4. Mammary development

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Abstract

Mammary development is a crucial component of milk yield potential in sows and it is therefore imperative to understand the mechanisms controlling it. There are three phases of rapid mammary development in swine, namely, from 90 days of age until puberty, during the last third of gestation and throughout lactation. Nutrition, endocrine status and management of gilts or sows during those periods can affect mammary development. More specifically, in growing gilts, feed restriction as of 90 days of age hinders mammary development and either supplying the phytoestrogen genistein or increasing circulating concentrations of prolactin stimulates mammogenesis. In late gestation, inhibition of relaxin or prolactin drastically diminishes mammary development and overly increasing dietary energy has a detrimental effect on mammogenesis. Recent results also suggest that feeding of the gestating sow can affect mammary development of her offspring once it reaches puberty. Various management factors such as litter size, nursing intensity and use or non-use of a teat in the previous lactation will have an impact on the amount of mammary tissue present at the end of lactation. At weaning, the process of mammary involution takes place, whereby there is a rapid and drastic regression in parenchymal tissue. This process of involution can also occur in early lactation when teats are not being regularly suckled, yet the impact of early involution on future mammary development and milk yield is not known. It is evident that much remains to be learned in order to develop the best management strategies for replacement gilts, and gestating and lactating sows that will maximize their mammary development, hence milk production.

Keywords: hormones, mammogenesis, milk yield, nutrition, sow

4.1 Introduction

Milk is the main energy source for piglets and is therefore essential for their growth and survival. However, sows cannot produce enough milk to sustain optimal growth of their litters. Indeed, it was shown that *ad libitum* access to nutrients, achieved via artificial rearing, during the pre-weaning phase results in dramatically heavier weaning weights of piglets compared with sow rearing (Harrell *et al.*, 1993). In that study, artificially-reared pigs weighed 53% more than sow-reared pigs at 21 days of age. More recently, it was also shown that providing supplemental milk to pre-weaning piglets significantly increases their weight at weaning (Miller *et al.*, 2012). The problem of inadequate milk intake

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by piglets was exacerbated with the use of hyperprolific sows. It is therefore imperative to develop management strategies that will increase sow milk yield. One crucial factor determining sow milking potential is the number of mammary cells that are present at the onset of lactation (Head and Williams, 1991) and this should receive more attention in terms of developing the best management practices to optimize mammary development in growing gilts and in gestating and lactating sows. Rapid mammary development occurs at three distinctive periods in the life of pigs and it is during these periods that it is possible to attempt to stimulate mammogenesis via management, nutritional and hormonal strategies. The present chapter summarizes what we know on the process of mammogenesis in swine and on the various factors that can affect it. More specifically, the impacts of nutrition, hormonal status and suckling of a teat on mammary development in swine will be covered as well as the process of mammary involution.

4.2 Ontogeny of mammary development

The mammary glands of swine are located in two parallel rows along the ventral body wall from the thoracic region to the inguinal area. The glands (thoracic, abdominal, or inguinal) are attached to the ventral body wall by adipose and connective tissue. Each gland is separate and distinct from adjoining glands (Turner, 1952) and it normally has one teat with two separate teat canals. Each of these canals leads to a small dilation of the sinus and eventually ramifies into its own section of alveolar-lobular tissue so that each teat opening has its own self-contained duct and glandular system (Hughes and Varley, 1980). Mammary tissue is derived from the ectoderm in the embryo and differentiation of the eventual udder first becomes apparent in the very early embryonic stage when two parallel ridges of ectoderm appear, these are known as 'milk lines'. Nodules along these milk lines form themselves into mammary buds, each of which being the progenitor of a teat. At birth, there is relatively little development of the duct system and mammary glands consist mainly of subcutaneous stromal tissue (Hughes and Varley, 1980). Accumulation of mammary tissue and mammary DNA, which is indicative of cell number, is slow until 90 days of age. The rate of accretion of mammary tissue and DNA then increases four- to sixfold (Sorensen *et al.*, 2002) so that by the time the gilt is mated, mammary glands are still very small but contain an extensive duct system with numerous budlike outgrowths (Turner, 1952). More recent data shows that puberty has a stimulatory effect on mammogenesis as parenchymal tissue mass (which contains the epithelial cell component of the gland) increases by 51% and extraparenchymal tissue mass (containing mainly adipose tissue) decreases by 16% in gilts that have reached puberty compared with gilts of a similar age that have not started cycling (Farmer *et al.*, 2004). Mammary stem cells provide the source of progenitor cells during all stages of mammary development (Borena *et al.*, 2013). Mammary adipose tissue, including that within the parenchymal and extraparenhymal tissues, is also important for development of the epithelial components of the gland (Hovey and Aimo, 2010). The role of mammary stem cells and adipose-derived stem cells in mammary development in swine has received only limited attention to date.

