Animal (2019), **13:S1,** pp s11–s19 © The Animal Consortium 2019
doi:10.1017/S1751731119000521

animal

Review: Mammary gland development in swine: embryo to early lactation

W. L. Hurley[†]

Department of Animal Sciences, University of Illinois, Urbana, IL 61801, USA

(Received 3 September 2018; Accepted 14 February 2019)

Milk production by the sow is a major factor limiting the growth and survival of her litter. Understanding the process of morphogenesis of the sow's mammary gland and the factors that regulate mammary development are important for designing successful management tools that may enhance milk production. Primordia of the mammary glands are first observable in the porcine embryo at approximately 23 days of gestation. The glands then progress through a series of morphologically distinct developmental stages such that, at birth, each mammary gland is composed of the teat, an organized fat pad and two separate lactiferous ducts each with a few ducts branching into the fat pad. The glands continue to grow slowly until about 90 days of age when the rate of growth increases significantly. The increased rate of mammary gland growth coincides with the appearance of large ovarian follicles and an increase in circulating estrogen. After puberty, the continued growth of the gland and elongation and branching of the duct system into the fat pad takes place in response to the elevated levels of estrogen occurring as part of the estrous cycles. After conception, parenchymal mass of each gland increases slowly during early pregnancy and then grows increasingly rapidly during the final trimester. This growth is in response to estrogen, progesterone, prolactin and relaxin. Lobuloalveolar development occurs primarily during late pregnancy. By parturition, the fat pad of the mammary gland has been replaced by colostrum-secreting epithelial cells that line the lumen of the alveoli, lobules and small ducts. All mammary glands develop during pregnancy, however, the extent of development is dependent on the location of the mammary gland on the sow's underline. The mammary glands undergo significant functional differentiation immediately before and after farrowing with the formation of colostrum and the transition through the stages of lactogenesis. Further growth of the glands during lactation is stimulated by milk removal. Individual glands may grow or transiently regress in response to the intensity of suckling during the initial days postpartum. *Attempts to enhance milk production by manipulation of mammary development at stages before lactation generally have met with limited success. A more in depth understanding of the processes regulating porcine mammary gland morphogenesis at all stages of development is needed to make further progress.*

Keywords: mammogenesis, fetal development, gilt, estrous cycles, pregnancy

Implications

The sow's milk production is a major limiting factor contributing to suboptimal growth and survival of the piglet. Implementation of management programs that enhance mammary development early in the sow's life may enhance subsequent lactation productivity. Mammary development starts in the early embryo and continues through a series of stages up to and through lactation. The rate of mammary growth is accelerated during several of these stages, offering an opportunity to manipulate the factors controlling this growth. Knowledge of the process of mammogenesis in swine is essential for designing programs aimed at increasing the sow's ability to provide for her piglets.

[†] E-mail: wlhurley@illinois.edu

Introduction

Milk production by the sow is a major limiting factor contributing to suboptimal growth and survival of her piglets (Boyd and Kensinger, 1998). The sow is not able to provide sufficient milk to achieve optimal piglet growth. Piglets raised on the sow have lower weaning weights than those provided with *ad libitum* access to nutrients (Harrell *et al.*, 1993). Providing supplemental milk to piglets sucking the sow increases litter weaning weights (Miller *et al.*, 2012). The limitations of sow milk production are further compounded by the use of hyperprolific sows that have been selected for larger litter size, without an accompanying increase in milk production (Silalahi *et al.*, 2017). There is a significant correlation between growth of a piglet and the mass and DNA of the individual gland that the piglet suckles

(Kim *et al*., 2000; Nielsen *et al*., 2001). This relationship underscores the importance of understanding the process of mammogenesis in swine. The current review provides an overview of the process of mammary gland development from the first rudiments of the mammary gland in the embryo through several developmental stages leading to early lactation. Mammary gland development during lactation and after weaning is reviewed by Farmer (2019).

Fetal mammary gland development

Prenatal morphogenesis of the mouse and rabbit mammary glands have been studied extensively and can be described with relative precision (Propper *et al*., 2013). Considerably fewer details of the embryonic and fetal development of the porcine mammary gland have been reported. The available literature indicates that the mammary gland of the piglet undergoes a similar sequence of morphological changes as does the mouse. The morphological development of the piglet mammary gland is a continuous process through the prenatal stages, where the transition from one stage to the next occurs over a period of days. As such, there is considerable variation in the day of gestation reported for the observations of each developmental stage.

The developing mammary gland is referred to as the mammary primordium during the embryonic phase. Alternative names are mammary anlage or mammary rudiment (Propper *et al*., 2013). The first recognizable structure in the sequence of mammary gland development is the mammary line, characterized by a thickening of the single layer of ectodermal cells extending from the base of the forelimb to the inguinal region, and lying lateral to the ventral mid-line (Turner, 1952). The mammary line is first recognizable at about 20 to 25 days of gestation (Turner, 1952; Marrable, 1971), which is shortly after implantation. Formation of the individual mammary primordia, called placodes, is recognized in the mouse as elliptical pseudostratified multilayered structures within the single-layered ectoderm of the mammary line (Propper *et al*., 2013). The placode stage has not been specifically identified in the piglet embryo, although similar structures were identified as a series of connected epithelial plaques along the mammary line at about 26 days of gestation (Marrable, 1971).

Ectodermal cells are drawn into the expanding mammary primordia as the cell mass continues to expand deeper into the mesoderm. This leads to the formation of the transitory hemispherical hillock stage. Continued sinking of the ectodermal mass into the mesoderm results in formation of a spherical or elongated spherical mass of ectodermal cells called the mammary bud, observed at 28 to 45 days of gestation. The mammary bud stage marks a critical point in the development of the mammary gland. For example, the mesodermal cells surrounding the bud begin to organize into concentric layers adjacent to the bud, forming what is referred to in the mouse as the primary mammary mesenchyme (Propper *et al*., 2013). Early drawings of the piglet mammary bud also include the recognition of this type of orientation of mesenchymal cells adjacent to the mammary bud (Turner, 1952). The next stage of mammary development in the mouse occurs when the core of ectodermal cells sinks further into the mesoderm developing a narrowed neck resulting in a structure referred to as the bulb stage in mice (Propper *et al*., 2013). Morphological descriptions of porcine embryonic mammary development do not specifically identify a bulb stage, however, the developing gland may indeed progress through a similar stage as observed in mice.

