

# Uterine and placental factors regulating conceptus growth in domestic animals<sup>1,2</sup>

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**ABSTRACT:** All mammalian 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). The ovine uterine gland knockout ewe model supports a primary role for endometrial glands and, by default, their secretions as essential for conceptus survival and development during the peri-implantation period of pregnancy. Endometrial adenogenesis, the process whereby glands develop in the uterus, is primarily a postnatal event in domestic and laboratory animals, as well as in humans. Endometrial adenogenesis involves differentiation and budding of glandular epithelium from luminal epithelium, followed by invagination and extensive tubular coiling and branching morphogenesis throughout uterine stroma to the myometrium. In sheep, pituitary prolactin acting on prolactin receptors expressed by uterine glandular epithelium regulates endometrial adenogenesis. In contrast, expression and functional activation of estrogen receptor  $\alpha$  in the uterus is a primary regulator of endometrial adenogenesis in the pig. In adult sheep and

pigs, extensive endometrial gland hyperplasia and hypertrophy occur during gestation, presumably to provide increasing histotrophic support for conceptus growth and development. A servomechanism has been proposed in sheep and pigs to regulate endometrial gland development and differentiated function during pregnancy that involves sequential actions of ovarian steroid hormones, pregnancy recognition signals, and lactogenic hormones from the pituitary and/or placenta. The fact that disruption of uterine development during critical organizational periods can alter the functional capacity and embryotrophic potential of the adult uterus reinforces the importance of understanding uterine developmental biology. Defects in endometrial gland morphogenesis during uterine growth and development may cause the unexplained, high rates of peri-implantation embryonic loss in domestic animals and humans. Knowledge of the basic mechanisms regulating uterine development is expected to suggest means to increase uterine capacity, litter size, and neonatal survival, as well as ameliorate certain types of infertility.

Key Words: Conceptus, Hormone, Pigs, Placenta, Sheep, Uterus

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## Introduction

Conceptus (embryo/fetus and associated extraembryonic membranes) growth and development is dependent initially on the endometrium and then on both the endometrium and placenta once implantation and then placentation are completed by midpregnancy. Histotrophic

nutrition is primarily derived from the secretions of uterine glands that bathe the conceptus and are absorbed by placental areolae. Areolae are unique placental structures in ruminants and pigs that develop over the mouth of each uterine gland as specialized areas for absorption and transport of uterine histotroph. Hematotrophic nutrition is derived from maternal blood and is, in large part, influenced by uterine blood flow. Following placentation, the requirement for histotroph remains critical in domestic animals with an epitheliochorial (pig) or synepitheliochorial (sheep, cattle, and goat) placenta. Histotrophic and hematotrophic nutrition influence conceptus development, onset of pregnancy recognition signals, and fetal-placental growth in the ungulate uterus. It is becoming clear that placental hormones act in a paracrine manner on the uterus to maximize production of histotroph. Knowledge of basic

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mechanisms regulating uterine development and function in the neonate and adult is necessary to develop biotechnological tools to increase uterine capacity and fetal growth, thereby enhancing reproductive efficiency and profitability of animal production agriculture. This review summarizes current information on the role of uterine and placental factors regulating conceptus growth in domestic animals, with particular emphasis on the sheep and pig.

### Uterine Factors Regulating Conceptus Growth

Most uterine factors that regulate conceptus growth in domestic animals emanate from the endometrial glands as histotroph or result from increases in the diameter and number of uterine blood vessels to allow increased blood flow to the uterus and hematotropic nutrition for the fetus. This section of the review specifically focuses on the function and developmental biology of uterine endometrial glands.

#### *The Functional Role of Endometrial Glands and Their Secretions*

All mammalian uteri contain endometrial glands that synthesize and secrete or transport a complex array of proteins and related substances termed histotroph (Wimsatt, 1950; Amoroso, 1952; Bazer, 1975), which is a complex mixture of enzymes, growth factors, cytokines, lymphokines, hormones, transport proteins, and other substances. The idea that uterine secretions nourish the developing conceptus was discussed by Aristotle in the third century BC, and by William Harvey in the 17th century. In 1882, Bonnett concluded that secretions of uterine glands were important for fetal well-being in ruminants (Bonnett, 1882). Evidence 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, implantation, and placentation (reviewed in Bazer et al., 1979; Roberts and Bazer, 1988; Bartol et al., 1999; Carson et al., 2000; Gray et al., 2001a). Recent studies of the uterine gland knockout (**UGKO**) ewe model revealed an essential role for endometrial glands and their secretions in normal estrous cycles and in peri-implantation conceptus survival and growth (Gray et al., 2000a, 2001b, 2002).

Continuous administration of a synthetic, nonmetabolizable progestin to neonatal ewes from birth to postnatal day (**PND**) 56 permanently ablated adenogenesis of glandular epithelium (**GE**) from luminal epithelium (**LE**) in the endometrium and produced a **UGKO** phenotype without altering development of myometrium or other Müllerian duct-derived female reproductive tract structures or the hypothalamic-pituitary-ovarian axis (Bartol et al., 1988b; Spencer et al., 1999c; Gray et al., 2000b, 2001b). These ewes do not exhibit normal 17-d estrous cycles due to the inability of the uterus to pro-

duce sufficient luteolytic pulses of  $\text{PGF}_{2\alpha}$ . The lack of superficial or ductal **GE**, coupled with an overall reduction in **LE** surface area, reduced the numbers of oxytocin receptors that could respond to oxytocin (Gray et al., 2000a; Spencer and Bazer, 2002).

Exogenous  $\text{PGF}_{2\alpha}$  induces luteolysis in **UGKO** ewes, and they display normal estrus mating behavior (Gray et al., 2000a, 2001b, 2002). However, adult **UGKO** ewes are unable to establish pregnancy (Gray et al., 2000a, 2001c, 2002), and transfer of normal hatched blastocysts into the uteri of timed recipient **UGKO** ewes failed to ameliorate this defect or to establish pregnancy (Gray et al., 2001c). Morphologically normal blastocysts are present in uterine flushes of bred **UGKO** ewes on d 6 or 9 after mating, but not on d 14 (Gray et al., 2001c, 2002). On d 14, uterine flushes of mated **UGKO** ewes contain either no conceptus or a severely growth-retarded tubular conceptus (Gray et al., 2001c). The peri-implantation period of pregnancy in sheep is marked by rapid elongation of the conceptus from a tubular to filamentous form between d 11 and 13 and production of interferon tau (**IFN $\tau$** ), the signal for maternal recognition of pregnancy (Spencer and Bazer, 2002). Although the growth-retarded conceptuses recovered from mated **UGKO** ewes produced little or no **IFN $\tau$** , the endometrium of **UGKO** ewes nonetheless responded to intrauterine infusions of recombinant ovine **IFN $\tau$**  with increased expression of **IFN $\tau$** -stimulated genes (Gray et al., 2002).