In pregnant gilts, quantitative development of the mammary glands is slow in the first two-thirds of gestation, while almost all accumulation of mammary tissue and DNA takes place in the last third (Hacker and Hill, 1972; Kensinger *et al.*, 1982; Sorensen *et al.*, 2002). Concentrations of DNA in mammary tissue increase dramatically during the last third of gestation (King *et al.*, 1996). Ji *et al.* (2006) also reported a significant increase in weight of mammary glands between days 45 and 112 of gestation, with accelerated mammary accretion occurring after day 75. Histologically, between days 45 and 75, the mammary tissue is primarily composed of adipose and stromal tissue, with elongating ducts and limited branching of ducts to form lobular structures (Figure 4.1; Ji *et al.*, 2006) that are similar to the terminal ductal lobular units identified in gilts in response to mammogenic hormones (Horigan *et al.*, 2009). During the period between days 75 and 112 glands undergo major histological changes as the adipose and stromal tissues are extensively replaced by lobuloalveolar tissue (Figure 4.1; Hacker and Hill, 1972; Ji *et al.*, 2006; Kensinger *et al.*, 1982). Ji *et al.* (2006) also reported a shift in mammary gland composition going from a high lipid content, reflective of the extensive adipose in the tissue, to a high protein content during the last third of gestation. Both histological changes and differences in DNA concentrations in mammary tissues from gilts indicate increased epithelial cell division between days 75 and 90 of gestation, with maximum cell concentrations present by day 90. Then, between days 90 and 105, there is an increase in cellular organelles associated with functional differentiation of the epithelia and

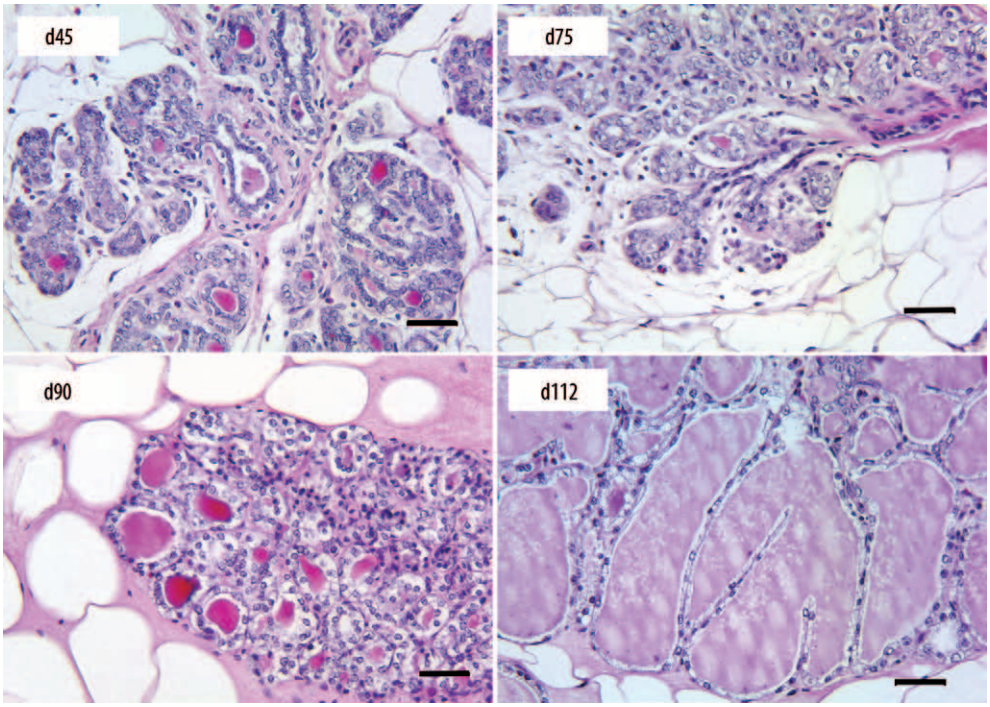

Figure 4.1. Stained histological sections of mammary tissue from pregnant gilts representing days 45, 75, 90 and 112 of gestation. Note terminal ductal lobular units in day 45 and day 75 images. Bar = 50 µm. Images are from gilts from the Ji et al. *(2006) study.*

abundant accumulation of secretion in the alveoli, indicating the onset of the lactogenic process (Kensinger *et al.,* 1982, 1986a). At the time of parturition, the lobules and alveoli are completely filled with secretion (Figure 4.1; Turner, 1952). Figure 4.2 illustrates the development of mammary tissue in pregnant gilts and a lactating sow. The location of the gland on the udder affects its development during gestation. The wet weight of middle glands (3rd, 4th and 5th pairs) is greater than that of posterior glands (6th, 7th and 8th pairs) on both day 102 and day 112 of gestation (Ji *et al.*, 2006).

These phenotypic changes in the mammary tissue during late gestation coincide with significant changes in mammary gene expression. In a study of the sow mammary transcriptome, a number of pathways and gene networks were found to change through the period between days 80 and 110 of gestation (Zhao *et al.*, 2013). For example, the increased synthesis of milk lipid in mammary cells in late gestation may be driven by activation of genes involved in fatty acid biosynthesis, the tricarboxylic acid cycle and glyoxylate and decarboxylase flux. These analyses also indicate that there may be a reduction in the degradation of essential amino acids and a reduction in other amino acid metabolic pathways in late gestation, consistent with a dramatic increase in mammary tissue protein deposition. Activation of genes associated with gap junctions, the mTOR signaling pathway (milk protein synthesis), and VEGF and MAPK signaling (blood flow regulation) all are consistent with known changes in mammary tissue function during late gestation (Zhao *et al.*, 2013).

