis (see Fig. 2 for timeline). Consequently, neonatal ungulates (e.g., sheep, cattle, and pigs) provide attractive models for the study of mechanisms regulating these processes (Gray et al., 2001[c]). Although endometrial adenogenesis is also a postnatal event in rodents, the adult rodent uterus does not contain the coiled, branched glands characteristic of endometria in most other mammals. Thus, ungulate and primate species may well provide more relevant models for the study of this process that affects the embryotrophic and functional capacity of the adult uterus in women as well as other mammals.

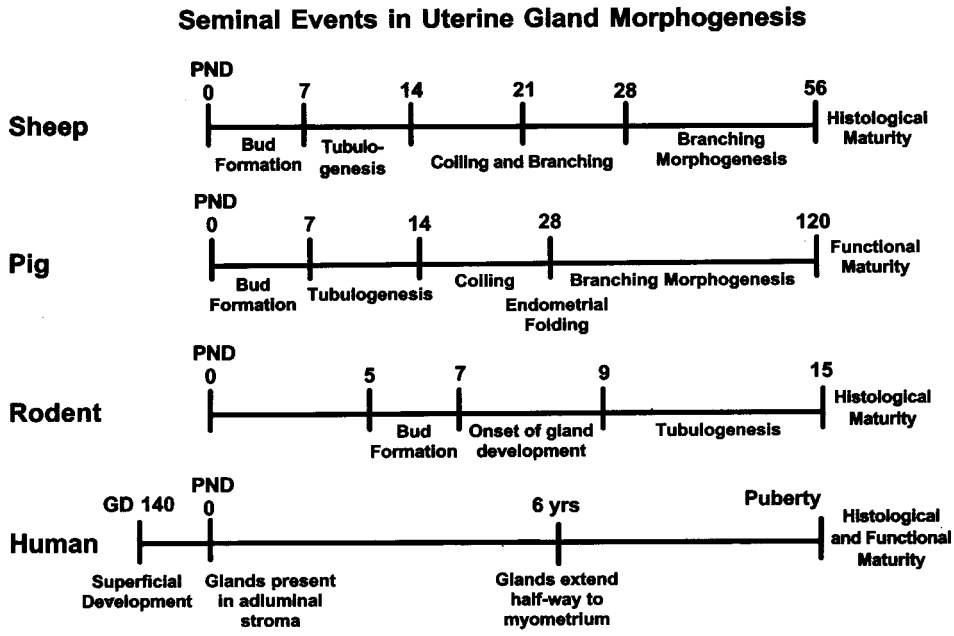

Figure 2. Comparative time line of the seminal events of endometrial gland morphogenesis in the sheep, pig, rodent and human. Postnatal day (PNDO) is the day of birth. GD, gestational day. Reprinted with permission from Gray et al. (2001[c]).

Sheep and cattle

The endometrium in adult sheep and cattle consists of a large number of raised aglandular caruncles and glandular intercaruncular areas (Wimsatt, 1950; Atkinson et al., 1984). Caruncular areas are the sites of superficial implantation and placentation (Wimsatt, 1950; Mossman, 1987). These ruminants have a synepitheliochorial type of placentation in which placental cotyledons fuse with endometrial caruncles to form placentomes which serve a primary role in fetal-maternal gas exchange and derivation of micronutrients by the placenta (Wimsatt, 1950; Wooding, 1982; Mossman, 1987). Intercaruncular endometrial areas contain large numbers of branched, coiled uterine glands which synthesize and secrete or transport a variety of enzymes, growth factors, cytokines, lymphokines, hormones, transport proteins and other substances, collectively termed "histotroph" (Amoroso, 1952; Bazer, 1975).

The dichotomous nature of the adult ruminant endometrium, consisting of both aglandular caruncular areas and glandular intercaruncular areas, makes it an excellent model for the study of mechanisms underlying establishment of divergent structural and functional areas within a single, mesodermally derived organ (Wiley et al., 1987). Uterine morphogenesis has been described in sheep (Davies, 1967; Bryden, 1969; Kennedy et al., 1974; Wiley et al., 1987; Bartol et al., 1988[a,b]; Gray et al., 2000[b]; Taylor et al., 2000, 2001) and, to a lesser extent, in cattle (Marion & Gier, 1971; Atkinson et al., 1984). The ewe and cow have gestation lengths of about 147 days and 284 days, respectively. Paramesonephric duct fusion occurs between gestational day (GD) 34 and 55 in sheep, is partial and produces a bicornuate uterus (Davies, 1967; Bryden, 1969; Wiley et al., 1987). By GD 90, raised aglandular nodules are present that are destined to become caruncles (Wiley et al., 1987; Bartol et al., 1988[a]). Endometrial gland development is first observed as shallow invaginations of LE in internodular clefts between GD 135 and 150 in sheep (Wiley et al., 1987) and on GD 250 in cattle (Marion & Gier, 1971; Atkinson et al., 1984).

Postnatal uterine morphogenesis in sheep and cattle involves the emergence and proliferation of endometrial glands, development of endometrial folds and, to a lesser extent, growth of endometrial caruncular areas and myometrium (Marion & Gier, 1971; Kennedy et al., 1974; Atkinson et al., 1984; Wiley et al., 1987; Bartol et al., 1988[b], 1999; Taylor et al., 2000). The progressive development of endometrial GE from the LE to the inner circular layer of myometrium is a coordinated event that involves bud formation, tubulogenesis, and coiling and branching morphogenesis. In sheep, endometrial gland genesis is initiated between birth (postnatal day or PND 0) and PND 7, when shallow epithelial invaginations appear along the LE in presumptive intercaruncular areas (Bartol et al., 1988[a]; Taylor et al., 2000). Between PNDs 7 and 14, nascent, budding glands proliferate and invaginate into the stroma, forming tubular structures that coil and branch by PND 21 (Taylor et al., 2000).

After PND 21, the majority of glandular morphogenetic activity involves branching morphogenesis of tubular and coiled endometrial glands to form terminal end bud-like structures in deeper stroma. By PND 56, the caruncular and intercaruncular endometrial areas are histoarchitecturally similar to those of the adult uterus. In UGKO ewes, the endometrium lacks a recognizable stratum spongiosum within the stroma that is characteristic of the normal stroma in intercaruncular glandular areas of the uterus (Spencer et al., 1999[a] ; Gray et al., 2000[a,b], 2001[a,b]). Thus, in sheep, development of GE appears to direct or permit differentiation of uterine stroma into subluminal stratum compactum and stratum spongiosum in intercaruncular areas of the endometrium.

Although the ovine uterine wall is histoarchitecturally mature by eight weeks after birth, final maturation and growth may not occur until puberty (Kennedy et al., 1974), or even the first pregnancy (Wimsatt ,1950; Stewart et al., 2000[a]). Extensive endometrial gland hyperplasia and hypertrophy occurs during each pregnancy (Wimsatt, 1950; Stewart et al., 2000[a]), presumably in response to increasing demands for histotrophic support by the growing fetoplacental unit (Bazer, 1975). After parturition in the ewe, intercaruncular endometrial LE remains intact, but contraction of many glands was observed in the days following parturition (O'Shea & Wright, 1984; CA Gray and TE Spencer, unpublished observations). Glandular regeneration commenced by Day 8 postpartum, and the glands were substantially regenerated by Day 15. In caruncles, regeneration of LE commenced after Day 8 and was not complete until Days 28 to 31 postpartum (Van Wyk et al., 1972[a,b]). Caruncular LE appeared to emanate from epithelia in the intercaruncular areas of the endometrium (Van Wyk et al., 1972[a,b]).

Overall, patterns of endometrial gland genesis and development in the neonatal and adult ovine uterus during pregnancy and after parturition are very similar to GE morphogenesis characteristic of the stages of mammogenesis, lactogenesis and involution of the mammary gland (Houdebine et al., 1985). However, the precise mechanisms and factors regulating endometrial gland morphogenesis and regeneration are likely to be different than that in the mammary gland given the differences in organ histoarchitecture and embryonic origin.

Pig

Transformation of the porcine uterine wall from histoarchitectural infancy to maturity occurs within 120 days of birth (Hadek & Getty, 1959; Bal & Getty, 1970; Erices & Schnurrbusch, 1979; Dyck, 1980; Dyck & Swierstra, 1983; Bartol et al., 1993; Spencer et al., 1993; Christenson et al., 1997; Tarleton et al., 1998). Uterine wall development in the neonatal pig is dramatic and includes appearance and proliferation of endometrial GE, organization of the stroma, development of endometrial folds, and growth of the myometrium. Overall patterns of endometrial morphogenesis

in the postnatal gilt are similar to those in the ewe (Bartol et al., 1999; Gray et al., 2001c). Endometrial glands are absent at birth but develop in a rapid, synchronous manner during early postnatal life (Spencer et al., 1993). Shallow, epithelial depressions can be observed on PND 0 which appear to represent primordial bud formations of GE that develop into the coiled, branched uterine glands characteristic of the adult porcine uterus. Endometrial adenogenesis is initiated when GE develops into simple epithelial tubes that extend radially from the luminal surface into the endometrial stroma. Eventually, tubular glands undergo coiling and branching within the stroma until they reach the adluminal border of the myometrium (Bal & Getty, 1970; Spencer et al., 1993; Tarleton et al., 1998, 1999). Mature uterine histoarchitecture is observed by PND 120 in crossbred gilts (Hadek & Getty, 1959; Bal & Getty, 1970; Erices & Schnurrbusch, 1979; Dyck, 1980; Dyck & Swierstra, 1983; Spencer et al., 1993; Christenson et al., 1997; Tarleton et al., 1998).

At birth, the porcine uterus consists of a simple, slightly corrugated columnar epithelium supported by unorganized stromal mesenchyme, encircled by a rudimentary myometrium (Spencer et al., 1993; Christenson et al., 1997; Tarleton et al., 1998). By PND 7, stromal zones, including a shallow stratum compactum and a deep stratum spongiosum, are evident, and distinct, simple, coiled, tubular glands are present throughout shallow stroma. By PND 14, many coiled tubular glands are apparent that extend approximately one-third of the distance from the LE to the myometrium, which has differentiated into inner circular and outer longitudinal layers. On PND 28, many of the coiled glands have obvious branches, and GE is present throughout the endometrial stroma. Additionally, well developed endometrial folds are apparent by PND 28, increasing uterine lumenal surface area. By PND 56, endometrial glandularity is dense and extensive (Spencer et al., 1993; Christenson et al., 1997; Tarleton et al., 1998). The porcine uterus is capable of supporting pregnancy by PND 120, indicating that it is functionally mature (Bartol et al., 1993).

Gestation in the pig lasts for approximately 114 days. On Day 30 of gestation, porcine uterine glands appear as simple, coiled, tubular structures with a narrow lumen (Perry & Crombie, 1982; Sinowatz et al., 1983). The simple columnar GE includes both ciliated and secretory cells. At mid-pregnancy, endometrial glands are highly dilated and filled with substantial amounts of uteroferrin which is a an acid phosphatase and indicator of secretory activity (Roberts & Bazer, 1988). Glandular secretory activity remains high in the last third of pregnancy. After parturition, uterine glands undergo rapid involution (Perry & Crombie, 1982). Areolae are unique placental structures which develop over the mouth of each uterine gland and provide specialized areas for absorption and transport of uterine histotroph (Dantzer, 1984). Indeed, the number of areolae in the placenta and, by inference, the number of uterine glands is directly related to birth weight of the fetus in the pig (Knight et al., 1977).

Horse

Gestation length in the horse is 335 to 340 days. Detailed descriptions of fetal organogenesis and neonatal morphogenesis of the equine uterus are few. Histology of the GD 100, 150 to 160, and 180 to 200 equine uterus was reported by Ginther (Ginther, 1979). On GD 100, the equine uterine wall is immature and composed of a simple LE supported by undifferentiated mesenchyme that is surrounded by a single longitudinal layer of smooth muscle cells. By GD 150 to 160, mitotic figures were described in the simple columnar LE, and endometrial folding was evident. Pronounced folding of the mucosa was apparent on GD 180 to 200. In some areas, the uterine epithelium was corrugated and contained slight invaginations into the stroma, but definitive GE was not present in the endometrium (Ginther, 1979). Equine endometrial gland development from birth to sexual maturity was described recently by Gerstenberg & Allen (1999). In marked contrast to observations for other large domestic animals, including sheep, cattle and pigs, endometrial glands in the neonatal mare are simple tubular structures that remain in a comparatively non-proliferative, juvenile state of development through the first pubertal estrus, but proliferate rapidly during the first diestrus. Observations suggest a unique role for progesterone in equine endometrial adenogenesis and glandular morphogenesis during gestation (Gerstenberg et al., 1999; Gerstenberg & Allen, 1999).The equine conceptus has a unique early association with the maternal endometrium, as it remains spherical and virtually unattached to the endometrium until Day 35 post-ovulation (Van Niekerk & Allen, 1975). Therefore, endometrial glands and their secretions are thought to be particularly important to support conceptus survival during this prolonged pre-implantation period (Samuel et al., 1977). Older mares often display a decreased ability to produce healthy foals as a result of degenerative changes in the endometrium termed endometrosis, a condition that affects the number and morphology of endometrial glands (Waelchi, 1990). These age-related degenerative changes cause deposition of fibrous tissue in the stroma and grouping of endometrial glands into "gland nests" (Kenney, 1978). Degenerated uterine glands display functional abnormalities, including an increase in ciliated cells, atypical mucus production, reduced expression of epidermal growth factor and the P19 lipocalin-like protein, and abnormal patterns of proliferation (Causey et al., 1997; Gerstenberg et al., 1999[a]; Stewart et al., 2000[b]). It has been suggested that abnormal endometrial glands present in uteri of mares exhibiting endometriosis may be responsible for observed decreases in fertility (Schoon et al., 1997; Gerstenberg et al., 1999). Postpartum changes in the equine endometrium include rapid degeneration of microcaruncles and uterine glands (Gomez-Cuetara et al., 1995). By Day 7 postpartum, endometrial histology is similar to that characteristic of normal proestrus, with cuboidal LE and edematous stroma. By Days 9 and 10 postpartum, endometrial histology is similar to that observed in the cycling mare.