Implantation in ruminants is a highly coordinated process that involves apposition, attachment, and adhesion of the conceptus trophoctoderm to **LE** (Guillomot, 1995). In sheep, the blastocyst enters the uterus on d 4 and hatches from the zona pellucida on d 9. Apposition of conceptus trophoctoderm and **LE** is initiated between d 10 to 14, followed by adhesion on d 15 and attachment on d 16 to 18. Elongation of spherical blastocysts to a filamentous form is thought to require transient attachment and adhesion of conceptus trophoctoderm to **LE**. Initially, the nonadhesive property of the **LE** appears to be partially due to apical expression of mucins, such as mucin glycoprotein-1, that sterically impair interactions between trophoctoderm and adhesive glycoproteins, such as integrins, due to their extensive glycosylation and extended extracellular structure (Johnson et al., 2001). Immunoreactive mucin glycoprotein-1 expression by **LE** decreases between d 9 and 17 of early pregnancy in normal (Johnson et al., 2001) and **UGKO** (Gray et al., 2002) ewes. Extracellular matrix and integrins are thought to be responsible for trophoctoderm attachment and adhesion to **LE** (Johnson et al., 2001; Burghardt et al., 2002). During the peri-implantation period of pregnancy in ewes, integrin subunits  $\alpha\text{v}$ ,  $\alpha\text{4}$ ,  $\alpha\text{5}$ ,  $\beta\text{1}$ ,  $\beta\text{3}$ , and  $\beta\text{5}$  are constitutively expressed on both conceptus trophoctoderm and the apical surface of **LE** (Johnson et al., 2001). Integrin expression on endometrial **LE** of **UGKO** ewes is not different from normal ewes (Gray et al., 2002). Further, expression of receptors for estrogen (**ER $\alpha$** ), progesterone (**P4**), and oxyto-

cin as well as several LE-specific genes are not different in the endometrium of UGKO and normal ewes (Gray et al., 2000a, 2002). Thus, by these measures, the endometrial LE does not appear to be defective in UGKO ewes. Available evidence strongly suggests that uterine glands and their secretions are essential for peri-implantation conceptus growth and survival.

Uterine flushes of UGKO ewes were analyzed for the presence of osteopontin (OPN) and glycosylated cell adhesion molecule one proteins, which are expressed by GE of the ovine uterus (Johnson et al., 1999; Spencer et al., 1999a) and are suggested to play a role in regulation of conceptus implantation. Uterine flushes of d 14 bred UGKO ewes contained lower amounts of OPN and glycosylated cell adhesion molecule-1 compared with d 14 pregnant ewes (Gray et al., 2002). Genomics and proteomics are being used to identify specific components of histotroph that are absent or diminished in the UGKO ewe (Spencer et al., 1999c). A better understanding of the components of histotroph may lead to the development of a better maturation medium for *in vitro* production of embryos. In addition, these important histotroph components will serve as useful markers of endometrial function and fertility in both domestic animals and humans.

Partial to complete UGKO phenotypes have also been produced in adult cows exposed from birth to a combination of P4 plus estradiol benzoate (Bartol et al., 1995, 1999). Pregnancy rates are reduced in adult heifers exposed neonatally to P4 plus estradiol benzoate with reduced endometrial gland numbers (Bartol et al., 1999). In the pig, the numbers of placental areolae are directly related to birth weight of the fetus (Knight et al., 1977; van Rens and van der Lende, 2002). Therefore, the success of endometrial gland morphogenesis in neonatal pigs also determines, in part, the embryotrophic and functional capacity of the adult uterus (Bartol et al., 1993, 1999).

Studies of UGKO ewes and neonatally estrogenized adult gilts strongly support the concept that mechanisms regulating postnatal uterine development and endometrial gland morphogenesis ultimately determine functional capacity and embryotrophic potential of the adult uterus (Bartol et al., 1993, 1999; Gray et al., 2001a). Uterine capacity is a complex, polygenic trait and is the most important factor limiting litter size in commercial pigs selected to have a high ovulation rate. In fact, 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; van Rens and van Der Lende, 2002). Accordingly, a rational approach to increasing litter size in commercial pigs would be to increase the number of endometrial glands and/or the length of the uterine horns, thereby decreasing early embryonic loss and enhancing the ability of the uterus to accommodate an increased number of fetuses. This approach to increasing litter size could be predicated on therapies targeting endometrial gland development in the neonate or dur-

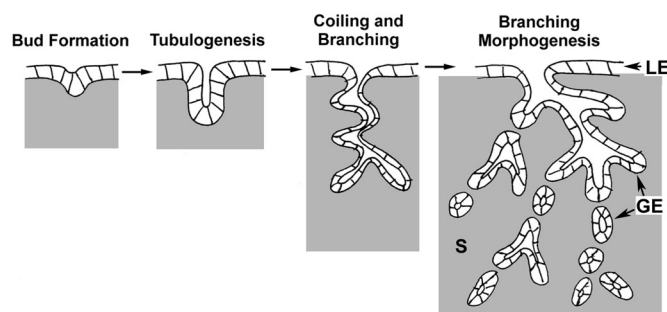
ing pregnancy in the adult. Hence, a complete understanding of the hormonal, cellular, and molecular mechanisms regulating postnatal uterine development and endometrial adenogenesis is critical to identifying mechanisms to increase functional capacity of the adult uterus.

### *Developmental Biology of the Uterus*

Although a functional role for endometrial glands has been established in most mammals, mechanisms regulating their development in domestic animals, laboratory animals, and humans are not well understood (Gray et al., 2001a). In all mammals, the uterus develops as a specialization of the paramesonephric or Müllerian ducts, which gives rise to the infundibula, oviducts, uterus, cervix, and anterior vagina. Morphogenetic events common to development of all uteri include: 1) differentiation and growth of the myometrium, 2) differentiation and morphogenesis of the endometrial glands, and 3) organization and stratification of endometrial stroma (Bartol et al., 1993, 1999; Gray et al., 2001a). Uterine development is initiated in the fetus, but is only completed postnatally with differentiation and development of the endometrial glands.

Uterine morphogenesis has been described in sheep (Wiley et al., 1987; Bartol et al., 1988a,b; Taylor et al., 2000, 2001). Paramesonephric duct fusion occurs between gestational d 34 and 55 in sheep, is partial, and produces a bicornuate uterus. By gestational d 90, raised aglandular uterine nodules will become caruncles. The dichotomous nature of the ruminant endometrium, consisting of both aglandular caruncular areas and glandular intercaruncular areas, makes it an excellent model for the study of mechanisms underlying the establishment of divergent structural and functional areas within a single, mesodermally derived organ (Wiley et al., 1987). Postnatal uterine morphogenesis in sheep involves differentiation and development of endometrial glands, development of endometrial folds, organization of intercaruncular endometrial stroma, and, to a lesser extent, growth of endometrial caruncular areas and myometrium (Wiley et al., 1987; Taylor et al., 2000).