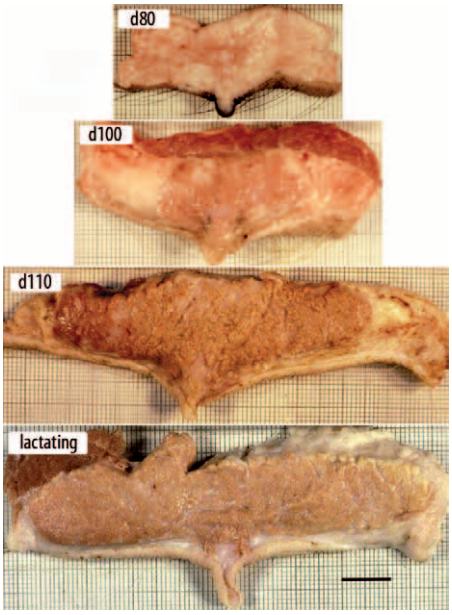

Figure 4.2. Transverse section of mammary glands from pregnant gilts during the last third of gestation and lactation. Images represent days 80, 100 and 110 of gestation and day 3 of lactation. Images from gestation are from the Hurley et al. *(1991) study. Bar = 2 cm.*

Mammary gland development does not stop at the end of gestation but it continues during lactation. In the nulliparous sow, the mammary gland consists of cell buds distributed among fat and connective tissue, whereas in the lactating gland, the connective tissue is largely displaced by glandular parenchyma. Mammary glands of the lactating sow are composed of a compound tubuloalveolar tissue with the secretory units arranged in lobules, which are lined by epithelial cells (lactocytes) that synthesize milk. The average weight of suckled mammary glands increases linearly from 381 g on day 5 of lactation up to 593 g on day 21 (57% increase). In first-parity sows, the increase in mammary volume during lactation is the consequence of both cellular hyperplasia and hypertrophy (Kim *et al.*, 1999a), whereas in multiparous sows, this appears to be mainly due to hypertrophy (Manjarin *et al.*, 2011). Mammary growth in lactation is related to the position of the gland on the udder, being greater for the five more anterior teat pairs than for more posterior teat pairs (Kim *et al.,* 2000), and there are indications that it may be related to the intensity of the post-ejection massage (Thodberg and Sorensen, 2006). Mammary development is also affected by parity because mammary gland wet weight increased by 63, 21 and 39% between day 113 of gestation and day 26 of lactation, for sows of parity 1, 2 and 4, respectively (Beyer *et al.*, 1994). Both cell division and cell differentiation contribute to milk production in early lactation in other species such as the goat (Knight and Peaker, 1984). While it is clear that the mammary gland of the sow grows during lactation, the extent to which an increase in the differentiation state of porcine mammary cells contributes to greater milk production has not been fully explored.

4.3 Control of mammogenesis

As noted above, mammary development occurs throughout many stages of growth and reproduction in swine. Various hormones are involved in the control of mammary development in swine during the post-pubertal period and pregnancy, the most important ones being estrogens, relaxin and prolactin. The essential role of estrogens is evidenced by the drastic effect of puberty onset on mammogenesis (Farmer *et al.*, 2004; Sorensen *et al.*, 2006). Farmer *et al.* (2004) reported a 51% increase in parenchymal tissue mass of cycling compared with non-cycling gilts of similar ages, which in turn led to increases in total parenchymal fat, protein and DNA.

During gestation, total plasma estrogen concentrations increase dramatically after day 75 in gilts (DeHoff *et al.*, 1986). Kensinger *et al.* (1986b,c) demonstrated that the drastic increase in metabolic activity of the mammary gland occurring in late gestation is associated with the increase in estrogens of fetal origin; indeed, mammary DNA was related to circulating concentrations of estrogen in sows on day 110 of gestation. An earlier study also showed that zearalenone, a mycotoxin with estrogen-like activities, affects mammary development. An increase in mammary glandular elements due to ductal hyperplasia was observed in sows receiving zearalenone (Chang *et al.*, 1979). Mammary development was even observed in some of the 7-day old piglets sucking the zearalenone treated sows (Chang *et al.*, 1979). Recently, an attempt was made to specifically stimulate mammary development in gilts by providing a dietary source of estrogen. When 2.3 g/d of the phytoestrogen genistein was added to the diet of growing

gilts from 90 to 183 days of age, there was an increase in mammary parenchymal DNA, indicative of hyperplasia, at the end of the treatment period (Farmer *et al.*, 2010a). The impact of providing a similar dose of genistein to gilts in the last third of pregnancy on their mammary development needs to be investigated.

In the cycling and pregnant animal, estrogen synergises with relaxin to stimulate mammary development. Relaxin is a polypeptide hormone produced by the corpora lutea of sows. Using a classical replacement therapy study with non-pregnant ovariectomized gilts, Winn *et al.* (1994) demonstrated that growth of mammary parenchymal tissue is stimulated by estrogen and relaxin. In a similar study of ovariectomized pregnant gilts, Hurley *et al.* (1991) clearly demonstrated that relaxin plays a major role in promoting mammary parenchymal growth in the last third of pregnancy. However, the potential effects of exogenous relaxin on mammogenesis of intact gestating gilts are not known. This mammogenic effect of relaxin may not carry over into lactation. Plasma relaxin concentrations were not different at 24 hours postpartum among sows of differing lactation performance, and the hormone was undetectable by 72 to 120 hours (Porter *et al.*, 1992).

Studies of the administration of exogenous growth hormone have shown varying results. Administration of recombinant porcine somatotropin in late lactation (days 12 to 29) was shown to increase milk yield in sows (Harkins *et al.*, 1989), while administration of porcine somatotropin from day 108 of gestation through day 28 of lactation did not result in increased milk production (Cromwell *et al.*, 1992). Neither of those studies evaluated mammary growth indicators. The potential stimulatory effect of growth hormone-releasing factor (GRF) on mammary development of lactating sows has also been studied. Administration of GRF in late gestation and throughout lactation decreased parenchymal weight, but increased parenchymal DNA concentration determined at day 30 of lactation (Farmer *et al.*, 1997). It is interesting to note that the effects of bovine somatotropin used in dairy cattle occur primarily after peak lactation has been reached (Peel and Bauman, 1987). The positive effects observed by Harkins *et al.* (1989) may have occurred primarily in the later period of treatment.