Rodents (mouse and rat)

Gestation length in mice and rats is 20 and 21 days, respectively. Paramesonephric duct fusion occurs on GD 15 to 16, is partial and produces a duplex uterus. At birth, uteri of mice and rats lack endometrial glands, and the uterus consists of a simple epithelium supported by undifferentiated mesenchyme. On PND 5, epithelial invaginations appear that represent formation of GE buds (Brody & Cunha, 1989). Genesis of endometrial glands is not observed until PND 7 and 9 in mice and rats, respectively (Branham et al., 1985). In the rat uterus, adenogenesis proceeds from PND 9 through PND 15 (Branham et al., 1985) and results in development of simple, tubular glands that, unlike ungulate endometrial glands, are neither tightly coiled nor extensively branched.

During the pre-implantation period of early pregnancy in these rodents, endometrial glands synthesize and secrete several proteins, such as LIF and calcitonin, required for establishment of uterine receptivity and embryo implantation (for reviews see Stewart & Cullinan, 1997; Armant et al., 2000; Carson et al., 2000; Bagchi et al., 2001). If a successful pregnancy occurs, endometrial glands are ablated by stromal decidualization in response to conceptus implantation. Information is not currently available on the fate of endometrial glands during decidualization or the histoarchitecture of endometrial gland regeneration after parturition during uterine involution in rodent species.

Humans and Menstruating Primates

Humans have a simplex uterus consisting of a single uterine body or corpus lacking uterine horns characteristic of species that possess a bicornuate uterus. Histologically, the adult human and primate endometrium is stratified into two zones, including the stratum functionalis and the stratum basalis (Padykula, 1989). The endometrial functionalis, which is lost during menses, is further subdivided into two parts. Zone I, which is lost almost entirely during menses, consists of LE and subadjacent stroma. Zone II consists of dense stroma surrounding the straight portions of endometrial glands (Padykula, 1989). The endometrial basalis is a dynamic, but structurally stable, compartment of the primate uterus that is not eroded during menstruation or at the end of gestation. This tissue functions as the germinal compartment of the endometrium in these species, and provides stem cells from which the functionalis regenerates within each cycle or after gestation. Histologically, the basalis includes endometrial Zone III, which contains loose stroma and the bodies of uterine glands, and endometrial Zone IV, where endometrial glands terminate and endometrial progenitor and stem cells are thought to reside (Padykula, 1989). The lower portions of the endometrial glands are retained within the stratum basalis (Bensley, 1951).

As in other mammals, the prenatal human uterus is formed by fusion of the paramesonephric ducts, which occurs prior to week eight of gestation

(O'Rahilly, 1973, 1989). As in rodents and ungulate species, the simple columnar epithelium of the undifferentiated uterine body gives rise to numerous invaginations that represent primordial GE buds (Koff , 1933; O'Rahilly, 1973, 1989). By 20 to 22 weeks of gestation the myometrium is well-defined, but endometrial gland development is very superficial (Koff, 1933; Rosa, 1955; Song, 1964). Endometrial histoarchitecture at birth resembles that of the adult, but is less developed. Neonatal endometrial LE is low columnar or cuboidal and GE is sparse and limited to the adluminal stroma (O'Rahilly, 1973; Valdes-Dapena, 1973). From birth to the onset of puberty, uterine glands develop slowly. By six years of age, endometrial glands extend from one-third to one-half of the distance to the myometrium. Mature uterine histoarchitecture is observed at puberty, with endometrial glands extending to the inner circular layer of the myometrium (Valdes-Dapena, 1973). Although initiated during fetal life, endometrial gland proliferation in the human uterus is completed postnatally, in a manner similar to that observed for domestic ungulates. Thus, genesis of endometrial glands in the human fetus and neonate involves differentiation of GE from LE and development of GE through endometrial stroma to the myometrium. This pattern of endometrial development is opposite to that observed for gland genesis in post-menstrual uteri of adult women and primates, where endometrial glands develop adluminally from the basalis during the proliferative phase (Padykula et al., 1984, 1989; Okulicz et al., 1997).

In contrast to non-menstruating primates, rodents and livestock, the pre-menopausal endometrium in adult humans and primates undergoes programmed, phasic changes that include menses, a proliferative phase during the follicular period, and a secretory phase during the luteal period (Hitschmann & Adler, 1908; Noyes et al., 1950; Wynn, 1989). With each menstrual cycle, the functionalis is eroded away during menstruation and regenerates from the basalis during the proliferative phase (Bartelmez et al., 1951). This process is primarily regulated by ovarian steroids (Padykula et al., 1984, 1989); however, responses to these hormones are cell- and endometrial zone-specific (Bensley, 1951; Ferency et al., 1979). As indicated previously, endometrial regeneration and growth of uterine glands during the proliferative period involves stromal and epithelial cells of the deep stratum basalis, which proliferate and organize adluminally to complete formation of the stratum functionalis (Okulicz et al., 1997). Interestingly, when compared to that of livestock and rodents, stromal layers in the primate endometrium are inverted. In menstruating primates, the more densely cellular or compact stroma (stratum compactum) is located in the stratum basalis, adjacent to the myometrium, while more loosely organized stroma (stratum spongiosum) is characteristic of the adluminal primate functionalis. In both adult ungulates and primates, available evidence supports the hypothesis that compact stroma, e.g. stratum compactum or basalis, supports endometrial gland genesis and tubule formation, whereas loose stroma, e.g. stratum spongiosum or

functionalis, supports coiling and branching morphogenesis of proliferating endometrial glands.

In the human uterus, during the proliferative phase, endometrial glands undergo extensive branching morphogenesis as the functionalis is reconstructed (Padykula et al. 1984, 1989; Okulicz et al., 1997). The early proliferative endometrium is thin, contains regenerating superficial epithelium of the functionalis, and endometrial glands that are narrow, short, straight and partially collapsed (Padykula et al., 1989). During the mid-proliferative phase, the glands elongate and become more tortuous. Proliferation is primarily observed in GE on Day 3, but by Day 5 proliferation of the endometrium is occurring in GE of the functionalis, as well as in the surrounding stroma (Okulicz et al., 1997). By the late proliferative phase, the glands are coiled tightly and are obviously branched. Tortuosity and branching of endometrial glands reach a maximum during the secretory phase by Day 8 post-ovulation. During this phase, glands increase in diameter and tortuosity, but do not proliferate (Okulicz et al., 1997). If pregnancy does not occur, endometrial glands regress late in the secretory phase, comensurate with luteolysis, and deteriorate during menses. Similar changes in endometrial histoarchitecture occur in menstruating primates (Fazleabas et al., 1993; Hild-Petito et al., 1994; Okulicz et al., 1997). The fate of and changes in uterine glands during pregnancy in the non-decidualizing area of the uterus have not been well described for either the human or other primates. Recent observations by Burton and Jauniaux (2001) support a primary role for secretions produced by endometrial glands in nutrition of the conceptus during the first trimester of human pregnancy.

Mechanisms Regulating Endometrial Adenogenesis

The process of uterine morphogenesis is governed by a variety of endocrine, cellular and molecular mechanisms, for which details remain to be defined (for reviews see Cunha, 1976; Cunha et al., 1983; Bartol et al., 1993, 1999; Gray et al., 2001[c]). Mechanisms regulating endometrial adenogenesis are similarly unclear. Based on

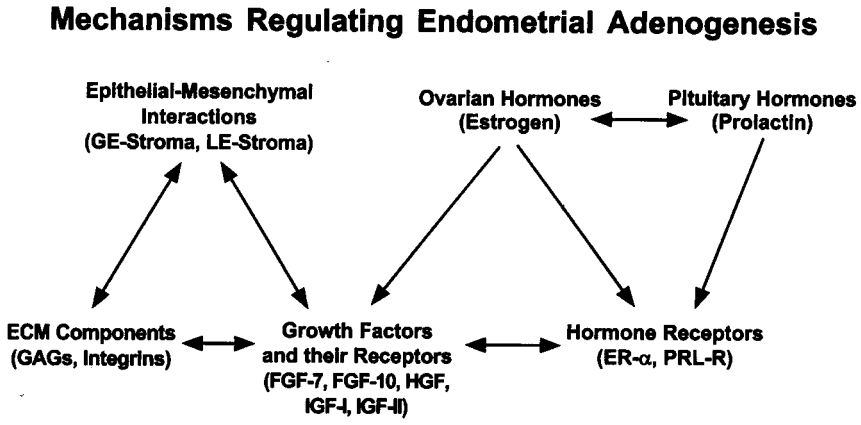

Figure 3. Schematic demonstrating the interactive mechanisms involved in endometrial adenogenesis. Reprinted with permission from Gray et al.(2001[c]).

regulatory mechanisms governing gland development in the uterus and other epithelial-mesenchymal organs, uterine adenogenesis is proposed to involve: (1) site-specific alterations in cell proliferation and movement; (2) paracrine, cell-cell and cell-ECM interactions; and (3) specific endocrine-, paracrine-, and juxtacrine-acting factors and their receptors (see Figure 3). Discovery and understanding of these mechanisms is important, as this knowledge will be useful to enhance uterine capacity and provide treatments for infertility resulting from dysgenesis or dysfunction of uterine glands.

Site-specific Alterations in Cell Proliferation

Differentiation and budding of endometrial GE from LE does not appear to require cell proliferation (Spencer et al., 1993). In developing lung epithelium, bud outgrowth and invagination does not involve localized cell proliferation (Nogawa et al., 1998), but does involve changes in ECM biochemistry in the basal lamina (Mollard et al., 1998). These results are supported by histochemical studies of the stromal-epithelial interface in neonatal ovine (Bartol et al., 1988[b]) and porcine uteri (Spencer et al., 1993). Progestin-induced ablation of endometrial gland genesis in the neonatal ovine uterus does not appear to involve specific suppression of epithelial cell proliferation (Gray et al., 2000[b]). However, evidence of focused proliferation of GE in tips of developing glands, documented in neonatal ovine and porcine uteri, as well as in the adult primate uterus, supports the idea that local microenvironmental conditions are important for gland proliferation (Bartol et al., 1988[a,b]; Padykula et al., 1989; Spencer et al., 1993; Okulicz et al., 1997; Taylor et al., 2000).

Epithelial-mesenchymal Interactions

Uterine development and function depends upon epithelial-mesenchymal interactions (Cunha, 1976; Cunha et al., 1983). These interactions provide local control and coordination of morphogenetically important cell behaviors including movement, adhesion, differentiation and proliferation (Bernfield et al., 1984; Sharpe & Ferguson, 1988). Tissue recombination studies in rodents clearly indicate that uterine mesenchyme directs and specifies patterns of epithelial development, whereas epithelium is required to support organization of endometrial stroma and myometrial differentiation (Cunha, 1976; Cunha et al., 1983, 1989). Results from studies of neonatal ovine (Gray et al., 2000[b]; Taylor et al., 2000, 2001), porcine (Bartol et al., 1993; Spencer et al., 1993) and rodent (Ogasawara et al., 1983) uteri, as well as of the regenerating post-menstrual primate uterus (Okulicz et al., 1997; Padykula et al., 1984, 1989), are consistent with the idea that uterine gland morphogenesis is supported and regulated through interactions between epithelium and stroma. It is through such interactions that developmentally critical tissue microenvironments, necessary to support and maintain spatially focused changes in cell behaviors

associated with gland genesis, are thought to evolve (Bartol et al., 1993, 1999). The concept that interactions between GE and stroma are required for endometrial morphogenesis and establishment of normal uterine histoarchitecture is supported by elegant tissue recombination studies involving the mouse uterus (Cunha, 1976; Cunha & Lung, 1979). In adult UGKO ewes (Spencer et al., 1999[a] ; Gray et al., 2000[a], 2001[a]) the endometrium is not only devoid of GE, but is generally thinner, with a more compact stroma that lacks characteristic zonation necessary for histological delineation of the stratum compactum and stratum spongiosum. This endometrial phenotype is proposed to be generated from the lack of stromal-epithelial interactions that normally occur during epithelial morphogenesis and cytodifferentiation, as well as stromal organization and development (Spencer et al., 1999[a]; Gray et al., 2000[b], 2001[a]).

Epithelial-mesenchymal interactions are mediated, in part, by changes in the composition and distribution of ECM components. Glycosaminoglycans (GAGs), oligosaccharide components of the ECM, can affect cell function directly and indirectly, by mediating access of growth factors and other molecules to their receptors or target cells. During adenogenesis in many tissues, including salivary glands, prostate, and uterus, sulfated GAGs, including chondroitins and heparans, become localized to morphogenetically inactive sites, such as the necks of glands, while non-sulfated GAGs, such as hyaluronic acid, accumulate in morphogenetically active sites, such as the tips of proliferating glands (Cunha & Lung, 1979; Bartol et al., 1988[b]; Bartol et al.,1993; Spencer et al., 1993). Although not investigated in the developing uterus, metalloproteinases and other factors that alter the biochemical nature of the basal lamina, affect both physical and chemical interactions between epithelium and underlying stroma. In this context, the ECM can affect patterns of branching morphogenesis through control of the cell cycle, apoptosis, and related changes in stromal and epithelial gene expression that define such developmental programs (Leliévre et al., 1996; Werb et al., 1996). Thus, elements of cooperative signaling pathways mediating uterine organization are likely to include cell-cell and cell-ECM interactions that facilitate the actions of insoluble signals from the ECM, as well as the actions of soluble hormones and growth factors (Leliévre et al., 1996).