The piglet reaches the status of fetus at approximately 36 days of gestation, when the main body systems have differentiated structurally and when sexual dimorphism is externally apparent (Marrable, 1971). The period between 36 and 55 days of gestation is considered as an allometric developmental phase, when the gland is developing at a rate faster than the fetus as a whole. Part of this phase includes the gradual ventral movement of the mammary primordia until they reach the mid-ventral line on either side of the fetus (Marrable, 1971). The next phases of porcine mammary development coincide with this allometric development and include the formation of a primary sprout emanating from the bud or bulb and the initial formation of the teat. These phases occur concurrently at approximately 40 to 60 days of gestation (Turner, 1952). The primary sprout is formed as an elongation of the distal end of the bud or bulb sinking further into the underlying mesenchyme. The teat is formed by the outward growth of the mesenchyme around the neck of the bulb. Two sprouts then branch off from the primary sprout and grow deeper into the mesenchyme (Turner, 1952). These will form the two lactiferous canals, each ultimately forming a separate milk producing gland (Hughes and Varley, 1980). The teat continues to form while the sprouts start branching to form a ductal tree. Canalization (formation of the lumen) of the primary sprout begins at approximately 85 days of gestation and starts to form the lumen of the ductal tree and lactiferous ducts. The mammary structures continue to grow throughout the remainder of gestation. The major structural elements of the mammary gland are formed by the time the piglet is born except for the secretory lobuloalveolar tissue.

Prepubertal mammary gland development

At birth, each mammary gland of the piglet is composed of the teat including the thick connective tissue base of the teat, an organized fat pad of adipose lobules and connective tissue, two lactiferous ducts, and a few ducts branching into the fat pad. These structures continue to grow during the period between birth and puberty. Growth of the parenchymal tissue is responsive to several factors during this period, including mammogenic hormones and nutrition. Understanding and manipulation of this tissue responsiveness is the rationale for much of the research conducted on mammary development during the prepubertal period in swine.

https://doi.org/10.1017/S1751731119000521 Published online by Cambridge University Press

Mammary gland tissue appears to be responsive to mammogenic hormones in the peripartal period. For example, female offspring born to sows fed zearalenone throughout gestation have signs of hyperestrogenism by 1 week after birth, including swollen vulva and early development of the mammary glands (Chang *et al*., 1979). Zearalenone is a mycotoxin with potent estrogenic activity. It is unclear from that study whether the precocious mammary development occurred before birth or while suckling the treated sows. Interestingly, Sorensen *et al*. (2002) observed a high proportion of dividing epithelial cells in a 5-day-old gilt. The possibility that the physiological environment experienced by the mammary gland of the pig early in the preweaning period may affect gland development later in the animal's life deserves greater attention.

In the absence of external stimuli, growth of mammary gland parenchyma and fat pad is normally slow until about 90 days of age (Sorensen *et al*., 2002). Thereafter, tissue growth increases rapidly until the time of puberty. Rate of accumulation of mammary wet weight increases by more than fivefold and DNA per gland increases nearly fourfold after day 90 compared with those rates before day 90 of age. This phase of rapid mammary gland growth occurs concurrently with an increase in circulating estrogen. The phase of rapid growth also coincides with increasing ovarian wet weight and number and size of ovarian follicles occurring after 70 days of age and continuing through puberty (Dyck and Swierstra, 1983). Urinary excretion of estrone increases during the period between about 96 and 154 days of age, then reaches a plateau that continues until shortly before the first estrus (Camous *et al*., 1985).

A number of studies have used plant-derived components that are considered to have estrogenic activity in order to enhance mammary gland development in prepubertal gilts (reviewed by Farmer, 2013; Farmer and Hurley, 2015). Flaxseed is a rich source of secoisolariciresinol diglycoside, the immediate precursor of the mammalian lignans, enterolactone and enterodiol, which can have estrogenic activity. Feeding flaxseed to gilts from 88 days of age through puberty results in changes in fatty acid profiles, however, has no effect on circulating estradiol or prolactin, nor any effect on mammary development. Expression of several genes involved in mammary epithelial cell proliferation also is not affected by flaxseed treatment. Interestingly, pubertal gilts whose dams are fed a flaxseed-supplemented diet during late gestation and lactation have greater parenchymal protein and tend to have greater parenchymal weight compared with gilts whose dams receive a control diet. Supplementation of diets with the soy phytoestrogen, genistein, starting at 90 days of age through day 183 results in increased parenchymal DNA, but has no effect on expression of mammary growth factor receptor genes or circulating concentrations of prolactin, estradiol, progesterone or IGF-1. Overall, feeding these weak estrogens to gilts during the prepubertal period has met with limited success in enhancing mammary gland growth.

Prolactin is another mammogenic hormone that affects mammary development in the prepubertal period. Plasma prolactin concentrations are elevated during the initial month after birth, decline after weaning and then slowly increase to reach constant levels for the remainder of the prepubertal period (13 to 15 ng/ml; Camous *et al*., 1985). Administration of recombinant porcine prolactin to prepubertal gilts from approximately 141 days (75 kg BW) to 170 days of age increases circulating levels of that hormone in a dose-dependent manner (Farmer and Palin, 2005). This period coincides with the middle portion of the pre-pubertal increase in growth rate of the mammary tissue (Sorensen *et al*., 2002). Mammary development is stimulated by prolactin treatment, as indicated histologically by the presence of distended alveolar and ductal lumina and the presence of secretory contents in the lumen (McLaughlin *et al*., 1997). Mammary parenchymal weight, parenchymal protein and DNA percentages, and total protein and DNA contents at day 170 of age are each increased and fat percentage decreased by prolactin treatment (Farmer and Palin, 2005). The level of messenger RNA for prolactin is decreased in both mammary parenchymal tissue and the anterior pituitary (Farmer and Palin, 2005). Level of messenger RNA for prolactin receptor in the anterior pituitary is not affected by prolactin treatment, whereas mammary parenchymal prolactin receptor messenger RNA is increased in prolactin-treated gilts. Mammary tissue levels of messenger RNA for signal transducer and activator of transduction 5A and 5B (STAT5A and STAT5B, respectively) are significantly increased in prolactin-treated gilts (Farmer and Palin, 2005). Extra-parenchymal tissue is unaffected by prolactin treatment. Neither of these studies administering exogenous prolactin observed a differential effect of prolactin dose, suggesting that even low doses of prolactin may be sufficient to enhance mammary development at this stage.