Prolactin is the hormone which has received most attention in terms of its effects on mammary development in swine. Prolactin affects mammogenesis in growing gilts. The first indication of this came from a trial where prolactin was provided to gilts in an attempt to affect their growth performance (McLaughlin *et al.*, 1997). These authors reported apparent mammary development with injections of 2 mg/d of recombinant porcine prolactin for 28 days, starting at 75 kg body weight. Mammary glands of treated gilts were characterized by distended alveolar and ductal lumina as well as the presence of secretory material. Yet, no measures of mammary composition were made. In a later experiment, injections of 4 mg/d of recombinant porcine prolactin to gilts for 29 days, as of 75 kg body weight, led to a 116% increase in mammary parenchymal tissue mass and a 160.9% increase in parenchymal DNA (Farmer and Palin, 2005). However, mammary secretions were also present, suggesting premature lactogenesis.

As early as 1945, there were indications that consumption of ergotized barley by late-pregnant sows had a detrimental effect on mammary development. Almost no mammary development was present in sows consuming the ergotized barley whereas all control sows had a normal mammary development (Nordskog and Clark, 1945). A negative impact of ergots on mammary development when fed for 8 days prior to farrowing was also reported more recently (Kopinski *et al.*, 2007). The normal prolactin surge associated with parturition in the sow occurs between about 2 days prepartum through several days postpartum (Dusza and Krzymowska, 1981). This is most interesting due to the finding that endotoxins have an inhibitory effect on prolactin secretion during the immediate postpartum period, thereby showing a potential relation between suppression of prolactin and insufficient milk yield in sows (Smith and Wagner, 1984). The first demonstration of the essential role of prolactin for mammary development in pregnant gilts was made over 10 years ago using the dopamine agonist bromocriptine to inhibit prolactin secretion (Farmer *et al.*, 2000). When feeding 10 mg of bromocriptine to gilts thrice daily from days 70 until 110 of gestation, mammary parenchymal tissue mass on day 110 of gestation was 581 g compared with 1,011 g for control animals, representing a 42.5% decrease (Figure 4.3).

It was subsequently shown that the specific time-window where prolactin exerts most of its stimulatory effect on mammary gland growth is from 90 to 109 days of gestation (Farmer and Petitclerc, 2003). Feeding 10 mg of bromocriptine thrice daily to gilts during that specific time period decreased total parenchymal mass by 46% (918.5 vs. 1,701.7 g) on day 110 of gestation but the treatment had no effect when given from days 50 to 69 or days 70 to 89 of gestation. Recent data showed that when creating a hyperprolactinemic state in that specific period of late-gestation, using the dopamine antagonist domperidone, there was a significant beneficial effect on secretory activity of mammary parenchyma and on mammary epithelial cell differentiation (VanKlompenberg *et al.*, 2013). Subsequent milk yield was also improved on days 14 and 21 of lactation, and piglet weight gain until weaning was increased by 21%. Yet, no measures of mammary composition were obtained.

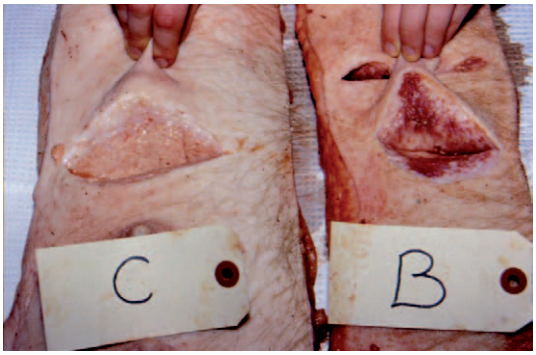

Figure 4.3. Transversal cut from the mammary gland of a control (C) or treated (B) gilt on day 110 of gestation. Treatment consisted of thrice daily feeding of 10 mg of the dopamine agonist bromocriptine from days 70 to 110 of gestation.

The degree of stimulation of prolactin secretion may also be an important consideration. King *et al.* (1996) administered high levels of porcine prolactin to first-litter gilts from day 102 of pregnancy through lactation. While concentrations of RNA and DNA in mammary tissue biopsies were not affected by prolactin administration, milk yield was reduced in sows given prolactin. Other evidence in that study suggested that the sows may have undergone premature lactogenesis with the elevated peripartum prolactin concentrations. In another study of administration of recombinant porcine prolactin from days 2 to 23 of lactation in third-parity sows, no significant effects on either milk yield or mammary composition were observed (Farmer *et al.*, 1999). This absence of effect could be due to the fact that mammary receptors for prolactin were already saturated in control animals, thereby preventing the exogenous prolactin from having any biological action (Farmer *et al.*, 1999).