Growth Factors

Communication between epithelium and stroma is facilitated by paracrine and autocrine pathways within uterine tissues that involve peptide growth factors and their receptors (Cooke et al., 1998; Kurita et al., 1998). Stromal-derived growth factors play important roles in epithelial proliferation, differentiation and branching morphogenesis in developing epitheliomesenchymal organs, including the uterus (Cooke et al.,1998; Gray et al., 2000[b]; Hom et al., 1998; Taylor et al., 2001). Interactions between growth factors and their receptors can involve elements of the ECM, which not only affect patterns of growth factor presentation to target cells, but

may also participate as elements of cell surface receptor complexes, as demonstrated for the fibroblast growth factor (FGF) family (McKeehan et al., 1998). Peptide growth factors implicated in uterine development include FGF-7, FGF-10, hepatocyte growth factor (HGF) and insulin-like growth factors one (IGF-I) and two (IGF-II) (Hom et al., 1998; Gu et al., 1999; Taylor et al., 2001).

Stromal growth factors

FGF-7 is an established paracrine growth factor that stimulates epithelial cell proliferation and differentiation (Rubin et al., 1995; Igarashi et al., 1998; Lu et al., 1999). FGF-10, isolated originally from rat lung mesenchyme, was determined to be essential for patterning of early events in branching morphogenesis (Yamasaki et al., 1996; Beer et al., 1997; Bellusci et al., 1997). HGF functions as a paracrine mediator of mesenchymal-epithelial interactions that govern mitogenic, motogenic and morphogenic behaviors of epithelia in developing lung and mammary tissues (Niranjan et al., 1995; Weidner et al., 1993; Ohmichi et al., 1998). In the developing neonatal ovine uterus, FGF-7, FGF-10, HGF and their epithelial receptors were identified as growth factor systems associated with endometrial morphogenesis (Gray et al., 2000[b]; Taylor et al., 2001). Although FGF-7 mRNA was constitutively expressed in uteri from PND 1 to 56, FGF-10 and HGF mRNA levels increased markedly after PND 21, a period characterized by coiling and branching development of endometrial glands in the neonatal ovine uterine wall (Taylor et al., 2001). Further, progestin-induced inhibition of endometrial adenogenesis in the neonatal ewe altered expression patterns of these paracrine-acting growth factors and/or their receptors (Gray et al., 2000[b]).

Insulin-like growth factors

In addition to their roles as mitogens, IGF-I and IGF-II induce cellular differentiation and promote the expression of differentiated functions in cells and tissues (Rotwein, 1991; Jones & Clemmons, 1995; Wang et al., 1999). These IGFs are, therefore, multifunctional regulators of cell proliferation, differentiation, and function that act through autocrine and/or paracrine mechanisms in many organ systems, including the uterus (Wang et al., 1999; Giudice et al., 1993; Thiet et al., 1994). In such tissues, IGFs can also regulate responses to steroid hormones. Examples include complex responses of the immature rodent uterus to estrogens (Ghahary & Murphy, 1989; Murphy & Ghahary, 1990; Murphy, 1991; Gu et al., 1999) and human endometrial proliferative growth responses to ovarian estradiol (Giudice et al., 1993). Null mutation of the IGF-I gene in mice was employed to demonstrate the critical role of this growth factor in normal development of the female reproductive tract (Baker et al., 1996), as well as its requirement for estrogen-induced uterine growth in cyclic female rodents (Adensanya et al., 1997). Mice lacking the

Type 2 IGF receptor (IGF-R) displayed delayed lung development and poor alveolar differentiation, indicating a functional role for this system in lung morphogenesis (Wang et al., 1994). In neonatal rodent and ovine uteri, the IGF system is involved in postnatal uterine morphogenesis and growth (Baker et al., 1996; Gu et al., 1999; Taylor et al., 2001). Gu et al. (1999) observed that IGF-I mRNA expression in the neonatal rat uterus was confined to stroma and myometrium and increased during the developmental period associated with uterine gland genesis. Expression of IGF-II was not detected, although it is present in endometrium from both nonpregnant and pregnant women (Guidice & Saleh, 1995).

In the neonatal ovine uterus, IGF-I and IGF-II are expressed in the stroma surrounding nascent and proliferating endometrial GE, which is both IGF-I receptor (IGF1R) and estrogen receptor-α (ER-α) positive (Taylor et al., 2000, 2001). Cross-talk between ER-α and IGF1R signaling pathways results in synergistic growth stimulation in a number of systems (Aronica & Katzenellenbogan, 1993; Smith, 1998). Activation of ER-α by growth factors like IGF-I involves the mitogen-activated protein kinase (MAPK) pathway via direct serine phosphorylation. In the developing neonatal ovine uterus, the morphogenetically active endometrial glands contained high levels of phosphorylated MAPK (Taylor et al., 2001[b]). In addition, estrogen increases IGF1R protein in the immature rat uterus (Ghahary & Murphy, 1989) and modulates IGF1R function by inducing tyrosine phosphorylation of IGF-I and insulin receptor substrate-1, which is followed by enhanced MAPK activation (Richards et al., 1996, 1998). Collectively, data can be interpreted to suggest that stromal IGF-I and IGF-II may stimulate proliferation of GE and support uterine gland genesis in the neonatal ovine uterine wall through such signaling pathways.

Steroids and Their Receptors

Jost (1973) established the concept that prenatal urogenital tract development in female mammals is an ovary-independent process. Since then, numerous studies have indicated that uterine development and endometrial adenogenesis proceeds normally in the absence of ovarian support for varying periods of time during early postnatal life. In the rat, circulating estrogens increase between PNDs 9 and 11 in association with endometrial adenogenesis (Döhler et al., 1975). However, early postnatal events in rat uterine development and endometrial adenogenesis are both ovary- (Clark & Gorski, 1970) and adrenal-independent (Ogasawara et al., 1983; Branham et al., 1995). In the neonatal pig, Tarleton et al., (1998) determined that ovariectomy at birth, while antiuterotrophic after PND 56, did not affect genesis of uterine glands or related endometrial morphogenetic events prior to PND 120. Similarly, ovariectomy of ewe lambs at birth did not affect patterns of uterine gland genesis on PND 14 (Bartol et al., 1988[b]), but there was reduced uterine weight after PND 28 (Kennedy et al., 1974). This finding suggests a role for

endogenous estrogens in branching morphogenesis, but not differentiation and budding of GE from LE or perhaps GE tubulogenesis.

In the neonatal ewe lamb, the ovary contains significant numbers of growing and vesicular ovarian follicles on PND 14 that are substantial by PND 28 and rapidly decline thereafter (Kennedy et al., 1974). Consistently, serum estradiol-17β was detected at relatively high levels in ewe lambs on PND 1, increased between PNDs 14 and 28, and then declined between PNDs 42 and 56 (Taylor et al., 2000). This pattern of circulating estrogen correlates with the ontogeny of endometrial gland development in the ewe lamb. However, the precise role of ovarian estrogens in ovine neonatal uterine development remains to be determined. In cyclic women and primates, estradiol-17β of ovarian origin is high during the proliferative phase of the menstrual cycle and was proposed to regulate endometrial morphogenesis (Padykula et al., 1984, 1989; Guidice et al., 1993; Okulicz et al., 1997).

Although endometrial adenogenesis is ovary-independent for some period after birth in neonatal rodents, pigs and sheep, genesis of endometrial glands in the neonatal porcine (Tarleton et al., 1999), rodent (Korach et al., 1988; Yamashita et al., 1989; Greco et al., 1991; Fishman et al., 1996) and ovine (Taylor et al., 2000) uteri involves coordinated changes in epithelial phenotype that are marked by ER-α expression in nascent and proliferating endometrial GE. Homozygous ER-α null mice (α ERKO) have hypoplastic uteri that contain all characteristic cell types in reduced proportions (Lubahn et al., 1993), including reduced uterine gland numbers (Curtis et al., 1999). Thus, ER-α expression is not essential for fetal murine uterine organogenesis, but is essential for normal postnatal uterine growth and development (Lubahn et al., 1993).

In the neonatal rat uterus, gland genesis occurs between PNDs 9 and 15, and treatment with estradiol-17β from PND 10 to 14 delayed the onset of gland genesis (Branham et al., 1985). Interestingly, treatment with estradiol-17β from PNDs 1 to 5 induced premature gland genesis, but ultimately reduced gland numbers between PNDs 15 to 26 (Branham et al., 1985). Mechanisms mediating these age-specific effects of estrogen on uterine gland genesis are not known, but could be due to estrogen-induced negative regulation of ER-α expression. Treatment of rats with the anti-estrogen tamoxifen, a mixed ER-α agonist/antagonist, from PNDs 1 to 5 or PNDs 10 to 14, elicited a dose-dependent inhibition of uterine gland genesis (Branham et al., 1985). Co-treatment of neonatal rats with ICI 182,780, a Type-II antiestrogen that is antiuterotrophic but not antiadenogenic in neonates of this species, inhibited characteristic tamoxifen-induced effects on endometrial gland development (Branham et al., 1996). Observations were interpreted to suggest that tamoxifen acts as an ER-α agonist in this physiological context. Compounds such as tamoxifen and estradiol-17β, both of which cause pronounced uterine hypertrophy in the rat when administered as uterine glands are developing postnatally, disrupt the normal process

of gland genesis. The first step in uterine gland development involves differentiation of GE from the LE and invagination of nascent gland buds into underlying stroma. Therefore, it is conceivable that the hypertrophic state induced by ER-α agonists or mixed agonists/antagonists in the endometrium prevents invagination of GE physically due to alterations in cell shape and associated changes in cell-cell, and cell-ECM relationships that would otherwise support this process (Leliévre et al., 1996; Werb et al., 1996). Under such conditions, epithelial cells could be unable to recognize and properly integrate and respond to signals that normally drive gland genesis (Branham et al., 1996; Leliévre et al., 1996; Werb et al., 1996). While this does not explain how endometrial gland genesis is initiated, it does provide an explanation of how the process might be disrupted through disorganization of local control mechanisms at the tissue level.

In the neonatal pig, administration of ICI 182,780 from birth inhibited endometrial adenogenesis on PND 14 (Tarleton et al., 1999). These results support the idea that uterine ER-α expression and changes in state of uterine ER-α activation, which may be species-specific, are important elements of the organizational program that determines patterns of uterine growth and endometrial morphogenesis. In this regard, elegant tissue recombination studies involving mouse uterine stroma and epithelium indicate that epithelial ER-α is neither necessary nor sufficient to mediate the mitogenic actions of estrogen (Cooke et al., 1997, 1998). In addition to direct ligand-dependent activation of epithelial ER-α, proliferative effects of estrogen on epithelium appear to be mediated primarily by stromal ER-á via production of paracrine-acting, stromal-derived growth factors such as epidermal growth factor (EGF) and IGF-I (Cooke et al., 1998).

In neonatal ewes, all uterine cell types are ER-α-positive on PND 1 (Taylor et al., 2000). Endometrial morphogenesis is accompanied by ER-α expression in emerging, proliferating and developing GE, as well as in the surrounding stroma. A requirement for ER-α in ovine uterine adenogenesis is supported by the finding that progestin-induced ablation of endometrial gland genesis in neonatal ewes involves suppression of epithelial ER-α expression (Gray et al., 2000[b]). Ablation of endometrial gland genesis in neonatal ewes treated with norgestomet from birth may reflect loss or attenuation of ER-α-dependent signaling. Ovarian estradiol-17β and growth factors, such as IGF-I, IGF-II and EGF, are also likely to be involved in mediation of endometrial adenogenesis characteristic of the proliferative phase of the menstrual cycle (Giudice et al., 1993; Guidice & Saleh, 1995). While critical experiments remain to be conducted, gland morphogenesis in the neonatal ovine endometrium is most likely an ER-α-dependent phenomenon not unlike that described for the neonatal pig (Bartol et al., 1999; Tarleton et al., 1998, 1999) and rat (Branham et al., 1985).

ER-α can be activated by estrogens, in a ligand-dependent manner, or by growth factor-coupled pathways, in a ligand-independent manner (Ignar-Trowbridge

et al., 1995; Smith, 1998). Transient transfection experiments indicate that ligand-independent ER-α activation can be induced by many factors including dopamine, EGF, transforming growth factor α (TGFα), heregulin, and IGF-I (Aronica & Katzenellenbogan, 1993; Ma et al., 1994; Ignar-Trowbridge et al., 1995; Pietras et al., 1995; Patrone et al., 1996). The EGF-like growth factor, heregulin, stimulated proliferation and progesterone receptor (PR) gene expression in an ER-α-dependent manner in MCF-7 cells (Pietras et al., 1995). In the neonatal ovine uterus and proliferative phase human endometrium, high levels of PR expression were detected in uterine epithelia that was attributed to ER-α activation (Snijders et al., 1992; Taylor et al., 2000). Progesterone levels in serum of neonatal ewes and follicular phase women are low or undetectable, and not likely to be sufficient to inhibit endometrial gland development (Taylor et al., 2000). The precise roles and significance of ligand-dependent and ligand-independent actions of ER-α in endometrial gland adenogenesis remain to be determined.