Several studies explored the effects of feeding regimens during the early and late prepubertal period in swine, as reviewed in Farmer (2013) and Farmer and Hurley (2015). Feeding regimens that include alternating phases of moderate (restricted) and maximal growth rates during the prepubertal period can negatively affect subsequent growth of mammary gland parenchymal tissue in the late stages of pregnancy. Mammary gland development during the early prepubertal period (birth to day 90 of age) does not appear to be impacted by feed restriction compared with *ad libitum* feeding. In contrast, several studies have demonstrated that *ad libitum* feeding during the later prepubertal period (day 90 of age to puberty) results in greater total mammary gland weight, but in a lower concentration of total tissue DNA. Results from these studies confirm that restrictive feeding during the later prepubertal period also restricts development of mammary parenchymal and extraparenchymal tissues. Furthermore, cycles of dietary restriction of gilts followed by over-allowance from the mid-prepubertal period through puberty also results in decreased parenchymal tissue at puberty. This negative effect on parenchymal development appears to carry over to the late stages of pregnancy. In spite of this effect on mammary parenchymal growth, there is no effect of the prepubertal restriction/over-allowance feeding

https://doi.org/10.1017/S1751731119000521 Published online by Cambridge University Press

regime on the growth rate of piglets suckling the treated sows. Dietary protein level fed during the period from day 90 of age to the first or second estrus also does not affect mammary development.

Postpubertal mammary gland development

The continued growth of the gland and elongation and branching of the duct system within the fat pad occurs after puberty in response to changing levels of hormones associated with the reproductive cycles, especially repeated cycles of increased estrogen. Research on the postpubertal stage of mammary development in the gilt is more limited than that for the prepubertal period. Most gilts that are to be used for reproductive purposes are bred shortly after reaching puberty. As a consequence, they undergo only a limited number of estrous cycles. Cycling gilts, as indicated by the presence of corpora lutea at slaughter, do have more total mammary DNA and RNA (parenchymal plus extra-parenchymal tissue) than non-cycling gilts. This observation is consistent with estrogen-stimulated ductal growth and elongation into the mammary fat pad (Sorensen *et al*., 2006). Gilts in estrus seem to have a higher proportion of mammary gland cells undergoing division than those in diestrus (Sorensen *et al*., 2002).

Administration of estrogen or progesterone for 3 days to intact 5-month-old Yucatan miniature gilts has no effect on the histological density of terminal ductal lobular units (TDLU) nor the relative stage of TDLU development (Horigan *et al*., 2009). Estrogen administration does increase the occurrence of terminal buds in mammary tissue. Terminal buds are capped structures observed at the end of elongating ducts or blind ended structures at the end of ductules within the TDLU (Horigan *et al*., 2009). Endogenous sources of the reproductive hormones need to be removed in order to study their effects on mammary development in virgin gilts. Ovariectomy is often used to remove the primary endogenous source of estrogen. Treatment with a dopamine agonist, such as 2-bromo-α-ergocryptine (bromocriptine), is often used to induce hypoprolactinemia, thus limiting the effects of endogenous prolactin. Treatment of ovariectomized virgin gilts with combinations of estrogen and/or progesterone and receiving bromocriptine or haloperidol (a dopamine antagonist; inducing hyperprolactinemia) provides an experimental model for the study of the interactions of mammogenic hormones on mammary development (Horigan *et al*., 2009). Ovariectomy with or without bromocriptine leads to regression of the mammary parenchymal structures. Treatment with bromocriptine alone inhibits formation of terminal buds and all TDLU compared with saline controls. Progesterone with or without haloperidol has no obvious effect compared with controls. Treatment with estrogen in all combinations of bromocriptine, haloperidol and progesterone results in a shift of TDLU development with fewer observed TDLU1 and more of the advanced TDLU2 stage structures. Estrogen plus haloperidol was found to be necessary to further shift the TDLU development to the third, more developed stage (Horigan *et al*., 2009), suggesting a synergy between estrogen and prolactin.

Administration of combinations of progesterone, estrogen and/or relaxin to ovariectomized virgin gilts for 10 days is used to distinguish the effects of these hormones (Winn *et al*., 1994). Gilts receiving no hormone or only progesterone have no apparent mammary gland development, indicating that this hormone does not affect mammary development in the absence of other hormones. Those gilts receiving estrogen or estrogen plus progesterone treatment have limited mammary development. As such, progesterone does not enhance the effect of estrogen alone. Gilts receiving only relaxin treatment have mammary development even more limited than those receiving only estrogen, suggesting that relaxin alone does not have a significant effect on mammary development (Winn *et al*., 1994). Treatment with relaxin plus progesterone results in slightly greater development than those receiving only relaxin (Winn *et al*., 1994). Finally, synergies among the hormones on mammary growth are clear in gilts receiving estrogen plus relaxin or estrogen plus relaxin and progesterone treatments, resulting in mammary tissue development similar to that found just before farrowing (Winn *et al*., 1994).