It would be of interest to find ways to increase circulating prolactin concentrations using feed additives that are not pharmaceutical agents so that they could be used in commercial swine operations. One possibility might be the plant extract silymarin (from milk-thistle) which was found to have hyperprolactinemic properties in rats (Capasso *et al.*, 2009) and hypergalactenemic actions in women (Di Pierro *et al.*, 2008) and cows (Tedesco *et al.*, 2004). In a recent study it was demonstrated that silymarin can increase prolactin concentrations in gestating sows, yet, the increase was not significant enough to have beneficial effects in terms of mammary development (Farmer *et al.*, 2014). More specifically, 4 g of silymarin was fed twice daily from 90 days until 110 days of gestation, leading to a 51.8% increase in circulating prolactin concentrations 4 days after the onset of treatment. However, this effect was no longer apparent 15 days later. The absence of beneficial effects on mammary development may be due to the fact that prolactin concentrations were not increased enough or for a long enough period of time. Indeed, in the study by VanKlompenberg *et al.* (2013), where a positive effect of increased prolactin concentrations was seen on secretory activity and epithelial cell differentiation of mammary tissue, prolactin concentrations were increased almost four-fold within 24 h of treatment and remained greater for 6 days. In the project using silymarin (Farmer *et al.*, 2014), no blood was obtained 24 h post-treatment so it is not known if prolactin concentrations peaked earlier. In any event, it is possible that a larger dose of silymarin could have had a greater effect. Yet, depending on the required duration of treatment, this would most likely not be economically feasible for producers to use on a regular basis.

4.4 Role of milk removal

Milk removal is also critical for mammary development and function during lactation. An accumulation of an autocrine feedback inhibitory factor(s) occurs in the mammary alveoli as part of the normal process of cellular secretion of milk components, inhibiting further secretion (Wilde *et al.*, 1995). If milk is not fully removed from a gland by the piglet, then the gland will reduce further milk secretion and eventually initiate the process of involution (discussed below). Suckling also stimulates secretion of prolactin and other hormones (Algers *et al.*, 1991; Spinka *et al.*, 1999), and weaning results in a rapid decline in plasma prolactin concentrations (Bevers *et al.*, 1978). The removal of the feedback

inhibitory factor and the stimulation of prolactin secretion can be expected to synergize to stimulate mammary growth, as well as maintain lactation function.

The effect of suckling and milk removal on mammary development in lactation can also be seen in studies in which mammary development during gestation is experimentally inhibited. Ovariectomizing pregnant gilts and providing progesterone replacement therapy without relaxin replacement results in impaired mammary development (Hurley *et al.*, 1991). When comparing the extent of mammary development in intact gilts at day 100 of gestation with that of gilts that were ovariectomized on day 100 and then mammary development estimated at day 110 (Hurley *et al.*, 1991), mammary glands seemed to have regressed in relaxin-deficient gilts between day 100 and 110, or at least did not develop further. Zaleski *et al.* (1996) studied the impact of relaxin in late gestation on subsequent lactation performance. Because relaxin-deficient gilts also do not undergo normal cervical softening associated with farrowing (O'Day *et al.*, 1989), the fetuses were removed by C-section at day 114 and gilts given healthy litters from normally farrowing sows. Piglet growth performance was determined over a 28-day lactation. Gilts deficient in relaxin throughout the period from day 80 to day 114, and therefore initiating lactation with a lower level of mammary development, might be expected to have significantly impaired litter growth. However, although litters suckling the relaxin-deficient gilts may have had delayed growth in early lactation, by lactation day 21 piglet weights were not different from that of controls (Zaleski *et al.*, 1996). This suggests that the lactating mammary gland of the sow has extensive growth potential even when prepartum development is impaired. Evidence is also presented in that study that extensive mammary growth may occur during the initial days of lactation as a consequence of suckling and milk removal.

The effect of milk removal on milk production can be seen from the effects of litter size, with greater total milk produced with a larger litter size (reviewed by King, 2000 also discussed in Chapter 8; Quesnel *et al*., 2015). Suckling intensity affects mammary growth during lactation as well as milk production. For example, litter size clearly impacts growth of total mass of mammary tissue during lactation (discussed below; Kim *et al.*, 1999c).

The effect of milk removal also can be seen from the effects of size of the piglet suckling a gland where larger piglets demand more milk or can remove more milk from the gland than smaller piglets (King, 2000). The developmental relationship between the piglet and the growth of the gland it suckles can be seen in the significant correlation that exists between the size of a mammary gland (either in terms of weight or DNA) and the growth of the piglet suckling that teat, being an estimate of milk yield (Kim *et al.*, 2000; Nielsen *et al.*, 2001). Furthermore, mammary parenchymal tissue of sows that have higher average piglet weight gains (i.e. 5.25 vs. 4.46 kg from days 2 to 21 of lactation) contains more DNA and more RNA per teat at the end of lactation than that of sows with lower piglet weight gains (Farmer *et al.*, 2010b).

A further demonstration of the effect that the size of the piglet has on the gland it suckles comes from studies where litters of one age are fostered to sows at a different stage of lactation. King *et al.* (1997) fostered 2-week old piglets on to day 2 lactating sows, resulting in greater milk yield between days 4-8 compared with controls. Conversely, fostering day

2 piglets on to 2-week lactating sows resulted in a decrease in milk yield compared with controls. Accounting for the close relationship between milk yield and mammary cell number (Boutinaud *et al.*, 2004), those observations indicate that the greater piglet size on the day 2 lactating sows stimulated a greater mammary development during the initial days of lactation to meet the demands of the piglets, while fostering smaller piglets onto an established mammary gland may result in regression of the tissue until it balances with the demands of the smaller piglets. In both cases, the effects on milk yield disappear by about 2 weeks, suggesting that the gland has reached a point of balance with the demands of the piglet. Another study of this effect used sows overexpressing a mammary-specific transgene (bovine alpha-lactalbumin; Marshall *et al.*, 2006). Fostering day 7 piglets on to day 2 lactating sows resulted in daily milk yield of transgenic sows increasing rapidly to a peak at day 9 and remaining higher than that of controls through day 15 of lactation. Fostering day 7 piglets onto day 2 non-transgenic sows had a more limited effect on milk yield.