Endometrial gland morphogenesis begins with GE bud formation from the LE, followed by tubulogenesis, and coiling and branching morphogenesis (Figure 1). In sheep, endometrial gland genesis is initiated between birth (PND 0) and PND 7, when shallow epithelial invaginations appear along the LE in presumptive intercaruncular areas. Between PND 7 and 14, nascent, budding glands proliferate and invaginate into the stroma, forming tubular structures that coil and branch by PND 21. 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 the deeper stroma. By PND 56, the caruncular and intercaruncular endometrial areas are histoarchitecturally mature. In UGKO ewes, the



**Figure 1.** General illustration of the process of endometrial gland development. Uterine glands originate as shallow gland buds from the luminal epithelium (LE) before undergoing invagination to form tubules. As the tubules progress through the stroma (S) toward the myometrium, they begin to coil and branch. The final stage of endometrial glandular epithelial (GE) differentiation is the process of branching morphogenesis, which does not occur in rodents. This process is similar to that occurring in epitheliomesenchymal organs, such as the lung, salivary gland, prostate, and mammary gland.

endometrium lacks a recognizable stratum spongiosum stroma, which is characteristic of the normal stroma in intercaruncular glandular areas of the uterus (Spencer et al., 1999c; Gray et al., 2000a,b, 2001b, 2002). Thus, development of GE is involved with differentiation of the stroma into stratum compactum and stratum spongiosum in intercaruncular areas of the ovine endometrium.

Although the ovine uterine wall is histoarchitecturally mature by PND 56, final maturation and growth may not occur until after puberty (Kennedy et al., 1974) and, perhaps, even after pregnancy (Wimsatt, 1950; Stewart et al., 2000). In sheep (Wimsatt, 1950; Stewart et al., 2000) and pigs (Sinowatz and Friess, 1977), endometrial glands undergo extensive hyperplasia and hypertrophy during pregnancy, presumably in response to placental hormones to meet increasing demands of the developing fetus for uterine histotroph (Bazer, 1975). The life cycle of endometrial glands in the uterus are strikingly similar to that of the mammary gland (Figure 2). These parallels in organ development suggest that the phenotypic capacity of the uterus may be affected by parity and can be improved epigenetically using hormones or other factors.

#### *Mechanisms Regulating Endometrial Gland Morphogenesis*

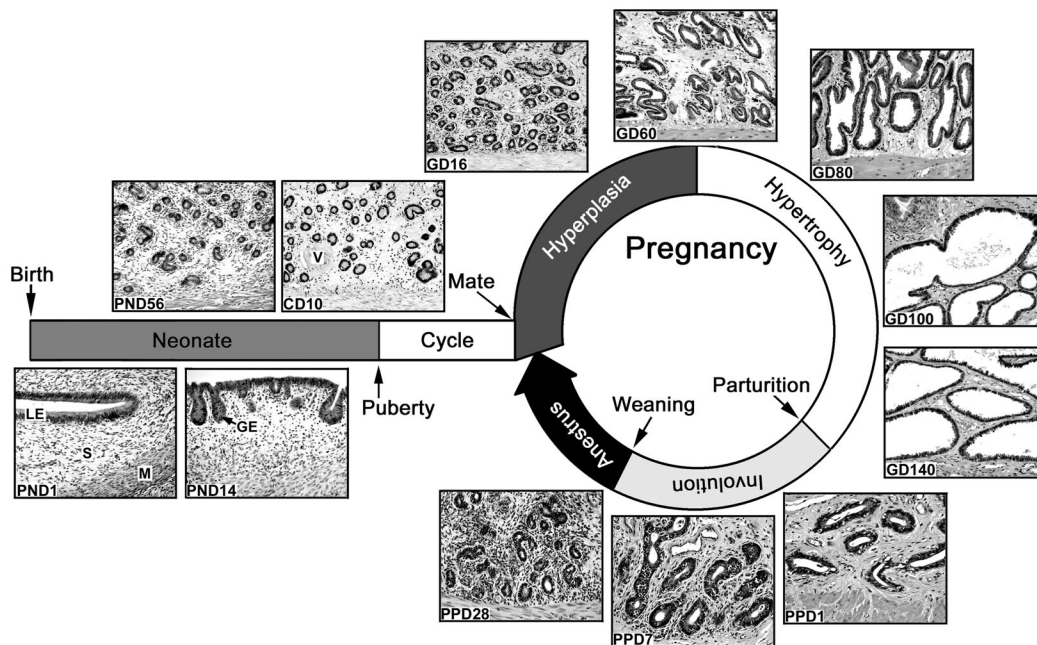
Uterine morphogenesis is governed by a variety of endocrine, cellular, and molecular mechanisms, many of which remain to be defined (for reviews, see Cunha et al., 1983; Bartol et al., 1993, 1999; Gray et al., 2001a). Based on regulatory mechanisms governing gland development in other epithelial-mesenchymal organs, uterine development and adenogenesis likely 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.

**Growth Factors.** The concept that interactions between epithelium and stroma are required for endometrial morphogenesis and establishment of normal uterine histoarchitecture is supported by tissue recombination studies involving the mouse uterus (Cunha, 1976; Cooke et al., 1998). Stromal-derived growth factors play important roles in epithelial proliferation, differentiation, and branching morphogenesis in many developing epitheliomesenchymal organs, including the uterus (Gray et al., 2000b; Taylor et al., 2001). Interactions between growth factors and their receptors can involve elements of the extracellular matrix, which not only affect patterns of growth factor presentation to target cells, but may also participate as elements of cell surface receptor complexes. In the neonatal ovine uterus, fibroblast growth factor-7 (FGF), FGF-10, hepatocyte growth factor, IGF-I and IGF-II, and the activin-follistatin system have been identified as candidate regulatory systems for endometrial adenogenesis (Gray et al., 2000b; Taylor et al., 2001; Carpenter et al., 2003c; Hayashi et al., 2003). Each of these stromal-derived growth factors has receptors that are expressed in the endometrial epithelia. Although many studies have promoted the concept that local growth factors predominantly regulated organ morphogenesis and differentiated function, recent evidence indicates that systemic growth factors, such as IGF-I, may be more important than previously thought for growth of the uterus and other tissues (Sato et al., 2002). Thus, uterine development is likely to be regulated by a carefully orchestrated network of growth factors and hormones from both local and systemic origins.