Mammary gland development during pregnancy

Mammary gland growth is limited during the first two-thirds of pregnancy. Mammary DNA content and tissue mass are not different between the second estrous cycle and the early stages of pregnancy (Sorensen *et al*., 2002), although duct development is slowly progressing during that period (Kensinger *et al*., 1982). The breakpoint for growth rate of mammary tissue has been estimated to occur at approximately day 74 of pregnancy (Ji *et al*., 2006). A significant increase in lobuloalveolar development occurs after day 75 (Kensinger *et al*., 1982). Parenchymal tissue DNA increases fourfold and total parenchymal DNA increases ninefold between days 75 and 105 of pregnancy (Weldon *et al*., 1991). Parenchymal tissue mass increases by over 200%, while parenchymal lipid decreases by nearly 70%. This latter observation suggests a decrease in adipocytes within the parenchymal tissue. The parenchyma is both getting larger and increasing in the density of epithelial structures. Extra-parenchymal tissue also increases by nearly 170% during this period (Weldon *et al*., 1991). Rate of accretion of tissue protein is 13-fold greater in the period of pregnancy after day 75 than before day 75 (Ji *et al*., 2006). Mammary gland parenchymal cross-sectional area increases by nearly fourfold between days 80 and 100, and then increases roughly another 30% between days 100 and 110 (Hurley *et al*., 1991). Growth rate of individual mammary glands varies according to location on the mammary line, with middle glands (glands 3, 4 and 5) achieving the largest tissue mass, followed by the anterior glands (glands 1 and 2) and the posterior glands (glands 6, 7 and 8) growing the slowest (Ji *et al*., 2006).

https://doi.org/10.1017/S1751731119000521 Published online by Cambridge University Press

Mammary development during pregnancy occurs in response to stimulation by a number of hormones. Of particular importance for the sow are progesterone, estrogen, prolactin and relaxin. Progesterone concentrations fluctuate during pregnancy, but they remain high until shortly before parturition (DeHoff *et al*., 1986; Eldridge-White *et al*., 1989). Serum progesterone concentrations in the sow decline rapidly between days 110 and 112 of gestation and then drop precipitously during the final days before farrowing (Eldridge-White *et al*., 1989). Fetal progesterone declines steadily from days 60 to 105 of pregnancy and then rapidly increases by day 112 (DeHoff *et al*., 1986). Circulating fetal progesterone concentrations are about fourfold greater than concentrations in maternal blood, consistent with the fetal hormone being available for conversion to estrogens by fetoplacental tissues. The major estrogens in the sow's blood during pregnancy are estrone, estrone sulfate, estradiol and estradiol sulfate, much of which are produced by conversion of estrogens produced by the fetus (DeHoff *et al*., 1986). Total plasma estrogen concentration remains low from early pregnancy through days 75 to 80 and then rises steadily to maximal concentrations by day 112 (Eldridge-White *et al*., 1989). Estrogen concentrations decline shortly after farrowing. Circulating concentrations of prolactin are moderately elevated during very early pregnancy and then remain low until about 2 days before parturition when there is a rapid surge of the hormone (Dusza and Krzymowska, 1981; DeHoff *et al*., 1986). Growth hormone concentrations in maternal blood remain constant throughout pregnancy, then have a rapid increase during the *peripartum* period (DeHoff *et al*., 1986). Blood concentrations of relaxin are low before day 80 of pregnancy, then increase about fourfold by day 110, followed by a greater than fivefold surge by approximately 20 h *prepartum* (Eldridge-White *et al*., 1989).

Induction of hypoprolactinemia in the first two-thirds of pregnancy does not result in an effect on mammary gland parenchymal tissue growth (reviewed in Farmer, 2013; Farmer and Hurley, 2015). It appears that the relatively low prolactin concentrations observed during pregnancy in the intact, non-treated gilt are sufficient to stimulate the limited mammary gland development which occurs during that period. In contrast, inducing hypoprolactinemia in pregnant gilts from days 90 to 110 of pregnancy significantly reduces mammary parenchymal tissue weight, total DNA, total RNA and protein percentage.

Efforts to induce hyperprolactinemia during late pregnancy in order to enhance mammary development and milk production have met with mixed results. Administration of porcine prolactin to multiparous sows from day 107 of pregnancy to day 2 of lactation has no effect on lactation performance (Crenshaw *et al*., 1989). Administration of silymarin, an extract of milk thistle, leads to a short-term increase in prolactin concentrations during late pregnancy, however, this effect on prolactin is not accompanied by a positive effect on mammary gland development (Farmer *et al*., 2014). Induction of hyperprolactinemia with the dopamine antagonist, domperidone, during late pregnancy increases mammary epithelial cell differentiation in late pregnancy and milk production during lactation, but not epithelial proliferation (VanKlompenberg *et al*., 2013). Administration of porcine prolactin to gilts from day 102 of pregnancy to the end of lactation is reported to have a negative effect on milk production (King *et al*., 1996). Exogenous prolactin during late pregnancy may induce premature lactogenesis disrupting the normal synchrony between birth of the litter and initiation of milk secretion. On the other hand, treatment of lactating sows with prolactin does not enhance milk production, possibly because prolactin receptors are already maximally saturated with endogenous prolactin concentrations (Farmer *et al*., 1999).

The role of growth hormone on porcine mammary development during pregnancy has not received as much attention as that of prolactin. Administration of growth hormone to gilts starting on day 108 of pregnancy and continuing to day 24 of lactation has no effect on milk yield or litter weaning weight (Cromwell *et al*., 1992). It is interesting to note that an effect of reducing circulating growth hormone on lactating rats can be observed only when circulating prolactin is also reduced (Flint *et al*., 1992). Characterization of an effect of growth hormone on mammary development and lactation in swine may also require the inhibition of the effect of prolactin.

A role for relaxin in mammary development in swine during pregnancy has been demonstrated using an ovariectomized pregnant gilt experimental model (Hurley *et al*., 1991). Relaxin is secreted by the corpora lutea in swine. The hormone requires concurrent or pre-exposure to estrogen to manifest its effects. The conceptus tissues provide the estrogen stimulation for this experimental model. Ovariectomy on days 80 or 100 of pregnancy with progesterone replacement therapy (to maintain pregnancy) dramatically reduces the extent of mammary development at days 100 or 110, respectively. Treatment of ovariectomized pregnant gilts with progesterone and relaxin results in mammary development similar to that observed in sham-ovariectomized control gilts. It is interesting to note that in gilts ovariectomized on day 100 of pregnancy, the mammary tissue cross-sectional area is decreased at day 110 compared with the area observed at day 100 (Hurley *et al*., 1991). This suggests that the mammary tissue undergoes an active regression in the absence of relaxin between days 100 and 110, even with continued exposure to endogenous estrogen from the conceptus. Results of morphometric analysis of mammary parenchymal tissue indicate an increase in parenchymal epithelial area and a decrease in stromal area in ovariectomized pregnant gilts receiving progesterone plus relaxin compared with those only receiving progesterone (Hurley *et al*., 1991). Prolactin concentrations were not determined in this relaxin-replacement therapy study.