Another example of how size of the piglet impacts development of the gland it suckles may be seen when comparing the mass of mammary glands at farrowing in first-parity gilts with the mass of glands at day 5 of lactation. An examination of data representing the mean DNA content of mammary tissue by gland location at day 5 of lactation (Kim *et al.*, 1999a) and the mean DNA content by gland location at day 0 (Ji *et al.*, 2006; Kim *et al.*, 2000) suggests that the gland develops over that 5-day period at the start of lactation by responding directly to the level of suckling demand of the piglet. In the case where piglet birth weight was held constant at the start of lactation and therefore the suckling intensity was similar across gland locations, the variation in mass among glands was reduced at day 5 compared with farrowing. Glands that are largest at farrowing (typically the middle glands; Ji *et al.*, 2006) may have excess tissue mass relative to the ability of the piglets to remove the milk and may undergo regression during the initial 5 days of lactation. In contrast, glands that are the smallest at farrowing (typically the posterior glands; Ji *et al.*, 2006) grow the most rapidly during the initial 5 days of lactation, although still remaining smaller than the anterior glands.

4.5 Nutritional impact on mammary development

Nutrition of swine in the growing, gestating or lactating periods can affect mammary development. A 34% feed restriction of growing gilts from 28 days (weaning) to 90 days of age had no significant impact on mammogenesis, whereas a 20% (Farmer *et al.*, 2004) or 26% (Sorensen *et al.*, 2006) feed restriction from 90 days of age until puberty reduced mammary parenchymal mass by 26.3 and 34.2%, respectively. The effect of feed restriction on mammogenesis is only seen as of 90 days of age, being the first period of rapid mammary development. High feeding levels from 90 days of age until puberty are therefore recommended to ensure optimal mammary development of growing gilts. On the other hand, reducing dietary crude protein from 18.7 to 14.4% in that same period does not affect mammogenesis (Farmer *et al.*, 2004) suggesting that total feed intake is more important than protein intake per se for mammary development of growing gilts. The impact of feeding flaxseed on mammary development of gilts was investigated

because of its high content of secoisolariciresinol diglycoside, which is a precursor for lignin formation, which in turn exhibits estrogenic activities (Adlercreutz *et al.*, 1987). Yet, dietary supplementation with 10% flaxseed from 88 days until 212 days of age did not lead to significant changes in mammary development on day 212 (Farmer *et al.*, 2007b).

Nutrition of growing gilts can also affect their mammary development at the end of gestation. Lyvers-Peffer and Rozeboom (2001) studied the effects of a growth-altering feeding regimen before puberty on mammary development at the end of gestation. They used dietary fiber (35% ground sunflower hulls) to achieve phases of moderate growth which alternated with phases of maximum growth. They reported that gilts on the moderate feeding regimen from 9 to 12 weeks and 15 to 20 weeks of age had less mammary parenchyma on day 110 of gestation than control gilts. In a later experiment using a similar approach, specific periods of diet deprivation (providing 70% of the protein and DE contents from the control diet) followed by over-allowance (providing 115% of the protein and DE contents from the control diet) in growing gilts did not have any beneficial effect on mammary development after puberty. In fact, this feeding regime led to a decrease in parenchymal tissue mass at puberty (Farmer *et al.*, 2012a). The same nutritional treatment also did not affect parenchymal mass at the end of gestation but led to a tendency for reduced percent protein in mammary parenchyma (Farmer *et al.*, 2012b).

Nutrition during pregnancy undoubtedly affects mammary development at the end of gestation. An early study where body composition of sows was altered by manipulating protein and energy intakes during gestation demonstrated that overly fat (36 mm backfat) and leaner gilts (24 mm backfat) had similar mammary weights at the end of gestation but there was a drastic reduction (approximately threefold) in mammary DNA concentration (i.e. cell number) in overly fat gilts compared with leaner gilts (Head and Williams, 1991). Yet, these body conditions are not representative of what is seen commercially and it is not known if such a difference in mammary DNA would be seen when comparing fat, average and lean gilts according to current standards. This is something which needs to be looked at in order to determine the ideal body condition required for optimal mammary development at the end of gestation. Increasing dietary energy (5.76 vs. 10.5 Mcal/ME) from day 75 of gestation until the end of gestation decreased mammary parenchymal weight and parenchymal DNA on day 105 of gestation (Weldon *et al.*, 1991). On the other hand, increasing protein intake (330 vs. 216 g CP/d) had no effects on mammogenesis (Weldon *et al.*, 1991). This finding was later corroborated by Kusina *et al.* (1999) who showed that lysine intakes of 4, 8 or 16 g/d from days 25 to 105 of gestation did not alter mammary development at the end of gestation. When using a period of diet deprivation (providing 70% of the protein and DE contents from the control diet) for the first 10 weeks of gestation, followed by a period of over-allowance (providing 115% of the protein and DE contents from the control diet) until the end of gestation, there was less parenchymal tissue at the end of gestation with no changes in parenchymal tissue composition (Farmer *et al.*, 2014b). The goal of that project was to look at the effect of compensatory feeding on mammary development yet, even though growth rate was increased in the over-feeding period, this increase was not large enough to compensate for the body weight loss in the restriction period during early gestation. A better adapted feeding regime needs to be developed to be able to truly assess the impact of compensatory feeding on

mammary development of gestating gilts. A recent report indicated that nutrition of sows in gestation and lactation can affect mammary development of their offspring. Indeed, dietary supplementation with 10% flaxseed from day 63 of gestation until the end of lactation increased mammary parenchymal mass of the offspring at puberty (Farmer and Palin, 2008). This is a first demonstration of such an in utero effect in swine and it leads to new avenues in terms of development of feeding strategies to enhance mammogenesis.