**Ovary and Estrogens.** Early postnatal events in rodent uterine development and endometrial adenogenesis are ovary- and adrenal-independent (see Gray et al., 2001a). In the neonatal pig, Tarleton et al. (1998) determined that ovariectomy at birth did not affect genesis of uterine glands or related endometrial morphogenetic events prior to PND 120, but did inhibit uterine weight after PND 60. Similarly, ovariectomy of ewe lambs at birth did not affect patterns of uterine gland genesis on PND 14 (Bartol et al., 1988b), but reduced uterine weight after PND 28 (Kennedy et al., 1974). However, recent results implicate ovarian factors as important regulators of endometrial adenogenesis in the ewe.

In spring-born Merino ewes, the ovary contains significant numbers of growing and antral ovarian follicles at birth (approximately 455 and 935 per ovary, respectively) and these increase in number by PND 28 (approximately 683 and 1,100 per ovary) and then decline in number by PND 84 (approximately 100 and 287 per ovary) (Kennedy et al., 1974). Recent studies of spring-born ewes indicated that circulating estradiol-17 $\beta$  levels were highest at birth and declined thereafter to very



**Figure 2.** Life cycle of uterine glands in sheep. At birth or postnatal day (PND) 0, the uterus is devoid of endometrial glandular epithelium and consists of endometrial luminal epithelium (LE) and stroma surrounded by the myometrium. Between PND 0 and PND 7, the endometrial glands (GE) differentiate and bud from the luminal epithelium. The gland buds form tubules by PND 14 and then coil and branch as they proliferate from the lumen to the myometrium. By PND 56, the uterus is histoarchitecturally mature as compared to that of adult cyclic ewes. During gestation, the endometrial glands undergo hyperplasia from gestational day (GD) 20 to 50 and then hypertrophy from GD 50 to GD 60. Maximal differentiated function of the endometrial glands occurs between GD 80 and GD 120. After parturition, the endometrial glands regress between postpartum d 1 (PPD 1) and PPD 28 during uterine involution. All photomicrographs are shown at the same magnification (20 $\times$ ). CD = cyclic day; M = myometrium; V = blood vessel.

low or undetectable levels between PND 7 and PND 56 (Carpenter et al., 2003a,c). Subsequently, spring-born ewes were ovariectomized on PND 7, and serum estradiol-17 $\beta$  levels were not different from sham control ewes between PND 7 and PND 56 (Carpenter et al., 2003c). Therefore, the ovaries of spring-born ewes do not appear to synthesize or secrete appreciable amounts of estrogens between birth and puberty, despite the large numbers of growing and antral follicles.

Ovariectomy of ewes on PND 7 reduced uterine wet weight by almost 50% on PND 56 (Carpenter et al., 2003c), and these ewes had fewer coiled and branched endometrial glands on PND 56 compared with sham control ewes. Thus, the ovary and an ovarian-derived factor(s) may influence coiling and branching morphogenesis of uterine glands after PND 14 in ewes. The ovarian factor(s) would presumably be secreted from the abundant growing and antral follicles in the ovary. Candidate ovarian factors include follistatin, activins, or inhibin (Carpenter et al., 2003c), as well as IGF. One may speculate that the coordinate activities of the activin-inhibin-follistatin system in the ovary and uterus is important in prolific breeds of ewes that possess an intrinsically high ovulation rate as well as enhanced uterine capacity to maintain large litters (Fahmy, 1996). Determination of the underlying ge-

netic basis of uterine capacity in these "outlier" breeds will also be useful and important to enable genetic selection for uterine capacity.

*Estrogen Receptors.* Although endometrial adenogenesis is an ovary- and apparently steroid-independent event in neonatal pigs, genesis of endometrial glands in rodent, porcine, and ovine uteri involves coordinated changes in epithelial phenotype that are marked by ER $\alpha$  expression in nascent and proliferating endometrial glands (Tarleton et al., 1999; Taylor et al., 2000; Gray et al., 2000b). Administration of ICI 182,780, a potent ER $\alpha$  antagonist, in the neonatal pig from birth, inhibited endometrial adenogenesis on PND 14 (Tarleton et al., 1999). Recently, neonatal ewes were treated with EM-800, a pure and potent ER $\alpha$  antagonist and antiestrogen, from birth to PND 56 without effects on uterine weight or horn length (Carpenter et al., 2003a). On PND 14, uteri from EM-800 ewes appeared histologically similar to control ewes, except for a slight reduction in coiled endometrial glands. However, on PND 56, the intercaruncular endometrium of EM-800 ewes had 44% fewer ductal gland invaginations and 22% fewer endometrial glands that were less coiled and branched. Although ER $\alpha$  does not regulate initial stages of endometrial adenogenesis in the neonatal ewe, it does appear to influence coiling and branching morphogenesis

of endometrial glands. Thus, the regulatory role of ER $\alpha$  in uterine development and endometrial adenogenesis is species- and developmental stage-specific. Inappropriate transient exposure of neonatal pigs to estrogens disrupts uterine development (Tarleton et al., 2003) and decreases litter size in the adult (Bartol et al., 1993, 1999). Therefore, estrogen-sensitive postnatal uterine organizational events are determinants of uterine size and functionality in pigs (Bartol et al., 1999; Tarleton et al., 2003).

**Prolactin.** Prolactin (**PRL**), a member of the helix bundle peptide hormone/cytokine superfamily, regulates growth and differentiation of a number of epitheliomesenchymal organs. In the mouse mammary gland, PRL and PRL receptor (**PRLR**) are required for the development and differentiation of the lobuloalveolar portion of epithelium (Horseman et al., 1997; Brisken et al., 1999). In the endometrium of adult sheep, humans, and primates, the PRLR gene is expressed exclusively by GE, and in ewes, increased PRLR expression during pregnancy correlates with hyperplasia and hypertrophy of endometrial glands (Stewart et al., 2000).

There is a primary role for pituitary PRL acting on PRLR in GE in the regulatory system controlling endometrial gland branching morphogenesis in the neonatal ovine uterus. In neonatal ewes, circulating levels of PRL are relatively high on PND 1, reach a maximum on PND 14, and then decline slightly to PND 56 (Taylor et al., 2000; Carpenter et al., 2003b). Expression of messenger RNA for both short and long PRLR is restricted to nascent GE buds on PND 7 and proliferating and differentiating GE from PNDs 14 to 56 (Taylor et al., 2000). Hyperprolactinemia, induced in neonatal ewes by treatment with recombinant ovine PRL from birth to PND 56, resulted in uteri with over 60% more endometrial glands (Carpenter et al., 2003c). Similarly, in adult ewes, intrauterine administration of placental lactogen (**PL**), a member of the PRL/GH family that activates the PRLR (Gertler and Djiane, 2002), stimulated proliferation of endometrial glands, particularly the coiled and branched glands found in the stratum spongiosum (Spencer et al., 1999b; Noel et al., 2003). On the other hand, hypoprolactinemia, induced in neonatal ewes by treatment with bromocryptine, a PRL secretion inhibitor, from birth to PND 56 (Carpenter et al., 2003b), reduced endometrial glands by 35%. Thus, PRL, acting via PRLR in GE, is a pivotal factor regulating endometrial adenogenesis in neonatal ewes. Determination of transcription factors that specify and regulate expression of the ovine PRLR gene in uterine GE will be key to understanding the cellular and molecular mechanisms regulating endometrial gland differentiation and morphogenesis. Clearly, PRL may be a useful therapeutic agent to increase endometrial gland development in the neonate, thereby increasing uterine function and capacity in the adult.