Prolactin

In other epitheliomesenchymal organs, prolactin (PRL) stimulates epithelial differentiation and development in a manner that may be facilitated by cooperative ECM signaling (Leliévre et al., 1996; Bole-Feysot et al., 1998; Freeman et al., 2000). In neonatal ewes, serum PRL concentrations are high at birth, increase between PNDs 1 and 14 and then decline to PND 56 (Ebling et al., 1989; Taylor et al., 2000). This pattern correlates well to the onset of endometrial gland proliferation in the developing uterine wall. Indeed, mRNAs for both the short and long PRL receptor (PRLR) proteins were found to be expressed in nascent and proliferating endometrial GE, and their relative levels of expression increased 7-fold between PNDs 7 and 56 (Taylor et al., 2000). In the adult endometrium of sheep, humans and primates (Jones et al., 1998; Frasor et al., 1999; Stewart et al., 2000[a]), the PRLR gene is also expressed exclusively by endometrial glands and, in ewes, increased PRLR expression during pregnancy correlates with hyperplasia and hypertrophy of endometrial glands (Stewart et al., 2000[a]). Expression of the PRLR gene is associated with GE in the deeper coiled and branched regions of endometrial glands of the neonatal and adult ovine endometrium. Whether PRL and the PRLR system play a role in terminal gland development, bud formation or branching morphogenesis in the neonatal ovine uterus remains to be determined. In the adult mouse, rabbit and pig, hyperprolactinemia stimulates uterine gland hyperplasia (Chilton et al., 1988; Young et al., 1990; Kelly et al., 1997). Similarly in sheep, intrauterine administration of placental lactogen (PL), a member of the PRL/growth hormone (GH) family, stimulated proliferation of endometrial glands, particularly the terminal ends of coiled, branched glands found in deeper stroma of adult ewes (Spencer et al., 1999[c]). Given the central role of PRLR in mammary gland morphogenesis and function (Houdebine et al., 1985; Brisken et

al., 1999), expression of PRLR in GE of the developing neonatal and adult ovine uterus may play a similar role.

Several lines of evidence support the idea that proliferation of uterine GE and genesis of uterine glands could involve PRLR-dependent, estrogen-independent activation of ER-α in developing endometrial GE. Prolactin can stimulate an increase in ER-α expression in the rat (Telleria et al., 1998). Activation of both short and long forms of the PRL-R stimulates MAPK signaling (Camarillo et al., 1997; Cheng et al., 2000). Thus, PRL stimulation of PRLR in developing uterine GE could activate the MAPK signaling cascade, resulting in serine phosphorylation, ligand-independent activation of ER-α, and up-regulation of ER-α, as well as IGF1R gene expression (Taylor et al., 2000, 2001). Other complementary pathways involving the PRLR system could also be involved in regulation of development and proliferation of uterine GE. In human endometrial stroma, PRLR mRNA expression is up-regulated by both IGF-I and estrogen (Tseng et al., 1998). Moreover, stimulation of human secretory phase endometrium with PRL increases interferon regulatory factor one (IRF-1) expression by GE (Dalrymple & Jabbour, 2000), which may increase cell proliferation (Yu-Lee et al., 1998). These data, taken together with the fact that PRLR gene expression has been documented in adult endometrium of sheep, humans, mice, rats and primates (Jones et al., 1998; Frasor et al., 1999; Dalrymple & Jabbour, 2000; Stewart et al., 2000[a]) in association with periods of hyperplasia and hypertrophy of GE, support the idea that this receptor system and its cognate ligands are important mechanistic components of the developmental program that regulates endometrial morphogenesis and uterine gland development in both neonatal and adult life.

Functional Role of Endometrial Glands

Endometrial glands are present in all mammalian uteri and produce secretions, termed histotroph (Amoroso, 1952; Bazer, 1975). Histotroph is complex and contains numerous binding and nutrient transport proteins, ions, mitogens, cytokines, lymphokines, glucose, enzymes, hormones, growth factors, protease inhibitors and mainly other substances (for reviews see Bazer, 1975; Bazer & First, 1983; Roberts & Bazer, 1988; Simmen & Simmen, 1990; Kane et al., 1997; Martal et al., 1997; Carson et al., 2000). Histotroph is proposed to be involved in trophoblast growth regulation, conceptus attachment and implantation, and perhaps immunological protection of the fetus (Fléchon et al., 1986; Roberts & Bazer, 1988; Geisert et al., 1992; Lee et al., 1998).

The components found in histotroph have been demonstrated to be essential in many species to support early conceptus survival and growth. In addition, histotroph from the endometrial glands has also been hypothesized to be an important supplement to hematotrophic nutrition during mid- to late pregnancy in domestic livestock (Wimsatt, 1950; Heap et al., 1979; Stewart et al., 2000[a]) as well as humans

(Burton & Jaunaiux, 2001). Compared to domestic livestock which exhibit superficial implantation and an epitheliochorial type of placentation, implantation in the human establishes a precocious and intimate apposition between the maternal and fetal tissues (Burton & Jauniaux, 2001). In the past it has been assumed that this relationship permits early onset of hematotrophic exchange. However, Burton and Jauniaux (2001) suggest that human pregnancy comprises two contrasting periods. During the first trimester there is little maternal bloodflow to the placenta, the oxygen tension within the feto-placental unit is low, and the uterine glands may provide much of the nutrient supply. At the start of the second trimester the maternal circulation within the intervillous space becomes fully established, the oxygen tension rises and hematotrophic nutrition becomes dominant. In addition, the endometrial glands remain functional in the human uterus throughout gestation (Graham J. Burton, personal communication).

Animal Models Illustrating Functional Importance of Endometrial Glands

In rodents, two specific substances only produced by the endometrial GE, leukemia inhibitory factor (LIF) and calcitonin, have been identified as necessary for implantation (Bhatt et al., 1991; Zhu et al., 1998[a]). In the sheep, findings from the UGKO ewe model indicates that components of histotroph are required for peri-implantation conceptus survival and development (Gray et al., 2000[a], 2001[a,b]). In humans and domestic livestock, models are needed to address the role of endometrial glands in uterine support of conceptus growth and development during mid- to late gestation.

Leukemia Inhibitory Factor (LIF)

In the rodent, the period of pre-implantation embryonic development is extremely short in comparison with domestic livestock and, in particular, the horse. The murine blastocyst enters the uterus, hatches from the zona pellucida, and begins to implant on Day 4 (Day 0=fertilization) (Finn & McLaren, 1967; Orcini & McLaren, 1967). Although the pre-implantation period is short, at least two factors produced only by the endometrial glands are required for successful establishment of uterine receptivity and conceptus implantation. One substance required for conceptus development that is present in mouse uterine secretions is LIF (for review see Stewart & Cullinan, 1997; Vogiagis & Salomonsen, 1999; Carson et al., 2000). LIF is a pleiotropic cytokine that exhibits a multitude of biological effects including modulation of cell proliferation and differentiation. In mice, LIF is expressed only in the endometrial glands and exhibits distinct temporal changes in expression during early pregnancy. A burst of LIF expression is detected on Day 1 of pregnancy that declines by Day 3 (Bhatt et al., 1991). A second burst of LIF expression occurs in the endometrial glands on Day 4 which is the beginning of implantation. After Day 4,

LIF expression is detected at low levels; a decline that is likely the result of gland ablation by decidualization. The increased circulating levels of estrogen on Days 3-4 of pregnancy is suggested to stimulate uterine LIF expression (Bhatt et al., 1991; Shen & Leder, 1992). Studies using LIF knockout (LIF-KO) mice have demonstrated that maternal LIF is necessary for normal implantation (Stewart et al., 1992). LIF-KO mice are viable and ovulate normally, but blastocysts fail to implant. Although LIF is expressed normally in pseudopregnant mice, the endometrium of LIF-KO mice is unable to undergo a decidual response naturally, or following artificial stimulation with intrauterine injection of paraffin oil (Stewart et al., 1992). Embryos from LIF-KO mice are viable and will implant in wild type mice. Recent evidence indicates that LIF from the endometrial glands is important for preparation of the uterus that is required for blastocyst activation (Carson et al., 2000). In particular, expression of heparin-binding EGF (HB-EGF) is not detected in LIF-KO mice (Song et al., 2000). In humans, maximal LIF expression is detected in the endometrial glands during the secretory phase of the menstrual cycle (Cullinan et al., 1996). LIF has also been identified in uterine flushings from rabbits (Yang et al., 1995), pigs (Anegon et al., 1994), sheep (Vogiagis et al., 1997), and western spotted skunk (Hirzel et al., 1999). LIF mRNA is expressed throughout the ovine estrous cycle and exhibits an increase at the beginning of implantation (Day 16). Passive immunization of ewes against LIF to neutralize its activity decreased pregnancy rates to 33.5%, even though LIF activity was only reduced and not ablated. Thus, LIF may be obligatory or facilitory for implantation in sheep (Vogiagis et al., 1997). Available evidence supports an essential role for LIF in embryo implantation across a wide variety of species.

Calcitonin

Calcitonin is a 32-amino acid peptide hormone previously thought to be produced exclusively by the parafollicular C cells of the thyroid gland as a regulator of calcium in bone and kidney cells (Foster, 1968). Calcitonin was identified as a gene expressed transiently by the endometrial glands of the uterus during the peri-implantation period (Ding et al., 1994). In rats, calcitonin expression increases in the endometrial glands on Day 2 of pregnancy and reaches a peak on Day 4 which is the day before implantation. On the day of implantation (Day 5), calcitonin expression declines and is undetectable by Day 6. In contrast to LIF expression in the endometrial glands, expression is progesterone-dependent (Zhu et al., 1998[a]). Using a delayed implantation rat model, Zhu et al., (1998[a]) demonstrated that levels of calcitonin remained high past Day 6, until the administration of estrogen to induce blastocyst implantation. Intrauterine injection of antisense oligonucleotides against calcitonin mRNAs suppressed calcitonin expression and dramatically reduced the number of implantation sites in rats (Zhu et al., 1998[b]). Therefore, calcitonin is an essential component of histotroph that is required for blastocyst implantation. Recent evidence indicates that calcitonin actions on the early embryo include modulation of

intracellular calcium oscillations (Armant et al., 2000). These may serve as a signaling event in embryonic development and a way for calcitonin to regulate trophoblast adhesion and/or integrin trafficking to modulate implantation events (Wang et al., 1998).

In the human, calcitonin expression is restricted to the endometrial epithelium of the midsecretory phase of the cycle, which closely overlaps the window of implantation (Kumar et al., 1998). In addition, progesterone regulates expression of calcitonin in human endometrium. Therefore, calcitonin is a unique marker of uterine receptivity in both the mouse and human.

Ovine uterine gland knockout (UGKO) model

The UGKO ewe is a novel model in which endometrial gland morphogenesis is epigenetically ablated by administration of a synthetic, non-metabolizable 19-norprogestin during the critical period of endometrial gland morphogenesis in the developing neonatal ewe (Spencer et al., 1999[a]; Gray et al., 2000[a]). The progestin specifically ablates development of the GE within the endometrium without altering development of the uterine myometrium or other Müllerian duct-derived female reproductive tract structures (Spencer et al., 1999; Gray et al., 2000[a], 2001[a,b]). UGKO ewes fail to cycle normally (Gray et al., 2000[a]). The estrous cycle of ewes is dependent on the production of luteolytic pulses of prostaglandin F2α (PGF) as a result of oxytocin binding of oxytocin receptors (OTRs) present on the uterine epithelia (McCracken et al., 1999). In the UGKO ewe, expression of hormone receptors (PR, ER-α and OTR) in the uterine LE is not different from that observed in normal cyclic ewes. Oxytocin challenges to measure uterine release of PGF found that UGKO ewes are unable to release a luteoltyic pulse of PGF (Gray et al., 2000[a]). Therefore, available evidence suggests that expression of OTR in the superficial GE in the uterine endometrium are necessary for cyclicity.

Despite repeated matings to fertile rams, adult UGKO ewes do not establish pregnancy (Gray et al., 2000[a], 2001[b,c]). Transfer of normal hatched blastocysts into the uteri of timed recipient UGKO ewes fails to ameliorate the pregnancy defect (Gray et al., 2001[c]). Normal blastocysts can be found in the uterine flushes of bred UGKO ewes on Days 6 or 9 post-mating, but not on Day 14 (Gray et al., 2001[b,c]). On Day 14, the uterine flush of bred UGKO ewes either contains no conceptus or a severely growth-retarded conceptus that has failed to properly elongate (Gray et al., 2001[c]).

Implantation in ruminants is a highly coordinated process that involves apposition, attachment, and adhesion of the endometrial lumenal epithelium (LE) and conceptus trophectoderm (Guillomot, 1995). The peri-implantation period is marked by rapid elongation of the ovine conceptus from a tubular to filamentous form on Day 13 and the production of large amounts of interferon tau (IFNτ), the signal for maternal recognition of pregnancy (Bazer et al., 1998). Elongation of the

conceptus is a developmental event that requires the uterus, because hatched blastocyts fail to elongate *in vitro* unless transferred into the uterus (Flèchon et al., 1986). In sheep, apposition of conceptus trophectoderm and lumenal epithelia (LE) is initiated on Day 14, followed quickly by adhesion on Day 15, and attachment on Days 16 to 18 (Guillomot et al., 1981; Guillomot, 1995). Adhesion of the conceptus trophectoderm to the LE is regulated by non-adhesive and adhesive factors (Burghardt et al., 1997; Johnson et al., 2001). The non-adhesive property of the LE is partially due to apical expression of mucins, such as Muc-1, that sterically impair interactions between the trophectoderm and adhesive glycoproteins, such as integrins, due to their extensive glycosylation and extended extracellular structure. Immunoreactive Muc-1 expression on the LE progressively decreases between Days 9 and 17 of early pregnancy in the ewe (Johnson et al., 2001). In the UGKO ewes, expression of Muc-1 on the LE is not different from that found in normal ewes (CA Gray & TE Spencer, unpublished observations). Therefore, failure of conceptus elongation in UGKO ewes is not due to aberrant expression of anti-adhesive proteins.