Nutrition during the last phase of rapid mammary accretion, namely lactation, also affects mammary development, yet there is very little information on the subject. Kim *et al.* (1999b) fed lactating primiparous sows four diets that were a combination of different protein (32 or 65 g lysine/d) and energy (12 or 17.5 Mcal ME/d) levels. Wet and dry weights of suckled mammary glands were positively affected by both energy and protein intakes. Results suggested that wet and dry mammary weights were maximized when sows consumed an average of 16.5 Mcal of ME and 950 g of crude protein per day, the latter being equivalent to 52.3 g of lysine daily. It is therefore apparent that nutrient intake during lactation is important for mammary development during that period.

4.6 Mammary involution

4.6.1 At weaning

The mammary gland is particularly interesting because it undergoes repeated cycles of growth, lactation and involution. Indeed, when piglets are weaned, there is an abrupt cessation of milk removal which leads to involution of the mammary glands. This process is characterized by rapid regression of the mammary parenchyma during at least the first 7 days post-weaning (Ford *et al.*, 2003). Changes in mammary tissue are quite dramatic (Figure 4.4). Parenchymal tissue wet weight and parenchymal DNA decrease by 68.8 and 66.8%, respectively, in those first 7 days. Significant changes are seen as early as in the first 2 days post-weaning, characterized by drastic decreases in cross-sectional area, wet weight per gland and parenchymal DNA. From days 2 to 4, regression of the mammary gland is minimal followed by additional significant declines until day 7 post-weaning (Ford *et al.*, 2003). The proportion of DNA per tissue mass was found not to be altered after weaning suggesting that the number of cells per milligram of wet tissue did not change during the involution process. However, other compositional changes were observed in mammary parenchyma after weaning, namely, a reduction in protein percentage and an increase in fat percentage. This is likely due to an increase of lipid within the tissue, which could very well reflect a transitory accumulation of milk lipid (Ford *et al.,* 2003). An earlier histological study also showed that weaned mammary glands become engorged in the first few days after weaning and that milk in the lumen then seems to be reabsorbed (Cross *et al.*, 1958).

Ford *et al.* (2003) suggested that mammary gland involution in weaned sows is achieved in three phases, namely: (1) from weaning until day 2; (2) from day 2 until day 4 or 5 post-weaning; and (3) from day 4 or 5 until at least day 7 post-weaning. In the initial phase, the sudden absence of milk removal leads to milk stasis and to an inhibition

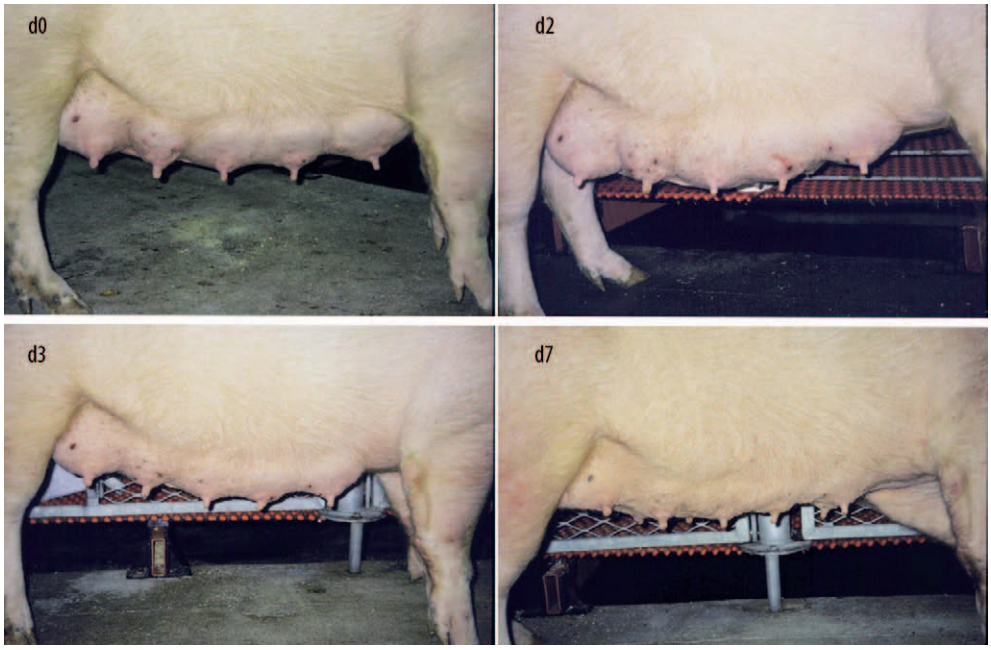

Figure 4.4. Mammary regression in a first-parity sow. Images are taken at weaning (d 0), and days 2, 3, and 7 post-weaning. Note the apparent engorgement of the glands on day 2 and the dramatic decrease in underline on day 3.

of milk secretion via accumulation of autocrine feedback inhibition of lactation in the alveolar lumen (Wilde *et al.*, 1995). A drastic loss of tissue fluid occurs alongside with an increase in tissue fat content and a considerable cell loss due to apoptosis. Cell loss during the involution process occurs at least in part through the expression of a set of genes that control apoptosis but also involves phagocytosis of apoptotic cells by macrophages (Motyl *et al.*, 2001). Mammary blood flow decreases by 40% within the initial 16 hours after weaning the litter (Renaudeau *et al.*, 2002). The second phase is characterized by more limited changes in mammary gland component mass, being consistent with the limited alterations in milk metabolites (Atwood and Hartmann, 1995). In the last phase of involution, only limited mammary secretions can be collected and they are very viscous (Atwood and Hartmann, 1995). There are very few alveolar structures remaining at this stage (Cross *et al.*, 1958) and there are final reductions in mammary parenchymal tissue and DNA (Ford *et al.*, 2003).