### Placental Factors Regulating Conceptus Growth

In domestic animals, the placenta produces a variety of steroid and protein hormones that act in a paracrine

manner on the endometrium to elicit changes in gene expression and development that support conceptus growth and development. This section of the review describes effects of placental lactogens in sheep and placental estrogens in pigs on endometrial support of conceptus growth.

#### *Servomechanism Regulating Endometrial Gland Morphogenesis and Function in Sheep*

In sheep, establishment and maintenance of pregnancy requires integration of endocrine and paracrine signals from the ovary, conceptus, and uterus (reviewed in Spencer and Bazer, 2002). Maintenance of pregnancy requires reciprocal communication between the conceptus and endometrium during implantation and synepitheliochorial placentation. In sheep, superficial implantation and placentation begins on d 15 to 16, but is not completed until d 50 to 60 of pregnancy (Wimsatt, 1950; Guillomot, 1995). During this period, the uterus grows and remodels substantially in order to accommodate rapid conceptus development and growth in the latter two-thirds of pregnancy. In addition to placentomal development in the caruncular areas of the endometrium and changes in uterine vascularity, the intercaruncular endometrial glands grow substantially in length (four-fold) and width (10-fold) and establish additional side-branchings during pregnancy (Wimsatt, 1950). During gestation, endometrial gland hyperplasia occurs between d 15 and 50 followed by hypertrophy to increase surface area that allows for maximal production of histotroph after d 60 (Stewart et al., 2000).

The pregnant ovine uterus is exposed sequentially to estrogen, P4, IFN $\gamma$ , PL, and placental GH. These hormones seem to regulate endometrial gland morphogenesis and differentiated secretory function (Spencer et al., 1999b; Noel et al., 2003). The placentae of a number of species, including rodents, humans, nonhuman primates, and sheep, secrete hormones structurally related to pituitary GH and PRL that are termed PL (Anthony et al., 1998; Gertler and Djiane, 2002). Ovine PL is produced by binucleate cells of the conceptus trophoctoderm beginning on d 16 of pregnancy, which is concomitant with the initiation of expression of uterine milk proteins (**UTMP**) by GE (Stewart et al., 2000). The UTMP are members of the serpin family of serine protease inhibitors (Ing and Roberts, 1989) and, along with OPN, an extracellular matrix protein (Johnson et al., 1999, 2001, 2003), serve as excellent markers for endometrial gland differentiation and overall uterine secretory capacity during pregnancy in ewes (Spencer et al., 1999b; Stewart et al., 2000). In maternal serum, PL can be detected as early as d 50, and it peaks between d 120 to 130 of gestation (Anthony et al., 1998). A homodimer of the PRLR, as well as a heterodimer of PRLR and GH receptor, transduces signals by ovine PL (Gertler and Djiane, 2002). In the ovine uterus, PL binding sites are specific to GE expressing PRLR (Noel et al., 2003). Temporal changes in circulating levels of

PL are correlated with endometrial gland hyperplasia and hypertrophy and increased production of UTMP and OPN during pregnancy (Johnson et al., 1999, 2003; Stewart et al., 2000). The ovine placenta also expresses GH between d 35 and 70 of gestation (LaCroix et al., 1996), which is correlated with the onset of GE hypertrophy and maximal increases in UTMP and OPN production by GE. These results suggest that members of the lactogenic and somatogenic hormone family play key roles in stimulating endometrial gland morphogenesis and differentiated function during pregnancy to facilitate conceptus growth and development.

Sequential exposure of the pregnant ovine endometrium to estrogen, P4, IFN $\gamma$ , PL, and placental GH constitutes a "servomechanism" that activates and maintains endometrial remodeling, secretory function, and uterine growth during gestation (Spencer et al., 1999b; Noel et al., 2003). Chronic treatment of ovariectomized ewes with P4 induces expression of UTMP and OPN by GE (Ing and Roberts, 1989; Spencer et al., 1999b; Noel et al., 2003). During early pregnancy, expression of the P4 declines to undetectable levels in uterine LE by d 11 and in GE by d 13 (Spencer and Bazer, 2002). Down-regulation of epithelial P4 appears to be required for progesterone induction of GE secretory gene expression (e.g., OPN and UTMP; Spencer et al., 1999b), because a combination of P4- and estrogen-increased ER $\alpha$  and P4 expression in the GE, which markedly inhibited expression of both OPN and UTMP. These results indicate that chronic effects of P4 down-regulated epithelial P4 expression to allow expression of UTMP and OPN. Indeed, the endometrial GE lacks detectable PR gene expression after d 16 of gestation (T. E. Spencer, unpublished results). Additional studies revealed that intra-uterine infusions of recombinant ovine PL or GH increased UTMP and OPN expression by uterine GE of P4-treated ewes, but only when the ewes were infused with IFN $\gamma$  between d 11 and 21, and then either PL or GH from d 16 to 29 (Spencer et al., 1999b). The increase in UTMP expression by endometrial GE was partly attributed to effects of PL and GH to increase the number of endometrial glands. Subsequently, intrauterine infusion of PL and GH into ewes treated with progesterone and IFN $\gamma$  was found to increase endometrial gland hypertrophy, an effect not observed in ewes infused with either PL or GH alone (Noel et al., 2003). The ability of PRL, PL, and GH to elicit similar effects on endometrial glands is not surprising since they are members of a unique hormone family based on genetic, structural, binding, receptor signal transduction, and function studies (Gertler and Djiane, 2002). These studies suggest that a developmentally programmed sequence of events, mediated by specific paracrine-acting factors at the conceptus-endometrial interface, stimulates both intercaruncular endometrial remodeling and differentiated function in order to increase production of histotroph for fetal-placental growth during gestation.

Leibovich et al. (2000) found that immunization of prolific and nonprolific ewes with recombinant ovine PL did not affect conception rates, but did increase lamb birth weights and ewe milk production. The antibody against PL in immunized ewes increased the levels of serum PL bioactivity through production of a binding type of antibody rather than a neutralizing or inactivating type of antibody. These findings support previous reports implicating a role(s) for PL in fetal growth and mammary gland development (Gertler and Djiane, 2002). The enhanced biological activity of PL in immunized ewes may increase uterine histotroph production, thereby enhancing uterine capacity in prolific ewes. Importantly, immunization of ewes against PL represents a novel practical technique for enhancing uterine and mammary gland capacity and improving reproductive and lactational efficiency.