Integrins are thought to be the dominant glycoproteins that regulate trophectoderm adhesion to the LE in both caruncular and intercaruncular areas. During the peri-implantation period of pregnancy in the ewe, integrin subunits αv, α4, α5, β1, β3 and β5 were constitutively expressed on the conceptus trophectoderm as well as the apical surface of uterine epithelia (Johnson et al., 2001). However, integrin expression in the Day 14 UGKO uterus is not different in the LE as compared to Day 14 pregnant normal ewes (Gray et al., 2001[d]; CA Gray & TE Spencer, unpublished results). Therefore, available evidence supports the hypothesis that the inability of the conceptus to survive and develop within the UGKO uterus is due to a lack of certain adhesion proteins and perhaps growth factors normally derived from the endometrial glands.

The endometrial glands of the ovine uterus produce two adhesion molecules, osteopontin (OPN) (Johnson et al., 1999[a,b], 2000) and gycosylation-dependent cell adhesion molecule (GlyCAM)-1 (Spencer et al., 1999[b]). These proteins are solely synthesized and secreted by the endometrial GE during the peri-implantation period and are thought to aid in conceptus elongation, adhesion, and attachment of conceptus to the LE (Johnson et al., 2001). Immunoreactive levels of OPN and GlyCAM-1 are much lower or absent in the uterine flushings of Day 14 bred UGKO ewes (Gray et al., 2001). Previous studies have found no differences in LE of steroid hormone receptors (Gray et al., 2000[a]) and LE-specific genes (Gray et al., 2001[c]) in UGKO compared to normal ewes. Thus, the LE of UGKO ewes does not appear to be the underlying cause of peri-implantation conceptus growth retardation and mortality. Rather, the absence of uterine gland secretions, or histotroph, in UGKO ewes appears to be responsible for the inability of the UGKO uterus to support conceptus survival development. Current efforts involve coupling the UGKO ewe model with genomic and proteomic strategies to identify specific secretions of the endometrial glands that are important for peri-implantation conceptus survival and growth.

Mechanisms Regulating Endometrial Gland Morphogenesis and Function in the Adult Uterus

Production of histotroph by the endometrium can be regulated by several different mechanisms. The most common regulators of histotroph production are the ovarian steroid hormones, estrogen and progesterone. In rodents, estrogen induces the production of many secretory proteins from the epithelium (Julian et al., 1992). Signals from the conceptus can also modulate the production of secretions by the uterus. In sheep, interactions between the maternal endometrium and the production of IFNτ by the trophectoderm cells of the conceptus results in simultaneous stimulation and inhibition of the secretion of proteins (Godkin et al., 1984; Vallet et al., 1987). Similarly, in the sow a large "dumping" of histotrophic secretions into the lumen of the endometrial glands occurs immediately following the release of estrogens from the conceptus on Day 11 of pregnancy (Geisert et al., 1982; Fazleabas et al., 1983). The hormonal, cellular and molecular mechanisms regulating uterine gland morphogenesis and function during gestation are not well understood in all species. In both the rabbit and pig, interactions between lactogenic hormones and ovarian steroids were proposed to constitute a "servomechanism" regulating endometrial function (Chilton et al., 1988; Young et al., 1990). Similarly, a hormonal servomechanism also appears to be operative in the ovine uterus that regulates endometrial gland differentiation and function during gestation (Spencer et al., 1999[c]).

Endometrial glands undergo a major period of remodeling and growth during pregnancy in sheep. The uterine glands increase in length (four-fold) and width (ten-fold) (Wimsatt, 1950; Stewart et al., 2000[a]). Thus, the GE undergoes intense cellular proliferation and greatly increases the secretory surface area.

Table 1. Hormones secreted by the ovine conceptus that are involved in a servomechanism. Sequential production of these placental hormones are necessary for maintenance of pregnancy in the sheep. IFNτ, interferon tau; PRL-R, prolactin receptor; GH-R, growth hormone-receptor.

Hormone	IFNτ	Placental Lactogen	Growth Hormone
Day	8-20	16-147	35-70
Produced By	Mononuclear cells	Binucleate cells	Syncytial cells
Binds To	Type I IFN receptor	Homodimer PRLR Heterodimer PRLR/GHR	Homodimer GHR
Function	Signal for maternal recognition of pregnancy	Stimulation of GE proliferation and differentiated function	
	Maintain corpus luteum (progesterone production)		
	Increased secretion of histotroph		

Established endometrial glands produce additional side-branchings during early pregnancy to provide even more glandular surface area for the production of histotroph (Wimsatt, 1950). During pregnancy in sheep (see Table 1), the endometrium is exposed sequentially to estrogen, progesterone, IFNτ, oPL and oGH, that may activate and maintain endometrial remodeling, secretory function, and uterine growth (Spencer et al., 1999[c]). Estrogen from ovulatory and non-ovulatory follicular waves increases expression of the PR in the endometrial epithelia (Spencer & Bazer, 1995; Bazer et al., 1998). Progesterone from the newly formed corpus luteum increases the expression of a number of unique progesterone-responsive genes in the endometrial LE and/or GE (Spencer et al., 1999[a]). However, continuous exposure of the endometrium to progesterone down-regulates expression of the PR in the endometrial LE and then GE (Spencer & Bazer, 1995; Spencer et al., 1999[c]). Between Days 11 and 15 of pregnancy, PR expression is down-regulated in the LE and superficial GE followed closely by the middle to deep GE. IFNτ serves as the signal for maternal recognition of pregnancy in ruminants, is produced by mononuclear cells of the conceptus trophectoderm between Days 8 to 21 in sheep (maximally on Days 14 to 16), and acts in a paracrine manner on the adult endometrium (Ashworth & Bazer, 1989). IFNτ maintains pregnancy by preventing development of the endometrial luteolytic mechanism to maintain the corpus luteum and maternal progesterone production (Bazer et al., 1998).

Progesterone down-regulation of PR expression in the GE is actually necessary for the onset of certain secretory genes, such as the uterine milk proteins (UTMP) and OPN, in the endometrial glands (Spencer et al., 1999[c]). Secretion of oPL by the binucleate cells of the conceptus trophectoderm begins on Day 16 of pregnancy, with peak secretion occurring from Days 120 to 130 of gestation (Kelly et al., 1974; Wooding et al., 1992). PL can bind to the long form of the PRLR (Schuler et al., 1997) as well as to a heterodimer of a PRLR and GH receptor (GHR) (Herman et al., 2000; Noel et al., 2001), which is expressed exclusively by GE and increases throughout gestation (Stewart et al., 2000[a]). Growth hormone is secreted by the ovine placenta on Days 35 to 70 of gestation and binds to endometrial GHR (Lacroix et al., 1996). Sequential intrauterine administration of ovine IFNô to ewes from Days 11 to 15 post-estrus, followed by ovine PL from Days 21 to 25, increased proliferation of GE in the deep stratum spongiosum. Further, infusion of oGH into the uterine lumen from Days 21 to 25 increased uterine gland density in the deep stratum spongiosum, and increased the size of endometrial glands in the shallow stratum spongiosum (Spencer et al., 1999[c]). These studies indicate that a developmentally programmed sequence of events, mediated by specific paracrine-acting factors at the conceptus-endometrial interface, ultimately supports both endometrial remodeling and induction or increases in uterine secretory activity (i.e., expression of uterine milk protein and OPN genes) during ovine gestation. Whether similar gestational servomechanisms regulate uterine gland development and function in other species remains to be determined. However, strategic manipulation of such mechanisms may offer therapeutic

schemes designed to improve uterine capacity, conceptus survival and reproductive health.

Concluding Remarks

Available data indicate that endometrial glands are required for normal uterine function. Mechanisms regulating endometrial development and the genesis, proliferation and function of endometrial glands are complex, and can be altered permanently by transient exposure of tissues to endocrine disrupting compounds during critical developmental periods, which are species-specific. The uterine organizational events that are subject to disruption under specific endocrine conditions or circumstances of xenobiotic exposure, must be defined if effective guidelines for optimizing reproductive development and uterine function are to be developed. Parallels in endometrial developmental biology between human, primate and ungulate species are significant. Consequently, domestic ungulates, including sheep, cattle and pigs, constitute practical and important models for studies aimed at understanding hormonal, ceullar and molecular mechanisms regulating endometrial development and uterine adenogenesis in the neonate and gland morphogenesis in the adult.

Studies of conceptus development in the ovine UGKO model, in which endometrial gland development was prevented by strategic endocrine disruption of early postnatal uterine organizational events, provides definitive evidence that peri-implantation conceptus survival and growth can be related directly to the presence and state of development of endometrial glands in the adult uterus. For instance, the number of areolae in the placenta and, by inference the number of uterine glands, is directly related to birth weight of the fetus in the pig. This finding supports the idea that one aspect controlling uterine capacity in pig is the number of endometrial glands. Therefore, strategies to enhance uterine adenogenesis in the neonate may be useful to enhance adult uterine capacity in livestock and humans. In humans and menstruating primates, regular, cyclical recrudescence of the endometrial functionalis offers repeated opportunities for developmental disruption. Such organizationally induced alterations in human endometrial gland formation and function may lead to infertility and early pregnancy loss. Consistent with observations in other species, endometrial glands and their secretions are critical for embryonic development in humans. Decreased expression of cell-surface and secretory proteins within the human uterine environment are correlated with abnormal uterine gland morphology that adversely affect uterine receptivity during the peri-implantation period and early stages of placentation (Lessey et al., 1992; Klentzeris et al., 1994; Dockery et al., 1996). An enhanced understanding of normal development will allow for the discovery of mechanisms that contribute to endometrial gland dysgenesis and

dysfunction leading to infertility as well as pathologies including adenomyosis, endometriosis and uterine cancer. Moreover, development of therapeutic strategies to enhance uterine adenogenesis and gland morphogenesis during gestation may be useful to increase uterine capacity, thereby enhancing reproductive success in terms of neonatal survivability and health. Future investigations into mechanisms regulating the process of uterine gland development and endometrial morphogenesis during gestation will undoubtedly provide insight into factors affecting embryonic survival and development in humans and livestock.

References

Adesanya O, Zhou J, Simpson S, Powell-Braxton L, Bondy CA. IGF-I is essential for estrogen-induced uterine growth. In: Program of 79th Annual Meeting of the Endocrine Society. Minneapolis, MN; 1997: Abstract P1-176.

Amoroso EC. Placentation. In: Parkes AS (ed.), Marshall's Physiology of Reproduction, 3rd edition, volume 2. London: Longmans Green; 1952:127-311.

Anegon I, Cuturi MC, Godard A, Moreau M, Terqui M, Martinat-Botte F, Soulillou JP. Presence of leukaemia inhibitory factor and interleukin 6 in porcine uterine secretions prior to conceptus attachment. Cytokine 1994;6:493-9.

Armant DR, Wang J, Liu Z. Intracellular signaling in the developing blastocyst as a consequence of the maternal-embryonic dialogue. Semin Reprod Med 2001; 18:273-87.

Aronica SM, Katzenellenbogen BS. Stimulation of estrogen receptor-mediated transcription and alteration in the phosphorylation state of the rat uterine estrogen receptor by estrogen, cyclic adenosine monophosphate, and insulin-like growth factor-I. Mol Endocrinol 1993;7:743-52.

Ashworth CJ, Bazer FW. Changes in ovine conceptus and endometrial function following asynchronous embryo transfer or administration of progesterone. Biol Reprod 1989;40:425-33.

Atkinson BA, King GJ, Amoroso EC. Development of the caruncular and intercaruncular regions in the bovine endometrium. Biol Reprod 1984;30:763-4.

Bagchi IC, Li Q, Cheon YP. Role of steroid hormone-regulated genes in implantation. Ann N Y Acad Sci 2001; 943:68-76.

Baker J, Hardy MP, Zhou J, Bondy C, Lupu F, Bellve AR, Efstratiadis A. Effects of an *Igf1* gene null mutation on mouse reproduction. Mol Endocrinol 1996;10: 903-18.

Bal HS, Getty R. Postnatal growth of the swine uterus from birth to six months. Growth 1970;34:15-30.

Bartelmez GW, Corner GW, Hartman CG. Cyclic changes in the endometrium of the rhesus monkey (*Macaca mulatta*). Contrib Embryol., Carnegie Institut. 1951;34:99-146.

Bartol FF, Johnson LL, Floyd JG, Wiley AA, Spencer TE, Buxton DF, Coleman DA. Neonatal exposure to progesterone and estradiol alters uterine morphology and luminal protein content in adult beef heifers. Theriogenology 1995;43:835-44.

Bartol FF, Wiley AA, Coleman DA, Wolfe DF, Riddel MG. Ovine uterine morphogenesis: effects of age and progestin administration and withdrawal on neonatal endometrial development and DNA synthesis. J Anim Sci 1988[b]; 66:3000-9.

Bartol FF, Wiley AA, Floyd JG, Ott TL, Bazer FW, Gray CA, Spencer TE. Uterine differentiation as a foundation for subsequent fertility. J Reprod Fertil Suppl 1999; 53:284-300.