As noted above, the concentrations of several mammogenic hormones are changing rapidly during the immediate *prepartum* period. This is a period where mammary tissue growth coincides with the process of lactogenesis. Analysis of tissue ultrastructure from late gestation gilts provides a framework for understanding the structural changes occurring in mammary tissue during the *peripartum* period.

https://doi.org/10.1017/S1751731119000521 Published online by Cambridge University Press

Mammary epithelial cells are relatively undifferentiated at day 90 of gestation (Kensinger *et al*., 1986), a period when the parenchymal tissue is rapidly growing (Ji *et al*., 2006). Epithelial cell differentiation has been initiated by day 105 and is further differentiated by day 112, as indicated by an increase in intracellular milk fat droplets and expanded rough endoplasmic reticulum (Kensinger *et al*., 1986). By several hours after birth of the last piglet, the epithelial cells have achieved the cellular polarity typical of a secretory tissue. In addition, the alveolar lumina appear to contain milk rather than colostrum. Removal of the mammary secretions by the piglet results in a rapid change in secretion composition after parturition (Hurley, 2015), which may explain the observation of milk in the alveolar lumen rather than colostrum. Ultrastructural differentiation of epithelial cells is complete by day 4 of lactation (Kensinger *et al*., 1986).

Diet deprivation during early pregnancy up to day 70, followed by over-allowance through the remainder of pregnancy, results in less total parenchymal tissue and less parenchymal fat, DNA and RNA at day 110 of pregnancy compared with gilts maintained on a control diet throughout pregnancy (reviewed in Farmer and Hurley, 2015). Composition of mammary parenchymal tissue is unaffected by dietary treatment. Piglet growth is not affected by dietary treatment. The expression of several selected genes is lower in mammary tissue of treated *v*. control gilts. Dietary protein level fed during pregnancy does not significantly affect mammary parenchymal development in primiparous gilts (reviewed in Farmer, 2013; Farmer and Hurley, 2015). On the other hand, level of dietary energy during pregnancy does affect mammary development. Parenchymal tissue weight is greater in gilts fed adequate energy *v*. those fed increased energy levels. Total gland parenchymal DNA, RNA and protein are greater in adequate energy *v*. increased energy. There is no interaction between level of energy and level of protein in their effects on mammary parenchyma.

Body condition of primigravid gilts correlates with the extent of mammary development at the end of pregnancy (Farmer *et al*., 2016a and 2016b). Concentrations of DNA and RNA in mammary parenchymal tissue at the end of pregnancy decrease as backfat depth increases. Extra-parenchymal tissue is greater in high backfat gilts than low backfat gilts at the end of pregnancy. Percentage of proliferating cells in mammary tissue is greater in high then low backfat gilts (Farmer *et al*., 2016b). Mammary development is negatively impacted in gilts with low backfat at the end of pregnancy, while development in gilts with medium and high (up to 26 mm) backfat is not impacted. Backfat thickness at mating, when maintained throughout pregnancy, also affects mammary development. Thinner gilts at mating have greater mammary parenchymal DNA and RNA concentrations than heavier gilts at day 110 of pregnancy (Farmer *et al*., 2016a and 2016b).

Peripartum mammary gland development

Circulating hormone concentrations undergo dramatic change during the *peripartum* period. In addition to the changing profiles of progesterone, estrogen and relaxin previously discussed, circulating concentrations of prolactin also undergo rapid change shortly before, during and after parturition. The *peripartum* prolactin surge begins about 2 days *prepartum* and extends through several days *postpartum* (Dusza and Krzymowska, 1981). Prolactin concentrations fluctuate during the initial hours after the start of parturition. Circulating prolactin concentrations decline slowly over the initial days *postpartum*, although remain significantly greater than those found during most of pregnancy (Dusza and Krzymowska, 1981).

Inhibition of the *peripartum* prolactin surge has profound effects on lactogenesis, milk secretion and continued development of the gland. Bromocriptine administered to pregnant gilts (oral; Farmer *et al*., 1998) or to pregnant multiparous sows (intramuscular; Whitacre and Trelfall, 1981) during the *prepartum* period up to parturition inhibits the *prepartum* prolactin surge and reduces circulating prolactin on the day of and the day after parturition. Milk production is inhibited to the point where piglets suckling treated sows must be removed from the study because of a lack of weight gain within the 1st day *postpartum*. Glands that are not regularly suckled regress (Kim *et al*., 2001). Feeding a diet containing high levels of sorghum ergot sclerotia to pregnant sows during the *prepartum* period also reduces blood prolactin, as well as lactation performance (Kopinski *et al*., 2007). Lower levels of the sorghum ergot have more limited responses. First parity sows are more sensitive to consumption of the sorghum ergot. First parity untreated sows have lower prolactin concentrations than multiparous sows, and therefore may be more susceptible to ergot inhibition (Kopinski *et al*., 2007).

Prepartum bromocriptine treatment can result in treated sows farrowing prematurely at 110.6 days of pregnancy *v*. 115.2 days for control sows (Whitacre and Trelfall, 1981). Treated sows often are unable to maintain piglets alive beyond 3 days *postpartum*. The lack of suckling-induced prolactin secretion after piglets are removed from the litter results in reduced *postpartum* circulating prolactin impacting further mammary gland growth and lactation performance. In addition, the limited development of the piglets and the mammary glands at day 110 of pregnancy may contribute to the unsuccessful lactation performance of these sows. Premature delivery of the piglets may impact suckling intensity, which in turn will impact the growth and milk production of the mammary gland (King, 2000).

Relaxin concentrations are also changing during the *peripartum* period. Maximal relaxin concentrations are reached several hours *prepartum* and then decline markedly by 48 h *postpartum* and are non-detectable by 72 to 120 h (Porter *et al*., 1992). The relaxin surge coincides with the *prepartum* surge of prolactin, the high estrogen concentrations and the declining progesterone concentrations. Administration of relaxin to intact or luteectomized pregnant gilts during late pregnancy can result in reduced lactation performance (based on percentage of piglets nursing and manual expression of milk) and a lower survival to weaning (Kertiles

https://doi.org/10.1017/S1751731119000521 Published online by Cambridge University Press

and Anderson, 1979). On the other hand, a deficiency of relaxin concentrations during lactation does not appear to impact lactation performance of sows (Porter *et al*., 1992).