#### *Estrogens and Endometrial Growth Factors in Pigs*

The major hormone produced by the placenta of the pig that acts on the endometrium is estrogen. Pig conceptuses secrete estrogens between d 10 and 15 of pregnancy, which are essential for establishment of pregnancy (Geisert et al., 1982). Estrogens, directly or indirectly, alter secretion of PGF $_{2\alpha}$  by the endometrium from an endocrine direction (towards uterine vasculature) to an exocrine direction (towards the uterine lumen). The PGF $_{2\alpha}$  sequestered in the uterine lumen is unavailable to exert a luteolytic effect on the corpus luteum. Additionally, an increase in selected histotroph components occurs in the uterine lumen immediately following the release of estrogens from the conceptus on d 11 of pregnancy (Geisert et al., 1982; Fazleabas et al., 1983). Placental estrogens also act on the endometrial epithelium in a paracrine manner to increase expression of specific growth factors, including IGF-I and FGF-7, which in turn act on the trophectoderm to stimulate cell proliferation and development.

*IGF-I.* Insulin-like growth factor-I is a pleiotropic growth factor required for postnatal uterine growth and embryo growth and development in the mouse (see Simmen et al., 1995 for review). In the porcine uterus, IGF-I is primarily expressed in the endometrial glands of both cyclic and pregnant pigs (Persson et al., 1997). Endometrial IGF-I gene expression increases during early pregnancy and peaks on d 12 to 13, which is coincidental with the production of estrogens by the elongating conceptus (Simmen et al., 1990, 1992). Treatment of either ovariectomized or cyclic gilts with estrogen increases IGF-I expression in the uterus (Simmen et al., 1990). Type-I IGF receptors were detected in the endometrium as well as in the embryo, suggesting paracrine and autocrine modes of action of IGF-I in the uterine microenvironment (Simmen et al., 1995).

*FGF-7.* Fibroblast growth factor-7, also known as keratinocyte growth factor (**KGF**), is an established paracrine mediator of hormone-regulated epithelial growth and differentiation (Rubin et al., 1995). In all organs

studied, FGF-7 was uniquely expressed in cells of mesenchymal origin. Intriguingly, expression of FGF-7 in the porcine uterus is exclusively in the endometrial LE and particularly abundant between d 12 and 15 of the estrous cycle and pregnancy (Ka et al., 2000). Endometrial FGF-7 messenger RNA levels were highest on d 12 in pregnant gilts and d 15 in cyclic gilts, and greater on d 12 of pregnancy than on d 12 of the estrous cycle. The FGF-7 protein was detected in the uterine flushes of both d 12 cyclic and pregnant gilts. The receptor for FGF-7, known as FGF receptor 2<sub>IIIb</sub> or KGF receptor, was detected in both endometrial epithelia and conceptus trophectoderm. Treatment of endometrial explants from d-9 cyclic gilts with estradiol-17 $\beta$  increased FGF-7 expression (Ka et al., 2001). Further, treatment of porcine trophectoderm cells with recombinant rat FGF-7 increased their proliferation, phosphorylated FGF receptor 2<sub>IIIb</sub>, activated the mitogen-activated protein kinase cascade, and increased expression of urokinase-type plasminogen activator, a marker for trophectoderm cell differentiation (Ka et al., 2001). Collectively, these results indicate that estrogen, the pregnancy recognition signal from the pig conceptus, increases uterine epithelial FGF-7 expression, and, in turn, FGF-7 stimulates the proliferation and differentiation of conceptus trophectoderm in pigs, which is the only species possessing a true epitheliochorial type of placentation besides the camel (Ka et al., 2000, 2001).

### Implications

Uterine capacity is a complex, polygenic trait that is an important factor limiting litter size in commercial pigs selected to have a high ovulation rate. A rational approach to increasing litter size in pigs may be to increase endometrial gland number and/or uterine horn length. Although uterine capacity is not an issue in ruminants with singleton or twin fetuses, the intraggression of genes that increase ovulation rate, such as the Boorola fecundity gene, into nonprolific sheep challenges uterine capacity. A potential solution to this problem is to permanently increase endometrial gland number and/or uterine horn length by administration of specific hormones and/or growth factors during critical organizational periods before puberty and during pregnancy. In this way, uterine capacity could be maximized in breeds that exhibit desirable production traits without resorting to lengthy genetic selection procedures that often negatively affect other desirable production traits.