Bartol FF, Wiley AA, Goodlett DR. Ovine uterine morphogenesis: histochemical aspects of endometrial development in the fetus and neonate. J Anim Sci 1988; 66:1303-13.

Bartol FF, Wiley AA, Spencer TE, Vallet JL, Christenson RK. Early uterine development in pigs. Reprod Fert Suppl 1993;48:99-116.

Bazer FW, First NL. Pregnancy and parturition. J Anim Sci 1983; (Suppl 2) 57: 425-460.

Bazer FW, Roberts RM, Thatcher WW. Actions of hormones on the uterus and effect of conceptus development. J Anim Sci (Suppl 2) 1979;49:35-45.

Bazer FW, Spencer TE, Ott TL. Endocrinology of the transition from recurring estrous cycles to establishment of pregnancy in subprimate mammals. In: Bazer FW, (ed.) The Endocrinology of Pregnancy. New Jersey: Humana Press; 1998:1-34.

Bazer FW. Uterine protein secretions: relationship to development of the conceptus. J Anim Sci 1975;41:1376-82.

Beer H-D, Florence C, Dammeier J, McGuire L, Werner S, Duan R. Mouse fibroblast growth factor 10: cDNA cloning, protein characterization, and regulation of mRNA expression. Oncogene 1997;15:2211-8.

Bell SC, Drife JO. Secretory proteins of the endometrium--potential markers for endometrial dysfunction. Baillieres Clin Obstet Gynaecol 1989;3:271-91.

Bell SC. Secretory endometrial/decidual proteins and their function in early pregnancy. J Reprod Fertil Suppl 1988;36:109-25.

Bellusci S, Grindley J, Emoto H, Itoh N, Hogan BLM. Fibroblast growth factor 10 (FGF10) and branching morphogenesis in the embryonic mouse lung. Development 1997;124:4867-78.

Bensley CM. Cyclic fluctuations in the rate of epithelial mitosis in the endometrium of the rhesus monkey. Contrib Embryol, Carnegie Instit 1951;34:89-98.

Bernfield MR, Banerjee SD, Koda JE, Rapraeger AC. Remodeling of the basement membrane: morphogenesis and maturation. Ciba Found Symp 1984;108:79.

Bhatt H, Brunet LJ, Stewart CL. Uterine expression of leukemia inhibitory factor coincides with the onset of blastocyst implantation. Proc Natl Acad Sci USA 1991; 88:11408-12.

Bole-Feysot C, Goffin V, Edery M, Binart N, Kelly PA. Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. Endocrine Rev 1998;19:225-68.

Bonnett R. Die Uterinmilch und ihre Bedeutung für die Fruct. *Beträge zur Biologie als Fetgabe dem Anatomen und Physiologen.* Th. von Bischoff, Stuttgart; 1882:221-63.

Branham WS, Fishman R, Streck RD, Medlock KL, De George JJ, Sheehan DM. ICI 182,780 inhibits endogenous estrogen-dependent rat uterine growth and tamoxifen-induced developmental toxicity. Biol Reprod 1996;54:160-7.

Branham WS, Sheehan DM. Ovarian and adrenal contributions to postnatal growth and differentiation of the rat uterus. Biol Reprod 1995;53:863-72.

Branham WS, Sheehan DM, Zehr DR. Medlock KL, Nelson CJ, Ridlon E. Inhibition of rat uterine gland genesis by tamoxifen. Endocrinology 1985;117:2238-48.

Branham WS, Sheehan DM, Zehr DR, Ridlon E, Nelson CJ. The postnatal ontogeny of rat uterine glands and age-related effects of 17β-estradiol. Endocrinology 1985; 117:2229-37.

Brisken C, Kaur S, Chavarria TE, Binart N, Sutherland RL, Weinberg RA, Kelly PA, Ormandy CJ. Prolactin controls mammary gland development via direct and indirect mechanisms. Dev Biol 1999;210:96-106.

Brody JR, Cunha GR. Histologic, morphometric, and immunocytochemical analysis of myometrial development in rats and mice: I. Normal development. Am J Anat 1989;186:1-20.

Bryden MM. Prenatal developmental anatomy of the sheep, with particular reference to the period of the embryo (11 to 34 days). DVM Thesis. Ithaca, NY: Cornell University; 1969.

Burghardt RC, Bowen JA, Newton GR, Bazer FW. Extracellular matrix and the implantation cascade in pigs. J Reprod Fertil Suppl 1997;52:151-64.

Burton GJ, Jaunaiux E. Maternal vascularization of the human placenta: does the embryo develop in a hypoxic environment. Gynecol Obstet Fertil 2001; 29: 503-8.

Camarillo IG, Linebaugh BE, Rillema JA. Differential tyrosyl-phosphorylation of multiple mitogen-activated protein kinase isoforms in response to prolactin in Nb2 lymphoma cells. Proc Soc Exp Biol Med 1997;215:198-202.

Carson DD, Bagchi I, Dey SK, Enders AC, Fazleabas AT, Lessey BA, Yoshinaga K. Embryo implantation. Dev Biol 2000; 223:217-37.

Causey RC, Ginn PS, LeBlanc MM. Mucus production of the equine endometrium: effects of cycle stage and susceptibility to equine endometritis. Pferdeheilkunde 1997;13:543 (abstract).

Cheng Y, Zhizhin I, Perlman RL, Mangoura D. Prolactin-induced cell proliferation in PC12 cells depends on JNK but not ERK activation. J Biol Chem 2000; 275:23326-32.

Chilton BS, Mani SK, Bullock DW. Servomechanism of prolactin and progesterone in regulating uterine gene expression. Mol Endocrinol 1988;2:1169-75.

Christenson RK, Bartol FF, Vallet JL, Wiley AA, Spencer TE. Comparative study of uterine morphogenesis and protein secretion in neonatal White crossbred and Meishan gilts. Biol Reprod 1997;56:1112-29.

Clark JH, Gorski J. Ontogeny of the estrogen receptor during early uterine development. Science 1970;169:76-8.

Cooke PS, Buchanan DL, Lubahn DB, Cunha GR. Mechanism of estrogen action: lessons from the estrogen receptor-alpha knockout mouse. Biol Reprod 1998;59:470-5.

Cooke PS, Buchanan DL, Young P, Setiawan T, Brody J, Korach KS, Taylor J, Lubahn DB, Cunha GR. Stromal estrogen receptors mediate mitogenic effects of estradiol on uterine epithelium. Proc Natl Acad Sci USA 1997; 94:6535-40.

Cullinan EB, Abbondanzo SJ, Anderson PS, Pollard JW, Lessey BA, Stewart CL. Leukemia inhibitory factor (LIF) and LIF receptor expression in human endometrium suggests a potential autocrine/paracrine function in regulating embryo implantation. Proc Natl Acad Sci U S A 1996;93:3115-20.

Cunha GR, Chung LWK, Shannon JM, Taguchi O, Fujii H. Hormone-induced morphogenesis and growth: role of mesenchymal-epithelial interactions. Recent Prog Horm Res 1983;39:559-95.

Cunha GR, Lung B. The importance of stroma in morphogenesis and functional activity of urogenital epithelium. In Vitro 1979;15:50-71.

Cunha GR. Stromal induction and specification of morphogenesis and cytodifferentiation of the epithelia of the Mullerian ducts and urogenital sinus during development of the uterus and vagina in mice. J Exp Zool 1976;196:361-70.

Cunha GR, Young P, Brody JR. Role of uterine epithelium in the development of myometrial smooth muscle cells. Biol Reprod 1989;40:861-71.

Curtis SW, Clark J, Myers P, Korach KS. Disruption of estrogen signaling does not prevent progesterone action in the estrogen receptor á knockout mouse uterus. Proc Natl Acad Sci USA 1999;96:3646-51.

Dalrymple A, Jabbour HN. Localization and signaling of the prolactin receptor in the uterus of the common marmoset monkey. J Clin Endocrinol Metab 2000; 85:1711-18.

Dantzer V. Scanning electron microscopy of the exposed surfaces of the porcine placenta. Acta Anat 1984;188:96-106.

Davies J. Comparative embryology. In: Wynn RM (ed.), Cellular Biology of the Uterus. New York: Appleton-Century Crofts; 1967:13-32.

Ding Y-Q, Zhu L-J, Bagchi MK, Bagchi IC. Progesterone stimulates calcitonin gene expression in the uterus during implantation. Endocrinology 1994;135:2265-74.

Dockery P, Pritchard K, Warren MA, Li TC, Cooke ID. Changes in nuclear morphology in the human endometrial glandular epithelium in women with unexplained infertility. Hum Reprod 1996;11:2251-6.

Döhler KD, Wuttke W. Changes with age in levels of serum gonadotropins, prolactin and gonadal steroids in prepubertal male and female rats. Endocrinology 1975; 97:898-907.

Dyck GW. Normal and abnormal development and puberty in gilts and boars. In: Morrow D (ed.), Current Therapy in Theriogenology. Philadelphia: Saunders Co.; 1980:1107-12.

Dyck GW, Swierstra EE. Growth of the reproductive tract of the gilts from birth to puberty. Can J Anim Sci 1983;63:81-7.

Ebling FJ, Wood RI, Suttie JM, Adel TE, Foster DL. Prenatal photoperiod influences neonatal prolactin secretion in the sheep. Endocrinology 1989;125:384-91.

Erices J, Schnurrbusch U. Uterus development in swine from birth to 8 months of age. Arch Exp Veterinarmed 1979;33:457-73.

Fazleabas AT, Donnelly KM, Hild-Petito S, Hausermann HM, Verhage HG. Secretory proteins of the baboon (Papio anubis) endometrium: regulation during the menstrual cycle and early pregnancy. Hum Reprod Update 1997;3:553-9.

Fazleabas AT, Donnelly KM, Mavrogianis PA, Verhage HG. Secretory and morphological changes in the baboon (*Papio anubis*) uterus and placenta during early pregnancy. Biol Reprod 1993;49:695-704.

Fazleabas AT, Geisert RD, Bazer FW, Roberts RM. The relationship between the release of plasminogen activator and estrogen by blastocysts and secretion of plasmin inhibitor by uterine endometrium in the pregnant pig. Biol Reprod 1983; 29:225-38.

Ferenczy A, Bertrand G, Gelfand MM. Proliferation kinetic of human endometrium during the normal menstrual cycle. Am J Obstet Gynecol 1979;133:859-67.

Finn CA, McLaren A. A study of the early stages of implantation. J Reprod Fertil 1967;13:259-67.

Fishman RB, Branham WS, Streck RD, Sheehan DM. Ontogeny of estrogen receptor messenger ribonucleic acid expression in the postnatal rat uterus. Biol Reprod 1996;55:1221-30.

Fléchon J-E, Guillomot M, Charlier M, Fléchon B, Martal J. Experimental studies on the elongation of the ewe blastocyst. Reprod Nutr Dev 1986;26:1017-24.

Foster GV. Calcitonin (Thyrocalcitonin). New Eng J Med 1968;279:349-60.

Frasor J, Gaspar CA, Donnelly KM, Gibori G, Fazleabas AT. Expression of prolactin and its receptor in the baboon uterus during the menstrual cycle and pregnancy. J Clin Endocrinol Metab 1999;84:3344-50.

Freeman ME, Kanyicska B, Lerant A, Nagy G. Prolactin: structure, function, and regulation of secretion. Physiol Rev 2000;80:1523-631.

Geisert RD, Renegar RH, Thatcher WW, Roberts RM, Bazer FW. Establishment of pregnancy in the pig: I. Interrelationships between preimplantation development of the pig blastocyst and uterine endometrial secretions. Biol Reprod 1982;27:925-41.

Gerstenberg C, Allen WR. Development of the equine endometrial glands from fetal life to ovarian cycling. J Reprod Fert 1999; Suppl 56:317-26.

Gerstenberg C, Allen WR, Stewart F. Cell proliferation patterns during development of the equine placenta. J Reprod Fertil 1999[a]; 117:143-52.

Gerstenberg C, Allen WR, Stewart F. Factors controlling epidermal growth factor (EGF) gene expression in the endometrium of the mare. Mol Reprod Devel 1999[b]; 53:255-65.

Ghahary A, Murphy LJ. Uterine insulin-like growth factor-I receptors: regulation by estrogen and variation throughout the estrous cycle. Endocrinology 1989;125: 597-604.

Ginther OJ. Placentation and Embryology. In: Ginther OJ (ed.), Reproductive Biology of the Mare, 1st edition. Ann Arbor, MI: McNaughton and Gunn, Inc. 1979:255-320.

Giudice LC, Dsupin BA, Jin IH, Vu TH, Hoffman AR. Differential expression of messenger ribonucleic acids encoding insulin-like growth factors and their receptors in human uterine endometrium and decidua. J Clin Endocrinol Metab 1993;76:1115-22.

Giudice LC, Saleh W. Growth factors in reproduction. Trends Endocrinol Metab 1995; 6:60-9.

Given RL, Enders AC. The endometrium of delayed and early implantation. In: Wynn RM, Jollie WP (eds), Biology of the Uterus, 2nd edition. New York: Plenum Medical Book Company; 1989:175-231.

Godkin JD, Bazer FW, Roberts RM. Ovine trophoblast protein 1, an early secreted blastocyst protein, binds specifically to uterine endometrium and affects protein secretion. Endocrinology 1984;114:120-30.

Goldring SR, Gorn AH, Yamin M, Krane SM, Wang JT. Characterization of the structural and functional properties of cloned calcitonin receptor cDNAs. Hormone Met Res 1993;25:477-80.