The reduced mammary development observed during late pregnancy in relaxin-deficient gilts (Hurley *et al*., 1991) provides an experimental model to explore the relative contribution of *prepartum v. postpartum* mammary development to lactation performance. Pregnant gilts ovariectomized on day 80 of gestation and administered progesterone from days 80 to 113 have been used to evaluate the effects of relaxin deficiency on subsequent lactation performance (Zaleski *et al*., 1996). Piglets were delivered by cesarean section, and gilts were given 1-day-old foster piglets that had received colostrum. Foster litters were allowed to suckle through day 28 of lactation. Mammary gland development at cesarean section was visually reduced in relaxin-deficient gilts compared with relaxin-replete gilts, consistent with observations of the earlier study (Hurley *et al*., 1991). Relaxin deficiency has no significant effect on time that piglets spent on the udder, piglet mortality, milk composition, mammary gland cross-sectional area at day 28 or sow weight change during lactation. Litter weights at day 21 of lactation in control (sham-ovariectomized) gilts tended to be heavier than litters from ovariectomized gilts receiving only progesterone or receiving progesterone and relaxin from day 80 to farrowing and were significantly greater than litters from ovariectomized gilts receiving progesterone and relaxin from day 100 to farrowing (Zaleski *et al*., 1996). Nevertheless, treated gilts were able to complete successful lactations in all cases. These observations indicate that suckling-stimulated mammary gland growth during lactation can at least partially overcome any effect on lactation performance caused by reduced *prepartum* mammary development. This is in contrast to the failure of lactation in sows that are prolactin deficient during the *peripartum* period (Whitacre and Trelfall, 1981; Farmer *et al*., 1998).

Mammary gland development during early lactation

The mammary glands undergo significant functional differentiation immediately before and after farrowing with the formation of colostrum and the passage through the two stages of lactogenesis (Pang and Hartmann, 2007). The initial stage is secretory differentiation, occurring in late pregnancy when the mammary epithelial cells differentiate to have the capacity to synthesize unique milk constituents. Secretory differentiation starts 8 to 10 days *prepartum* in the sow based on changes in mammary cell structure and metabolism (Kensinger *et al*., 1982), and on increasing concentrations of lactose found in the urine (Hartmann *et al*., 1984). Secretory activation is the second stage, when copious milk secretion begins. Secretory activation is associated with rapid changes in concentrations of many milk components. Earlier studies suggested that secretory activation begins 2 or 3 days *prepartum* (Kensinger *et al*., 1986). However, a careful evaluation of piglet growth during the initial hours *postpartum* shows that piglet weight change occurs in two phases, an initial increase reaching a plateau at about 20 h, followed by a sustained increase starting at about 34 h *postpartum* (Theil *et al*., 2014). This latter observation is consistent with copious milk secretion being initiated approximately a day and a half *postpartum*.

Substantial growth of the mammary gland occurs during lactation in swine (Kim *et al*., 1999). For example, total gland DNA increases by 82% between the day of farrowing and day 21 of lactation (Kim *et al*., 1999 and 2001). Growth of the glands during lactation is stimulated by milk removal (Farmer and Hurley, 2015). Milk synthesis and mammary tissue growth during lactation both are tied to systemic hormonal and local mammary responses to milk removal. The extent of mammary development during lactation is dependent on the degree of milk removal demand, also referred to as suckling intensity (King, 2000; Hurley, 2001). This intensity is determined by factors such as frequency and completeness of milk removal, number and size of suckling offspring, and hormones released in response to mammary stimulation (King, 2000; Hurley, 2001).

A strong teat preference by piglets and coordinated suckling pattern are established soon after parturition in some swine breeds such as the Yorkshire × Landrace crosses used in much of the research discussed in this review. These factors result in a close relationship between growth of a piglet and growth of the individual gland that the piglet suckles (Kim *et al*., 2000; Nielsen *et al*., 2001).

Mammary gland development during pregnancy occurs in response to systemic hormones, which could be expected to regulate growth of all the glands comparably. Size of a gland at parturition is determined in part by position on the ventral surface of the mother. It also might be expected that a differential developmental pattern would occur in individual glands after parturition and during the initial days of lactation dependent on the size of the piglet and location of the gland. Comparing the mean DNA content of mammary tissue by gland location at day 5 of lactation (Kim *et al*., 1999) with the mean DNA content by gland location at the day of farrowing (Kim *et al*., 2000) gives a picture of mammary growth during the initial days of lactation. The standardization of litter size and piglet birth weight (1.34 to 1.49 kg) provides a consistent suckling intensity across gland locations on the sow. The mammary glands with the least total DNA at parturition (the numbers 1, 6 and 7 glands) grow rapidly during the initial 5 days of lactation when receiving the same suckling intensity as the other gland locations, although these glands are still on average the smallest at day 5. The numbers 2 and 3 glands are intermediate in total DNA at parturition and experience little growth until day 5. Finally, the largest glands at parturition, the numbers 4 and 5, may have excess milk producing capacity relative to the demands of the piglets suckling those glands through day 5, and the glands may undergo regression by day 5 *postpartum*. The suckling-induced mammary gland growth of all glands occurring after day 5 of lactation continues up to peak lactation (Kim *et al*., 1999). Individual glands may grow or transiently regress in response to the demand of

https://doi.org/10.1017/S1751731119000521 Published online by Cambridge University Press

the suckling piglet during the initial days *postpartum*. The relationship between gland size and suckling intensity it receives during the days after parturition has not been thoroughly studied. Enhancement of gland size at parturition by endocrine, nutritional or any other means may only have significant benefit if suckling intensity is increased in early lactation. Fostering older piglets onto a sow in early lactation does result in greater milk production during that period (reviewed in Farmer and Hurley, 2015). Further discussion of mammary gland development during lactation is extensively reviewed by Farmer (2019).