### Literature Cited

- Amoroso, E. C. 1952. Placentation. Pages 127–311 in Marshall's Physiology of Reproduction. Vol. 2. A. S. Parkes, ed. Longmans Green, London, U.K.
- Anthony, R. V., S. W. Limesand, M. D. Fanning, and R. Liang. 1998. Placental lactogen and growth hormone: Regulation and action. Pages 461–490 in The Endocrinology of Pregnancy. Vol. 1. F. W. Bazer, ed. Humana Press Inc., Totowa, NJ.
- Bartol, F. F., L. L. Johnson, J. G. Floyd, A. A. Wiley, T. E. Spencer, D. F. Buxton, and D. A. Coleman. 1995. Neonatal exposure to progesterone and estradiol alters uterine morphology and luminal protein content in adult beef heifers. *Theriogenology* 43:835–844.
- Bartol F. F., A. A. Wiley, D. A. Coleman, D. F. Wolfe, and M. G. Riddel. 1988a. Ovine uterine morphogenesis: Effects of age and progestin administration and withdrawal on neonatal endometrial development and DNA synthesis. *J. Anim. Sci.* 66:3000–3009.
- Bartol, F. F., A. A. Wiley, J. G. Floyd, T. L. Ott, F. W. Bazer, C. A. Gray, and T. E. Spencer. 1999. Uterine differentiation as a foundation for subsequent fertility. *J. Reprod. Fertil.* 54(Suppl.):287–302.
- Bartol, F. F., A. A. Wiley, and D. R. Goodlett. 1988b. Ovine uterine morphogenesis: Histochemical aspects of endometrial development in the fetus and neonate. *J. Anim. Sci.* 66:1303–1313.
- Bartol, F. F., A. A. Wiley, T. E. Spencer, J. L. Vallet, and R. K. Christenson. 1993. Early uterine development in pigs. *Reprod. Fert.* 48(Suppl. 1):99–116.
- Bazer, F. W. 1975. Uterine protein secretions: Relationship to development of the conceptus. *J. Anim. Sci.* 41:1376–1382.
- Bazer, F. W., R. M. Roberts, and W. W. Thatcher. 1979. Actions of hormones on the uterus and effect of conceptus development. *J. Anim. Sci.* 49(Suppl. 2):35–45.
- Bonnett, R. 1882. Die Uterinmilch und ihre Bedeutung für die Frucht. Pages 221–263 in Beiträge zur Biologie als Fetgabe dem Anatomen und Physiologen. Th. von Bischoff, Stuttgart, Germany.
- Brisken, C., S. Kaur, T. E. Chavarria, N. Binart, R. L. Sutherland, R. A. Weinberg, P. A. Kelly, and C. J. Ormandy. 1999. Prolactin controls mammary gland development via direct and indirect mechanisms. *Dev. Biol.* 210:96–106.
- Burghardt, R. C., G. A. Johnson, L. A. Jaeger, H. Ka, J. E. Garlow, T. E. Spencer, and F. W. Bazer. 2002. Integrins and extracellular matrix proteins at the maternal-fetal interface in domestic animals. *Cells Tissues Organs* 171:202–217.
- Carpenter, K. D., C. A. Gray, T. M. Bryan, T. H. Welsh, Jr., and T. E. Spencer. 2003a. Estrogen and anti-estrogen effects on neonatal ovine uterine development. *Biol. Reprod.* 69:708–717.
- Carpenter, K. D., C. A. Gray, S. Noel, F. W. Bazer, A. Gertler, and T. E. Spencer. 2003b. Prolactin regulation of neonatal ovine uterine gland morphogenesis. *Endocrinology* 144:110–120.
- Carpenter, K. D., K. Hayashi, and T. E. Spencer. 2003c. Ovarian regulation of endometrial gland morphogenesis and activin-follistatin system in the neonatal ovine uterus. *Biol. Reprod.* 69:851–860.
- Carson, D. D., I. Bagchi, S. K. Dey, A. C. Enders, A. T. Fazleabas, B. A. Lessey, and K. Yoshinaga. 2000. Embryo implantation. *Dev. Biol.* 223:217–237.
- Cooke, P. S., D. L. Buchanan, D. B. Lubahn, and G. R. Cunha. 1998. Mechanism of estrogen action: Lessons from the estrogen receptor—alpha knockout mouse. *Biol. Reprod.* 59:470–475.
- Cunha, G. R. 1976. 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.* 196:361–370.
- Cunha, G. R., L. W. K. Chung, J. M. Shannon, O. Taguchi, and H. Fujii. 1983. Hormone-induced morphogenesis and growth: Role of mesenchymal-epithelial interactions. *Recent Prog. Horm. Res.* 39:559–595.
- Fahmy, M. H. 1996. Prolific Sheep. CAB International, Wallingford, Oxon, U.K.
- Fazleabas, A. T., R. D. Geisert, F. W. Bazer, and R. M. Roberts. 1983. 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.* 29:225–238.
- Geisert, R. D., R. H. Renegar, W. W. Thatcher, R. M. Roberts, and F. W. Bazer. 1982. Establishment of pregnancy in the pig: I. Interrelationships between preimplantation development of