Gomez-Cuetara C, Flores JM, Sanchez J, Rodriguez A, Sanchez MA. Histological changes in the uterus during postpartum in the mare. Anat Histol Embryol 1995;24:19-23.

Gray CA, Bartol FF, Tarleton BJ, Wiley AA, Johnson GA, Bazer FW, Spencer TE. Developmental biology of uterine glands. Biol Reprod 2001[c];65:1311-23.

Gray CA, 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[a];62:448-56.

Gray CA, Bazer FW, Spencer TE. Effects of neonatal progestin exposure on female reproductive tract structure and function in the adult ewe. Biol Reprod 2001[a]; 64:797-804.

Gray CA, Johnson GA, Burghardt RC, Bazer FW, Spencer TE. The defect in conceptus elongation in uterine gland knockout (UGKO) ewes is due to an absence of endometrial glands, but not differences in expression of lumenal epithelial adhesion molecules. Biol Reprod 2001[d]; 64 (suppl. 1):188 (Abstract 218).

Gray CA, Taylor KM, Bazer FW, Spencer TE. Mechanisms regulating norgestomet inhibition of endometrial gland morphogenesis in the neonatal ovine uterus. Mol Reprod Dev 2000[b];57:67-78.

Gray CA, Taylor KM, Ramsey WS, Hill JR, Bazer FW, Bartol FF, Spencer TE. Endometrial glands are required for pre-implantation conceptus elongation and survival. Biol Reprod 2001[b];64:1608-13.

Greco TL, Furlow JD, Duello TM, Gorski J. Immunodetection of estrogen receptors in fetal and neonatal female mouse reproductive tracts. Endocrinology 1991; 129:1326-32.

Gu Y, Branham WS, Sheehan DM, Webb PJ, Moland CL, Streck RD. Tissue-specific expression of messenger ribonucleic acids for insulin-like growth factors and insulin-like growth factor-binding proteins during perinatal development of the rat uterus. Biol Reprod 1999;60:1172-82.

Guillomot M. Cellular interactions during implantation in domestic ruminants. J Reprod Fertil 1995;49:39-51.

Guillomot M, Fléchon J-E, Wintenberger-Torres S. Conceptus attachment in the ewe: an ultrastructural study. Placenta 1981;2:169-82.

Hadek R, Getty R. The changing morphology in the uterus of the growing pig. Amer J Vet Res 1959;20:573-7.

Harvey MB, Leco KJ, Arcellana-Panililo MY, Zhang X, Edwards DR, Schultz GA. Proteinase expression in early mouse embryos is regulated by leukaemia inhibitory factor. Development 1995;121:1005-14.

Heap RB, Flint AP, Gadsby JG. Role of embryonic signals in the establishment of pregnancy. Brit Med Bull 1979;35:129-35.

Herman A, Bignon C, Daniel N, Grosclaude J, Gertler A, Djiane J. Functional heterodimerization of prolactin and growth hormone receptors by ovine placental lactogen. J Biol Chem 2000;275:6295-301.

Hild-Petito S, Verhage HG, Fazleabas AT. Characterization, localization, and regulation of receptors for insulin-like growth factor I in the baboon uterus during the cycle and pregnancy. Biol Reprod 1994;50:791-801.

Hirzel DJ, Wang J, Das SK, Dey SK, Mead RA. Changes in uterine expression of leukemia inhibitory factor during pregnancy in the western spotted skunk. Biol Reprod 1999; 60:484-92.

Hitschmann F, Adler L. Der Bu der Uterusschleimhaut des geschlechtsreifen Weibes mit besonderer Berucksichtigung der Menstruation. Monatscchr Geburtshilfe Gynaekol 1908;27:1-82.

Hom YK, Young P, Thomson AA, Cunha GR. Keratinocyte growth factor injected into female mouse neonates stimulates uterine and vaginal epithelial growth. Endocrinology 1998;139:3772-9.

Houdebine LM, Djiane J, Dusanter-Fourt I, Martel P, Kelly PA, Devinoy E, Servely JL. Hormonal action controlling mammary activity. J Dairy Sci 1985; 68: 489-500.

Igarashi M, Finch PW, Aaronson SA. Characterization of recombinant human fibroblast growth factor (FGF)-10 reveals functional similarities with keratinocyte growth factor (FGF-7). J Biol Chem 1998;273:13230-5.

Ignar-Trowbridge DM, Pimentel M, Teng CT, Korach KS, McLachlan JA. Cross talk between peptide growth factor and estrogen receptor signaling systems. Environ Health Perspect 1995;103:35-8.

Johnson GA, Bazer FW, Jaeger LA, Ka H, Garlow JE, Pfarrar C, Spencer TE, Burghardt RC. Muc-1, integrin, and osteopontin expression during the implantation cascade in sheep. Biol Reprod 2001;65:820-8.

Johnson GA, Burghardt RC, Spencer TE, Newton GR, Ott TL, Bazer FW. Ovine osteopontin: II. Osteopontin and αvβ3 integrin expression in the uterus and conceptus during the periimplantation period. Biol Reprod 1999[b];61:892-9.

Johnson GA, Spencer TE, Burghardt RC, Bazer FW. Ovine osteopontin: I. Cloning and expression of messenger ribonucleic acid in the uterus during the periimplantation period. Biol Reprod 1999[a];61:884-91.

Johnson GA, Spencer TE, Burghardt RC, Taylor KM, Gray CA, Bazer FW. Effects of progesterone and interferon-tau on osteopontin gene expression in the ovine uterus. Biol Reprod 2000;62:622-7.

Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. Endocrine Rev 1995;16:3-34.

Jones RL, Critchley HO, Brooks J, Jabbour HN, McNeilly AS. Localization and temporal expression of prolactin receptor in human endometrium. J Clin Endocrinol Metab 1998;83:258-62.

Jost A. Studies on sex differentiation in mammals. Rec Prog Horm Res 1973; 29:1-41.

Julian J, Carson DD, Glasser SR. Polarized rat uterine epithelium *in vitro*: constitutive expression of estrogen-induced proteins. Endocrinology 1992;130:79-87.

Kane MT, Morgan PM, Coonan C. Peptide growth factors and preimplantation development. Hum Reprod Update 1997;3:137-57.

Kelly MA, Rubinstein M, Asa SL, Zhang G, Saez C, Bunzow JR, Allen RG, Hnasko R, Ben-Jonathan N, Grandy DK, Low MJ. Pituitary lactotroph hyperplasia and chronic hyperprolactinemia in dopamine D2 receptor-deficient mice. Neuron 1997;19:103-13.

Kelly PA, Robertson HA, Friesen HG. Temporal pattern of placental lactogen and progesterone secretion in sheep. Nature 1974;248:435-7.

Kennedy JP, Worthington CA, Cole ER. The post-natal development of the ovary and uterus of the merino lamb. J Reprod Fertil 1974;36:275-82.

Kenney RM. Cyclic and pathologic changes of the mare endometrium as detected by biopsy, with a note on early embryonic death. J Am Vet Med Assoc 1978; 172:241-62.

King GJ, Atkinson BA, Robertson HA. Development of the intercaruncular areas during early gestation and establishment of the bovine placenta. J Reprod Fertil 1981;61:469-74.

Klentzeris LD, Bulmer JN, Seppala M, Li TC, Warren MA, Cooke ID. Placental protein 14 in cycles with normal and retarded endometrial differentiation. Hum Reprod 1994;9:394-8.

Knight JW, Bazer FW, Thatcher WW, Franke DE, Wallace HD. Conceptus development in intact and unilaterally hysterectomized-ovariectomized gilts: interrelations among hormonal status, placental development, fetal fluids and fetal growth. J Anim Sci 1977;44:620-37.

Koff AK. Development of the vagina in the human fetus. Contr Embryol Carneg Inst 1933;24:59-90.

Korach KS, Horigome T, Tomooka Y, Yamashita S, Newbold RR, McLachlan JA. Immunodetection of estrogen receptor in epithelial and stromal tissues of neonatal mouse uterus. Proc Natl Acad Sci USA 1988;85:3334-7.

Kumar S, Zhu LJ, Polihronis M, Cameron ST, Baird DT, Schatz F, Dua A, Ying YK, Bagchi MK, Bagchi IC. Progesterone induces calcitonin gene expression in human endometrium within the putative window of implantation. J Clin Endocrinol Metab 1998;83:4443-50.

Kurita T, Young P, Brody JR, Lydon JP, O'Malley BW, Cunha G. Stromal progesterone receptors mediate the inhibitory effects of progesterone on estrogen-induced uterine epithelial cell deoxyribonucleic acid synthesis. Endocrinology 1998;139:4708-13.

Lacroix MC, Devinoy E, Servely JL, Puissant C, Kann G. Expression of the growth hormone gene in ovine placenta: detection and cellular localization of the protein. Endocrinology 1996;137:4886-92.

Lee RSF, Wheeler TT, Peterson AJ. Large-format, two-dimensional polyacrylamide gel electrophoresis of ovine periimplantation uterine luminal fluid proteins: identification of aldose reductase, cytoplasmic actin, and transferrin as conceptus-synthesized proteins. Biol Reprod 1998;59:743-52.

Leliévre S, Weaver VM, Bissell MJ. Extracellular matrix signaling from the cellular membrane skeleton to the nuclear skeleton: A model of gene regulation. Rec Prog Horm Res 1996;51:417-32.

Lessey BA, Damjanovich L, Coutifaris C, Castelbaum A, Albelda SM, Buck CA. Integrin adhesion molecules in the human endometrium. Correlation with the normal and abnormal menstrual cycle. J Clin Invest 1992:90:188-95.

Lu W, Luo Y, Kan M, McKeehan WL. Fibroblast growth factor-10 a second candidate stromal to epithelial cell andromedin in prostate. J Biol Chem 1999;274:12827-34.

Lubahn DB, Moyer JS, Golding TS, Couse JF, Korach KS, Smithies O. Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. Proc Natl Acad Sci USA 1993; 90:11162-6.

Ma ZQ, Santagati S, Patrone C, Pollio G, Vegeto E, Maggi A. Insulin-like growth factors activate estrogen receptor to control the growth and differentiation of the human neuroblastoma cell line SK-ER3. Mol Endocrinol 1994;8:910-8.

Marion GB, Gier HT. Ovarian and uterine embryogenesis and morphology of the non-pregnant female mammal. J Anim Sci 1971;32(Suppl 1):24-47.

Martal J, Chene N, Camous S, Huynh L, Lantier F, Hermier P, L'Haridon R, Charpigny G, Charlier M, Chaouat G. Recent developments and potentialities for reducing embryo mortality in ruminants: the role of IFN-ô and other cytokines in early pregnancy. Reprod Fertil Dev 1997;9:355-80.

McCracken JA, Custer EE, Lamsa JC. Luteolysis: a neuroendocrine-mediated event. Physiol Rev 1999;78:263-323.

McKeehan WL, Wang F, Kan M. The heparan sulfate-fibroblast growth factor family: diversity of structure and function. Prog Nucleic Acid Res Mol Biol 1998;59:135-76.

Mollard R, Dziadek M. A correlation between epithelial proliferation rates, basement membrane component localization patterns, and morphogenetic potential in the embryonic mouse lung. Am J Respir Cell Mol Biol 1998;19:71-82.

Mossman HA. Vertebrate Fetal Membranes. New Brunswick, NJ: Rutgers University Press; 1987.

Murphy LJ. Estrogen induction of insulin-like growth factors and myc proto-oncogene expression in the uterus. J Steroid Biochem 1991;40:223-30.

Murphy LJ, Ghahary A. Uterine insulin-like growth factor-1: regulation of expression and its role in estrogen-induced uterine proliferation. Endocr Rev 1990;11:443-53.

Niranjan B, Buluwela L, Yant J, Perusinghe N, Atherton A, Phippard D, Dale T, Gusterson B, Kamalati T. HGF/SF: a potent cytokine for mammary growth, morphogenesis and development. Development 1995;121:2897-908.

Noel SD, Herman A, Gertler A, Burghardt RC, Bazer FW, Spencer TE. Evidence for ovine placental lactogen binding to a prolactin receptor and growth hormone receptor heterodimer in ovine endometrial glands. Biol Reprod 2001;64(suppl. 1):30 (Abstract 4).

Nogawa H, Morita K, Cardoso WV. Bud formation precedes the appearance of differential cell proliferation during branching morphogenesis of mouse lung epithelium *in vitro*. Dev Dyn 1998;213:228-35.

Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. Fertil Steril 1950; 1:3-25.

Ogasawara Y, Okamoto S, Kitamura Y, Matsumoto K. Proliferative pattern of uterine cells from birth to adulthood in intact, neonatally castrated and/or adrenalectomized mice, assayed by incorporation of [^{125}I] iododeoxyruridine. Endocrinology 1983;113:582-7.

Ohmichi H, Koshimizu U, Matsumoto K, Nakamura T. Hepatocyte growth factor (HGF) acts as a mesenchyme-derived morphogenic factor during fetal lung development. Development 1998;125:1315-24.

Okulicz WC, Ace CI, Scarrell R. Zonal changes in proliferation in the rhesus endometrium during the late secretory phase and menses. Proc Soc Exp Biol Med 1997;214:132-8.

O' Rahilly R. Prenatal human development. In. RM Wynn and WP Jollie (eds.), Biology of the Uterus. New York: Plenum Publishing Corporation; 1989:35-56.

O'Rahilly R. The embryology and anatomy of the uterus. In: HJ Norris, AT Hertig and MR Abell (eds.), The Uterus. Baltimore, MD: Williams and Wilkins Company. 1973:17-39.