Conclusion

The mammary gland of the gilt undergoes a series of developmental stages beginning during the early embryonic period. Characterization of the morphogenesis of the mammary glands in the embryo and fetus has not been completely described. In addition, information about the factors controlling that development is limited. The impact of hormonal and metabolic environments experienced by the neonatal porcine mammary gland on its subsequent development and lactation function have not been explored. The mammary gland is growing isometrically during the initial 3 months of postnatal life, followed by a period of rapid allometric growth up to puberty. This latter period of prepubertal mammary development presents an opportunity to externally enhance the growth through hormonal or nutritional means. Information about the postpubertal period of mammary development is also limited, in part because production gilts are bred after only a very few estrous cycles. After conception, the gland grows slowly through the first two-thirds of pregnancy. By about days 75 to 80 of pregnancy the gland begins growing rapidly in response to stimulation by several hormones, including estrogen, relaxin and prolactin. Inhibiting these hormones can compromise development in late pregnancy. While development of the mammary gland is essential for successful lactation, even a gland that is developmentally compromised during late pregnancy may successfully lactate in response to sucking stimulus. This underscores the important role of suckling and milk removal in stimulating mammary development after parturition.

Acknowledgments

The author wishes to acknowledge the many collaborators that he has had over the years in performing research on mammary development and lactation in swine.

Declaration of interest

The author has no conflicts of interest to disclose.

Ethics statement

Not applicable to this review paper.

Software and data repository resources

None of the data were deposited in an official repository.

References

Boyd RD and Kensinger RS 1998. Metabolic precursors for milk synthesis. In The lactating sow (ed. MWA Verstegen, PJ Moughan and JW Schrama), pp. 71–95. Wageningen Academic Publishers, Wageningen, The Netherlands.

Camous S, Prunier A and Pelletier J 1985. Plasma prolactin, LH, FSH and estrogen excretion patterns in gilts during sexual development. Journal of Animal Science 60, 1308–1317.

Chang K, Kurtz HJ and Mirocha CJ 1979. Effects of the mycotoxin zearalenone on swine reproduction. American Journal of Veterinary Research 40, 1260–1267.

Crenshaw TD, Grieshop CM, McMurtry JP and Schricker BR 1989. Exogenous porcine prolactin injections did not alter sow lactation performance. Journal of Animal Science 67(suppl. 1), 258 (abstr).

Cromwell GL, Stahly TS, Edgerton LA, Monegue HJ, Burnell TW, Schenck BC and Schricker BR 1992. Recombinant porcine somatotropin for sows during late gestation and throughout lactation. Journal of Animal Science 70, 1404–1416.

DeHoff MH, Stoner CS, Bazer FW, Collier RJ, Kraeling RR and Buonomo FC 1986. Temporal changes in steroids, prolactin and growth hormone in pregnant and pseudopregnant gilts during mammogenesis and lactogenesis. Domestic Animal Endocrinology 3, 95–105.

Dusza L and Krzymowska H 1981. Plasma prolactin levels in sows during pregnancy, parturition and early lactation. Journal of Reproduction and Fertility 61, 131–134.

Dyck GW and Swierstra EE 1983. Growth of the reproductive tract of the gilt from birth to puberty. Canadian Journal of Animal Science 63, 81–87.

Eldridge-White R, Easter RA, Heaton DM, O'Day MB, Petersen GC, Shanks RD, Tarbell MK and Sherwood OD 1989. Hormonal control of the cervix in pregnant gilts. I. Changes in physical properties of the cervix correlate temporally with elevated serum levels of estrogen and relaxin. Endocrinology 125, 2996–3003.

Farmer C 2013. Review: Mammary development in swine: effects of hormonal status, nutrition and management. Canadian Journal of Animal Science 93, 1–7.

Farmer C 2019. Review: Mammary development in lactating sows: the importance of suckling. Animal, 13 s20−s25. https://doi.org/10.1017/S1751731118003464.

Farmer C, Comi M, Duarte CRA, Vignola M, Charagu P and Palin M-F 2016b. Differences in body condition of gilts that are maintained from mating to the end of gestation affect mammary development. Journal of Animal Science 94, 3206–3214.

Farmer C, Duarte CRA, Vignola M and Palin M-F 2016a. Body condition of gilts at the end of gestation affects their mammary development. Journal of Animal Science 94, 1897–1905.

Farmer C and Hurley WL 2015. Mammary development. In The gestating and lactating sow (ed. C Farmer), pp. 73–94. Wageningen Academic Publishers, Wageningen, The Netherlands.

Farmer C, Lapointe J and Palin M-F 2014. Effects of the plant extract silymarin on prolactin concentrations, mammary gland development, and oxidative stress in gestating gilts. Journal of Animal Science 92, 2922–2930.

Farmer C and Palin M-F 2005. Exogenous prolactin stimulates mammary development and alters expression of prolactin-related genes in prepubertal gilts. Journal of Animal Science 83, 825–832.

Farmer C, Robert S and Rushen J 1998. Bromocriptine given orally to periparturient or lactating sows inhibits milk production. Journal of Animal Science 76, 750–757.

Farmer C, Sorensen MT, Robert S and Petitclerc D 1999. Administering exogenous porcine prolactin to lactating sows: milk yield, mammary gland composition, and endocrine and behavioral responses. Journal of Animal Science 77, 1851–1859.

Flint DJ, Tonner E, Beattie J and Panton D 1992. Investigation of the mechanism of action of growth hormone in stimulating lactation in the rat. Journal of Endocrinology 134, 337–383.

Harrell RJ, Thomas MJ and Boyd BD 1993. Limitations of sow milk yield on baby piglet growth. In Proceedings of the Cornell Nutrition Conference for Feed Manufacturers, 19–21 October 1993, Ithaca, NY, pp. 156–164.

Hartmann PE, Whitely JL and Willcox DL 1984. Lactose in plasma during lactogenesis, established lactation and weaning in sows. Journal of Physiology 347, 453–463.

Horigan KC, Trott JF, Barndollar AS, Scudder JM, Blauwiekel RM and Hovey RC 2009. Hormone interactions confer specific proliferative and histomorphogenic responses in the porcine mammary gland. Domestic Animal Endocrinology 37, 124–138.

Hughes PE and Varley MA 1980. Lactation. In Reproduction in the pig (ed. PE Hughes and MA Varley), pp. 136–158. The Butterworth Group, London, UK.