- the pig blastocyst and uterine endometrial secretions. *Biol. Reprod.* 27:925–941.
- Gertler, A., and J. Djiane. 2002. Mechanism of ruminant placental lactogen action: Molecular and in vivo studies. *Mol. Genet. Metab.* 75:189–201.
- Gray, C. A., F. F. Bartol, B. J. Tarleton, A. A. Wiley, G. A. Johnson, F. W. Bazer, and T. E. Spencer. 2001a. Developmental biology of uterine glands. *Biol. Reprod.* 65:1311–1323.
- Gray, C. A., F. F. Bartol, K. M. Taylor, A. A. Wiley, W. S. Ramsey, T. L. Ott, F. W. Bazer, and T. E. Spencer. 2000a. Ovine uterine gland knock-out model: Effects of gland ablation on the estrous cycle. *Biol. Reprod.* 62:448–456.
- Gray, C. A., F. W. Bazer, and T. E. Spencer. 2001b. Effects of neonatal progesterin exposure on female reproductive tract structure and function in the adult ewe. *Biol. Reprod.* 64:797–804.
- Gray, C. A., R. C. Burghardt, G. A. Johnson, F. W. Bazer, and T. E. Spencer. 2002. Evidence that an absence of endometrial gland secretions in uterine gland knockout (UGKO) ewes compromises conceptus survival and elongation. *Reproduction* 124:289–300.
- Gray, C. A., K. M. Taylor, F. W. Bazer, and T. E. Spencer. 2000b. Mechanisms regulating norgestomet inhibition of endometrial gland morphogenesis in the neonatal ovine uterus. *Mol. Reprod. Dev.* 57:67–78.
- Gray, C. A., K. M. Taylor, W. S. Ramsey, J. R. Hill, F. W. Bazer, F. F. Bartol, and T. E. Spencer. 2001c. Endometrial glands are required for pre-implantation conceptus elongation and survival. *Biol. Reprod.* 64:1608–1613.
- Guillomot, M. 1995. Cellular interactions during implantation in domestic ruminants. *J. Reprod. Fertil.* 49:39–51.
- Hayashi, K., K. D. Carpenter, C. A. Gray, and T. E. Spencer. 2003. The activin-follistatin system in the neonatal ovine uterus. *Biol. Reprod.* 69:851–860.
- Horseman, N. D., W. Zhao, E. Montecino-Rodriguez, M. Tanaka, K. Nakashima, S. J. Engle, F. Smith, E. Markoff, and K. Dorshkind. 1997. Defective mammopoiesis, but normal hematopoiesis, in mice with a targeted disruption of the prolactin gene. *EMBO J.* 16:6926–6935.
- Ing, N. H., and R. M. Roberts. 1989. The major progesterone-modulated proteins secreted into the sheep uterus are members of the serpin superfamily of serine protease inhibitors. *J. Biol. Chem.* 264:3372–3379.
- Johnson, G. A., F. W. Bazer, L. A. Jaeger, H. Ka, J. E. Garlow, C. Pfarrar, T. E. Spencer, and R. C. Burghardt. 2001. Muc-1, integrin, and osteopontin expression during the implantation cascade in sheep. *Biol. Reprod.* 65:820–828.
- Johnson, G. A., R. C. Burghardt, M. M. Joyce, T. E. Spencer, F. W. Bazer, C. Pfarrer, and C. A. Gray. 2003. Osteopontin expression in uterine stroma indicates a decidualization-like differentiation during ovine pregnancy. *Biol. Reprod.* 68:1951–1958.
- Johnson, G. A., R. C. Burghardt, T. E. Spencer, G. R. Newton, T. L. Ott, and F. W. Bazer. 1999. Ovine osteopontin: II. Osteopontin and  $\alpha v \beta 3$  integrin expression in the uterus and conceptus during the periimplantation period. *Biol. Reprod.* 61:892–899.
- Ka, H., L. A. Jaeger, G. A. Johnson, T. E. Spencer, and F. W. Bazer. 2001. Keratinocyte growth factor is up-regulated by estrogen in the porcine uterine endometrium and functions in trophectodermal cell proliferation and differentiation. *Endocrinology* 143:2303–2310.
- Ka, H., T. E. Spencer, G. A. Johnson, and F. W. Bazer. 2000. Keratinocyte growth factor: Expression by endometrial epithelia of the porcine uterus. *Biol. Reprod.* 62:1772–1778.
- Kennedy, J. P., C. A. Worthington, and E. R. Cole. 1974. The post-natal development of the ovary and uterus of the merino lamb. *J. Reprod. Fertil.* 36:275–282.
- Knight, J. W., F. W. Bazer, W. W. Thatcher, D. E. Franke, and H. D. Wallace. 1977. Conceptus development in intact and unilaterally hysterectomized-ovariectomized gilts: Interrelations among hormonal status, placental development, fetal fluids and fetal growth. *J. Anim. Sci.* 44:620–637.
- Lacroix, M. C., E. Devino, J. L. Servely, C. Puissant, and G. Kann. 1996. Expression of the growth hormone gene in ovine placenta: Detection and cellular localization of the protein. *Endocrinology* 137:4886–4892.
- Leibovich, H., A. Gertler, F. W. Bazer, and E. Gootwine. 2000. Active immunization of ewes against ovine placental lactogen increases birth weight of lambs and milk production with no adverse effect on conception rate. *Anim. Reprod. Sci.* 64:33–47.
- Noel, S., A. Herman, G. A. Johnson, C. A. Gray, M. D. Stewart, F. W. Bazer, A. Gertler, and T. E. Spencer. 2003. Ovine placental lactogen specifically binds endometrial glands of the ovine uterus. *Biol. Reprod.* 68:772–780.
- Persson, E., L. Sahlin, B. Masironi, V. Dantzer, H. Eriksson, and H. Rodriguez-Martinez. 1997. Insulin-like growth factor-I in the porcine endometrium and placenta: Localization and concentration in relation to steroid influence during early pregnancy. *Anim. Reprod. Sci.* 46:261–281.
- Roberts, R. M., and F. W. Bazer. 1988. The function of uterine secretions. *J. Reprod. Fertil.* 82:875–892.
- Rubin, J. S., D. P. Bottaro, M. Chedid, T. Miki, D. Ron, G. Cheon, W. G. Taylor, E. Fortney, H. Sakata, P. W. Finch, and W. J. LaRochelle. 1995. Keratinocyte growth factor. *Cell Biol. Int.* 19:399–411.
- Sato, T., G. Wang, M. P. Hardy, T. Kurita, G. R. Cunha, and P. S. Cooke. 2002. Role of systemic and local IGF-I in the effects of estrogen on growth and epithelial proliferation of mouse uterus. *Endocrinology* 143:2673–2679.
- Simmen, F. A., R. C. M. Simmen, R. D. Geisert, F. Martinat-Butte, F. W. Bazer, and M. Terqui. 1992. Differential expression during the estrous cycle and pre- and post-implantation conceptus development, of messenger ribonucleic acids encoding components of the pig uterine insulin-like growth factor system. *Endocrinology* 130:1547–1556.
- Simmen, R. C. M., M. L. Green, and F. A. Simmen. 1995. IGF system in periimplantation uterus and embryonic development. Pages 185–204 in *Molecular and Cellular Aspects of Periimplantation Processes*. Vol. 1. S.K. Dey, ed. Springer-Verlag, Inc., New York, NY.
- Simmen, R. C. M., F. A. Simmen, A. Hofig, S. J. Farmer, and F. W. Bazer. 1990. Hormonal regulation of insulin-like growth factor gene expression in pig uterus. *Endocrinology* 127:2166–2174.
- Sinowatz, F., and A. E. Friess. 1983. Uterine glands of the pig during pregnancy. An ultrastructural and cytochemical study. *Anat. Embryol. (Berlin)* 166:121–134.
- Spencer, T. E., F. F. Bartol, F. W. Bazer, G. A. Johnson, and M. M. Joyce. 1999a. Identification and characterization of glycosylation-dependent cell adhesion molecule 1-like protein expression in the ovine uterus. *Biol. Reprod.* 60:241–250.
- Spencer, T. E., and F. W. Bazer. 2002. Biology of progesterone action during pregnancy recognition and maintenance of pregnancy. *Frontiers in Bioscience* 7:d1879–1898.
- Spencer, T. E., C. A. Gray, G. A. Johnson, K. M. Taylor, A. Gertler, E. Gootwine, T. L. Ott, and F. W. Bazer. 1999b. Effects of recombinant ovine interferon tau, placental lactogen, and growth hormone on the ovine uterus. *Biol. Reprod.* 61:1409–1418.
- Spencer, T. E., C. A. Gray, M. J. Joyce, G. Jenster, C. G. Wood, F. W. Bazer, A. A. Wiley, and F. F. Bartol. 1999c. Discovery and characterization of genes expressed in the endometrial epithelium using the ovine uterine gland knockout model. *Endocrinology* 140:4070–4080.
- Stewart, M. D., G. A. Johnson, C.A. Gray, L. A. Schuler, R. C. Burghardt, M. M. Joyce, F. W. Bazer, and T. E. Spencer. 2000. Prolactin receptor and UTMP expression in the ovine endometrium during the estrous cycle and pregnancy. *Biol. Reprod.* 62:1779–1789.
- Tarleton, B. J., T. D. Braden, A. A. Wiley, and F. F. Bartol. 2003. Estrogen-induced disruption of neonatal porcine uterine development alters adult uterine function. *Biol. Reprod.* 68:1387–1393.

- Tarleton, B. J., A. A. Wiley, and F. F. Bartol. 1999. Endometrial development and adenogenesis in the neonatal pig: Effects of estradiol valerate and the antiestrogen ICI 182,780. *Biol. Reprod.* 61:253–263.
- Tarleton, B. J., A. A. Wiley, T. E. Spencer, A. G. Moss, and F. F. Bartol. 1998. Ovary-independent estrogen receptor expression in neonatal porcine endometrium. *Biol. Reprod.* 58:1009–1019.
- Taylor, K. M., C. Chen, C. A. Gray, F. W. Bazer, and T. E. Spencer. 2001. 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.* 64:1236–1246.
- Taylor, K. M., C. A. Gray, M. M. Joyce, M. D. Stewart, F. W. Bazer, and T. E. Spencer. 2000. Neonatal ovine uterine development involves alterations in expression of receptors for estrogen, progesterone, and prolactin. *Biol. Reprod.* 63:1192–1204.
- van Rens, B. T., and T. van der Lende. 2002. Piglet and placental traits at term in relation to the estrogen receptor genotype in gilts. *Theriogenology* 57:1651–1667.
- Wiley, A. A., F. F. Bartol, and D. H. Barron. 1987. Histogenesis of the ovine uterus. *J. Anim. Sci.* 64:1262–1269.
- Wimsatt, W. A. 1950. New histological observations on the placenta of the sheep. *Am. J. Anat.* 87:391–436.