Orcini MW, McLaren A. Loss of the zona pellucida in mice and the effect of tubal ligation and ovariectomy. J Reprod Fertil 1967;13:485-99.

O'Shea JD, Wright PJ. Involution and regeneration of the endometrium following parturition in the ewe. Cell Tissue Res 1984;236:477-85.

Padykula HA. Regeneration in the primate uterus: the role of stem cells. In: Biology of the Uterus. Wynn RM, Jollie WP. Plenum Medical Book Company: New York, 1989:279-87.

Padykula HA, Coles LG, McCracken JA, King NW Jr, Longcope C, Kaiserman-Abramof IR. A zonal pattern of cell proliferation and differentiation in the rhesus endometrium during the estrogen surge. Biol Reprod 1984;31:1103-18.

Padykula HA, Coles LG, Okulicz WC, Rapaport SI, McCracken JA, King, Jr NW, Longcope C, Kaiserman-Abramof IR. The basalis of the primate endometrium: a bifunctional germinal compartment. Biol Reprod 1989;40:681-90.

Patrone C, Ma ZQ, Pollio G, Agrati P, Parker MG, Maggi A. Cross-coupling between insulin and estrogen receptor in human neuroblastoma cells. Mol Endocrinol 1996; 10:499-507.

Perry JS, Crombie PR. Ultrastructure of the uterine glands of the pig. J Anat 1982; 134:339-50.

Pietras RJ, Arboleda J, Reese DM, Wongvipat N, Pegram MD, Ramos L, Gorman CM, Parker MG, Sliwkowski MX, Slamon DJ. HER-2 tyrosine kinase pathway targets estrogen receptor and promotes hormone-independent growth in human breast cancer cells. Oncogene 1995;10:2435-46.

Renfree MB. Diapause, pregnancy, and parturition in Australian marsupials. J Exp Zool 1993;266:450-62.

Richards RG, DiAugustine RP, Petrusz P, Clark GC, Sebastian J. Estradiol stimulates tyrosine phosphorylation of the insulin-like growth factor-1 receptor and insulin receptor substrate-1 in the uterus. Proc Natl Acad Sci USA 1996; 93:12002-7.

Richards RG, Walker MP, Sebastian J, DiAugustine RP. Insulin-like growth factor-1 (IGF-1) receptor-insulin receptor substrate complexes in the uterus. Altered signaling response to estradiol in the IGF-1(m/m) mouse. J Biol Chem 1998; 273:11962-9.

Roberts RM, Bazer FW. The function of uterine secretions. J Reprod Fertil 1988; 82:875-92.

Rosa P. Endocrinologie sexuelle du foetus feminin. Paris: Masson, 1955.

Rotwein P. Structure, evolution, expression and regulation of insulin-like growth factors I and II. Growth Factors 1991;5:3-18.

Rowson LEA, Moor RM. Embryo transfer in the sheep: the significance of synchronizing oestrus in the donor and recipient animal. J Reprod Fertil 1966;11:207-12.

Rubin JS, Bottaro DP, Chedid M, Miki T, Ron D, Cheon G, Taylor WG, Fortney E, Sakata H, Finch PW, LaRochelle WJ. Keratinocyte growth factor. Cell Biol Int 1995;19:399-411.

Samuel CA, Allen WR, Steven DH. Studies on the equine placenta III. Ultrastructure of the uterine glands and the overlying trophoblast. J Reprod Fert 1977;51:433-7.

Schoon HA, Schoon D, Klug E. The endometrial biopsy in the mare with regards to clinical correlation. Pferdeheilekunde 1997;13:453.

Schuler LA, Nagel RJ, Gao J, Horseman ND, Kessler MA. Prolactin receptor heterogeneity in bovine fetal and maternal tissues. Endocrinology 1997;3187-94.

Sharpe PM, Ferguson MW. Mesenchymal influences on epithelial differentiation in developing systems. J Cell Sci Suppl 1988;10:195-230.

Shen MM, Leder P. Leukemia inhibitory factor is expressed by the preimplantation uterus and selectively blocks primitive ectoderm formation in vitro. Proc Nat Acad Sci USA 1992;89:8240-4.

Simmen RCM, Simmen FA. Regulation of uterine and conceptus secretory activity in the pig. J Reprod Fert Suppl 1990;40:279-92.

Sinowatz F, Friess AE. Uterine glands of the pig during pregnancy. An ultrastructural and cytochemical study. Anat Embryol (Berl) 1983;166:121-34.

Smith CL. Cross-talk between peptide growth factor and estrogen receptor signaling pathways. Biol Reprod 1998;58:627-32.

Snijders MP, de Goeij AF, Debets-Te Baerts MJ, Rousch MJ, Koudstaal J, Bosman FT. Immunocytochemical analysis of oestrogen receptors and progesterone receptors in the human uterus throughout the menstrual cycle and after the menopause. J Reprod Fertil 1992; 94:363-71.

Song H, Lim H, Das SK, Paria BC, Dey SK. Dysregulation of EGF family of growth factor 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 2000;14:1147-61.

Song J. The Human Uterus: Morphogenesis and Embryological Basis for Cancer. Charles C Thomas: Springfield, Il; 1964.

Spencer TE, Bartol FF, Bazer FW, Johnson GA, Joyce MM. Identification and characterization of glycosylation-dependent cell adhesion molecule 1-like protein expression in the ovine uterus. Biol Reprod 1999[b]; 60:241-50.

Spencer TE, Bartol FF, Wiley AA, Coleman DA, Wolfe DF. Neonatal porcine endometrial development involves coordinated changes in DNA synthesis, glycosaminoglycan distribution, and ^{3}H-glucosamine labeling. Biol Reprod 1993;46:729-40.

Spencer TE, Bazer FW. Temporal and spatial regulation of uterine receptors for estrogen and progesterone during the estrous cycle and early pregnancy in ewes. Biol Reprod 1995;53:1527-44.

Spencer TE, Gray CA, Johnson GA, Taylor KM, Gertler A, Gootwine E, Ott TL, Bazer FW. Effects of recombinant ovine interferon tau, placental lactogen, and growth hormone on the ovine uterus. Biol Reprod 1999[c]; 61:1409-18.

Spencer TE, Gray CA, Joyce MJ, Jenster G, Wood CG, Bazer FW, Wiley AA, Bartol FF. Discovery and characterization of genes expressed in the endometrial epithelium using the ovine uterine gland knockout model. Endocrinology 1999[a];140: 4070-80.

Stewart CL, Cullinan EB. Preimplantation development of the mammalian embryo and its regulation by growth factors. Dev Genet 1997;21:91-101.

Stewart CL, Kaspar P, Brunet LJ, Bhatt H, Gadi Inder, Köntgen, Abbondanzo SJ. Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. Nature 1992;359:76-9.

Stewart F, Kennedy MW, Suire S. A novel uterine lipocalin supporting pregnancy in equids. Cell Molecular Life Sci 2000[b];57:1373-8.

Stewart MD, Johnson GA, Gray CA, Schuler LA, Burghardt RC, Joyce MM, Bazer FW, Spencer TE. Prolactin receptor and UTMP expression in the ovine endometrium during the estrous cycle and pregnancy. Biol Reprod 2000[a];62:1779-89.

Tarleton BJ, Wiley AA, Bartol FF. Endometrial development and adenogenesis in the neonatal pig: effects of estradiol valerate and the antiestrogen ICI 182,780. Biol Reprod 1999;61:253-63.

Tarleton BJ, Wiley AA, Spencer TE, Moss AG, Bartol FF. Ovary-independent estrogen receptor expression in neonatal porcine endometrium. Biol Reprod 1998;58:1009-19.

Taylor KM, Chen C, Gray CA, Bazer FW, Spencer TE. Expression of mRNAs for fibroblast growth factors 7 and 10, hepatocyte growth factor and insulin-like growth factors and their receptors in the neonatal ovine uterus. Biol Reprod 2001;64:1236-46.

Taylor KM, Gray CA, Joyce MM, Stewart MD, Bazer FW, Spencer TE. Neonatal ovine uterine development involves alterations in expression of receptors for estrogen, progesterone, and prolactin. Biol Reprod 2000;63:1192-204.

Telleria CM, Zhong L, Deb S, Srivastava RK, Park KS, Sugino N, Park-Sarge OK, Gibori G. Differential expression of the estrogen receptors alpha and beta in the rat corpus luteum of pregnancy: regulation by prolactin and placental lactogens. Endocrinology 1998;139:2432-42.

Thiet MP, Osathanondh R, Yeh J. Localization and timing of appearance of insulin, insulin-like growth factor-I, and their receptors in the human fetal mullerian tract. Am J Obstet Gynecol 1994;170:152-6.

Tseng L, Zhu HH. Progestin, estrogen, and insulin-like growth factor-I stimulate the prolactin receptor mRNA in human endometrial stromal cells. J Soc Gynecol Investig 1998;5:149-55.

Valdes-Dapena MA. The development of the uterus in late fetal life, infancy, and childhood. In: HJ Norris, AT Hertig and MR Abell (eds), The Uterus. Baltimore: Williams & Wilkins Company. 1973:40-67.

Vallet JL, Bazer FW, Roberts RM. The effect of ovine trophoblast protein-one on endometrial protein secretion and cyclic nucleotides. Biol Reprod 1987;37:1307-16.

Van Niekerk CH, Allen WR. Early embryonic development in the horse. J Reprod Fertil Suppl 1975;23:495-8.

Van Wyk LC, Van Niekerk CH, Belonje PC. Involution of the post partum uterus of the ewe. J S Afr Vet Assoc 1972[a];43:19-26.

Van Wyk LC, Van Niekerk CH, Belonje PC. Further observations on the involution of the post partum uterus of the ewe. J S Afr Vet Assoc 1972[b];43:29-33.

Vogiagis D, Fry RC, Sandeman RM, Salamonsen LA. Leukaemia inhibitory factor in endometrium during the oestrous cycle and early pregnancy and in ovariectomize steroid-treated ewes. J Reprod Fertil 1997;109:279-88.

Vogiagis D, Salamonsen LA. The role of leukaemia inhibitory factor in the establishment of pregnancy. J Endocrinol 1999;160:181-90.

Waelchi RO. Endometrial biopsy in mares under nonuniform breeding management conditions: prognostic value and relationship with age. Can Vet J 1990;31:379-84.

Wang HS, Chard T. IGFs and IGF-binding proteins in the regulation of human ovarian and endometrial function. J Endocrinol 1999;16:1-13.

Wang J, Rout UK, Bagchi IC, Armant DR. Expression of calcitonin receptors in mouse preimplantation embryos and their function in the regulation of blastocyst differentiation by calcitonin. Development 1998;125:4293-302.

Wang ZQ, Fung MR, Barlow DP, Wagner EF. Regulation of embryonic growth and lysosomal targeting by the imprinted Igf2/Mpr gene. Nature 1994;372:464-67.

Weidner KM, Sachs M, Birchmeier W. The Met receptor tyrosine kinase transduces motility, proliferation, and morphogenic signals of scatter factor/hepatocyte growth factor in epithelial cells. J Cell Biol 1993;121:145-54.

Werb Z, Sympson CJ, Alexander CM, Thomasset N, Lund LR, MacAuley A, Ashkenas J, Bissell MJ. Extracellular matrix remodeling and the regulation of epithelial-stromal interactions during differentiation and involution. Kidney Int Suppl 1996;54:S68-S74.

Wiley AA, Bartol FF, Barron DH. Histogenesis of the ovine uterus. J Anim Sci 1987; 64:1262-9.

Wimsatt WA. New histological observations on the placenta of the sheep. Am J Anat 1950;87:391-436.

Wooding FB. The role of the binucleate cell in ruminant placental structure. J Reprod Fertil Suppl 1982;31:31-9.

Wooding FB, Morgan G, Forsyth IA, Butcher G, Hutchings A, Billingsley SA, Gluckman PD. Light and electron microscopic studies of cellular localization of oPL with monoclonal and polyclonal antibodies. J Histochem Cytochem 1992;40:1001-9.

Wynn RM. The human endometrium: cyclic and gestational changes. In: Biology of the Uterus. Wynn RM, Jollie WP. Plenum Medical Book Company: New York, 1989:289-332.

Yamasaki M, Miyake A, Tagashira S, Itoh N. Structure and expression of the rat mRNA encoding a novel member of the fibroblast growth factor family. J Biol Chem 1996;271:15918-21.

Yamashita S, Newbold RR, McLachlan JA, Korach KS. Developmental pattern of estrogen receptor expression in female mouse genital tracts. Endocrinology 1989; 125;2888-96.

Yang Z-M, Le S-P, Chen D-B, Yasukawa K, Harper MJK. Expression patterns of leukaemia inhibitory factor receptor (LIFR) and the gp130 receptor component in rabbit uterus during early pregnancy. J Reprod Fertil 1995;103:249-55.

Young KH, Kraeling RR, Bazer FW. Effect of pregnancy and exogenous ovarian steroids on endometrial prolactin receptor ontogeny and uterine secretory response in pigs. Biol Reprod 1990;43:592-9.

Yu-Lee LY, Luo G, Book ML, Morris SM. Lactogenic hormone signal transduction. Biol Reprod 1998;58:295-301.

Zhu LJ, Bagchi MK, Bagchi IC. Attenuation of calcitonin gene expression in pregnant rat uterus leads to a block in embryonic implantation. Endocrinology 1998[a]; 139:330-9.

Zhu LJ, Cullinan-Bove K, Polihronis M, Bagchi MK, Bacgchi IC. Calcitonin is a progesterone-regulated marker that forecasts the receptive state of endometrium during implantation. Endocrinology 1998[b];139:3923-34.