Hurley WL 2001. Mammary gland growth in the lactating sow. Livestock Production Science 70, 149–157.

Hurley WL 2015. Composition of sow colostrum and milk. In The gestating and lactating sow (ed. C Farmer), pp. 193–229. Wageningen Academic Publishers, Wageningen, The Netherlands.

Hurley WL, Doane RM, O'Day-Bowman MB, Winn RJ, Mojonnier LE and Sherwood OD 1991. Effect of relaxin on mammary development in ovariectomized pregnant gilts. Endocrinology 128, 1285–1290.

Ji F, Hurley WL and Kim SW 2006. Characterization of mammary gland development in pregnant gilts. Journal of Animal Science 84, 579–587.

Kensinger RS, Collier RJ and Bazer FW 1986. Ultrastructural changes in porcine mammary tissue during lactogenesis. Journal of Anatomy 145, 49–59.

Kensinger RS, Collier RJ, Bazer FW, Ducsay CA and Becker HN 1982. Nucleic acid, metabolic and histological changes in gilt mammary tissue during pregnancy and lactogenesis. Journal of Animal Science 54, 1297–1308.

Kertiles LP and Anderson LL 1979. Effect of relaxin on cervical dilation, parturition and lactation in the pig. Biology of Reproduction 21, 57–68.

Kim SW, Easter A and Hurley WL 2001. The regression of unsuckled mammary glands during lactation in sows: the influence of lactation stag, dietary nutrients, and litter size. Journal of Animal Science 79, 2659–2668.

Kim SW, Hurley WL, Han IK and Easter RA 1999. Changes in tissue composition associated with mammary gland growth during lactation in sows. Journal of Animal Science 77, 2510–2516.

Kim SW, Hurley WL, Han IK and Easter RA 2000. Growth of nursing pigs related to the characteristics of nursed mammary glands. Journal of Animal Science 78, 1313–1318.

King RH 2000. Factors that influence milk production in well-fed sows. Journal of Animal Science 78 (suppl. 3), 19–25.

King RH, Pettigrew JE, McNamara JP, McMurtry JP, Henderson TL, Hathaway MR and Sower AF 1996. The effect of exogenous prolactin on lactation performance of first-litter sows given protein-deficient diets during the first pregnancy. Animal Reproduction Science 41, 37–50.

Kopinski JS, Blaney BJ, McVeigh JF and Murray S-A 2007. Feeding sorghum ergot (*Claviceps Africana*) to sows before farrowing inhibits milk production. Australian Veterinary Journal 85, 169–176.

Marrable AW 1971. The embryonic pig. Pitman Medical, London, UK.

McLaughlin CL, Byatt JC, Curran DF, Veenhuizen JJ, McGrath MF, Buonomo FC, Hintz RL and Baile CA 1997. Growth performance, endocrine, and metabolite responses of finishing hogs to porcine prolactin. Journal of Animal Science 75, 959–967.

Miller YJ, Collins AM, Smits RJ, Thomson PC and Holyoake PK 2012. Providing supplemental milk to piglets preweaning improves the growth but not survival of gilt progeny compared with sow progeny. Journal of Animal Science 90, 5078–5085.

Nielsen OL, Pedersen AR and Sorensen MT 2001. Relationships between piglet growth rate and mammary gland size of the sow. Livestock Production Science 67, 273–279.

Pang WW and Hartmann PE 2007. Initiation of human lactation: secretory differentiation and secretory activation. Journal of Mammary Gland Biology and Neoplasia 12, 211–221.

Porter DG, Friendship RM, Ryan PL and Wasnidge C 1992. Relaxin is not associated with poor milk yield in the postpartum sow. Canadian Journal of Veterinary Science 56, 204–207.

Propper AY, Howsard BA and Veltmaat JM 2013. Prenatal morphogenesis of mammary glands in mouse and rabbit. Journal of Mammary Gland Biology and Neoplasia 18, 93–104.

Silalahi P, Tribout T, Billon Y, Gogue J and Bidanel JP 2017. Estimation of the effects of selection on French Large White sow and piglet performance during the sucking period. Journal of Animal Science 95, 4333–4343.

Sorensen MT, Farmer C, Vestergaard M, Purup S and Sejrsen K 2006. Mammary development in prepubertal gilts fed restrictively or ad libitum in two subperiods between weaning and puberty. Livestock Science 99, 249–255.

Sorensen MT, Sejrsen K and Purup S 2002. Mammary gland development in gilts. Livestock Production Science 75, 143–148.

Theil PK, Lauridsen C and Quesnel H 2014. Neonatal piglet survival: impact of sow nutrition around parturition on fetal glycogen deposition and production and composition of colostrum and transient milk. Animal 8, 1021–1030.

Turner CW 1952. The anatomy of the mammary glands of swine. In The mammary gland. I. The anatomy of the udder of cattle and domestic animals (ed. CW Turner), pp. 279–314. Lucas Brothers Publishers, Columbia, MO, USA.

VanKlompenberg MK, Manjarin R, Trott JF, McMicking HF and Hovey RC 2013. Late gestational hyperprolactinemia accelerates mammary epithelial differentiation that leads to increased milk yield. Journal of Animal Science 91, 1102–1111.

Weldon WC, Thulin AJ, MacDougal OA, Johnston LJ, Miller ER and Tucker HA 1991. Effects of increased dietary energy and protein during late gestation on mammary development in gilts. Journal of Animal Science 69, 194–200.

Whitacre MD and Threlfall WR 1981. Effects of ergocryptine on plasma prolactin, luteinizing hormone, and progesterone in the periparturient sow. American Journal of Veterinary Research 42, 1538–1541.

Winn RJ, Baker MD, Merle CA and Sherwood OD 1994. Individual and combined effects of relaxin, estrogen, and progesterone in ovariectomized gilts II. Effects on mammary development. Endocrinology 135, 1250–1255.

Zaleski HM, Winn RJ, Jennings RL and Sherwood OD 1996. Effects of relaxin on lactational performance in ovariectomized gilts. Biology of Reproduction 55, 671–675.

https://doi.org/10.1017/S1751731119000521 Published online by Cambridge University Press