The process of mammary involution is affected by the duration of lactation. When comparing lactation lengths of 22 and 44 days, the Na/K ratio in milk was greater at 44 days indicating that mammary epithelial cell tight junctions became leaky as lactation advanced (Farmer *et al.*, 2007a). The disruption in mammary epithelial integrity coupled with an increase in plasma lactose concentrations suggests that the process of involution started before weaning on day 44 of lactation. The onset of involution could be related to a reduced suckling intensity because piglets that were weaned at 44 days had access to

creep feed as of day 22 of lactation. Alternatively, this period marks the declining phase after peak lactation in sows. The declining phase of lactation in ruminants is associated with a loss of mammary epithelial cells (Stefanon *et al.*, 2002).

4.6.2 During lactation

Unsuckled mammary glands in early lactation regress at a similar rate and follow a similar pattern of regression than mammary glands that regress post-weaning (Kim *et al.,* 2001). Mammary gland tissue weight decreased by 2/3 within the first 7 to 10 days of lactation when the gland was not suckled whereas the rate of regression was much slower thereafter (Kim *et al.*, 2001). In accordance, mammary glands that are not suckled during lactation do not show further loss of parenchymal tissue after weaning (Ford *et al.*, 2003). When comparing litter sizes of 6 to 12 piglets, there were no differences in composition or size of unsuckled mammary glands on day 21 of lactation indicating an absence of litter size effect on regression (Kim *et al.*, 2001). On the other hand, the rate of regression of unsuckled mammary glands is affected by dietary nutrient level during lactation. Kim *et al.* (2001) reported that wet weight of unsuckled glands was 91% greater on day 5 of lactation in sows fed a high energy (17.5 vs. 12 Mcal ME/day) high-protein (65 vs. 32 g of lysine/day) diet compared with sows fed a low energy-low protein diet. The rate of regression of unsuckled glands therefore appears to be slowest under dietary conditions which promote mammary growth. Nevertheless, the impact of the extent and rapidity of regression of unsuckled mammary glands during lactation on their future development and milk yield is not known.

The degree to which mammary gland involution is reversible is particularly important in swine because of the common practice of cross-fostering piglets. Results from Kim *et al.* (2001) showing extensive loss of wet weight and DNA in unsuckled glands suggest that the loss of lactation function would not be reversible after the initial several days of lactation. In 2005, Theil *et al.* specifically addressed that question by blinding teats for either 24 or 72 h post-farrowing in order to prevent suckling by piglets. Mammary development throughout lactation of glands blinded for 24 h was similar to that of regularly-suckled glands, whereas that of teats blinded for 72 h was lesser as of day 6 of lactation. Regression of unsuckled mammary glands during early lactation was found to be reversible (gland rescue) during the first 24 h but to be irreversible after 3 days (Theil *et al.*, 2005). Milk production from the rescued glands that were unsuckled during 24 h remained lower throughout lactation. This was further demonstrated by the fact that piglets cross-fostered later than 24 h after the foster sow farrowed weighed 900 g less at weaning than control piglets (Thorup, 1998). Suckling intensity is also important for mammary regression. Theil *et al.* (2006) compared no suckling, transient suckling (until 12 to 14 h postpartum) or regular suckling of mammary glands and observed that regularly-suckled glands maintained lactation whereas transiently-suckled and non-suckled glands regressed during lactation.

4.7 Management strategies that can affect mammary development

Litter size is known to affect milk yield (King, 2000) and it also has an impact on mammary development (Kim *et al.*, 1999c). First-parity sows with a larger litter size (12 pigs) were shown to have a greater total mammary mass on day 21 of lactation than sows with a smaller litter size (6 pigs), however, the weight of each individual gland was lower in the larger litter size (Kim *et al.*, 1999c). Wet and dry weights of total nursed mammary glands increased linearly with litter size and doubling the litter size from 6 to 12 pigs led to a 65% increase in total mammary wet weight, a 67% increase in total mammary DNA, and a 63% increase in total protein. These increases result from the greater number of lactating glands since within individual nursed glands wet and dry weights as well as the amounts of dry fat-free tissue, protein, DNA and fat decreased as litter size increased. Results suggested that there are no differences in terms of cell density or cell size between nursed glands from sows with differing litter size, rather there was greater mass of parenchymal tissue in glands from sows with smaller litter sizes. An important finding was that an increase in the size of mammary glands or in the amount of mammary protein had a positive impact on piglet weight gain. This supports the importance of maximizing mammary growth via feeding and other strategies during lactation.

Another management aspect which affects mammary development is teat use or lactational history. That is, the extent to which a gland grows and lactates in one lactation may impact that gland's growth and function in the subsequent lactation. Ford *et al.* (2003) noted that mammary glands that were suckled during lactation were larger than non-suckled glands at the end of the involution process, suggesting a possible beneficial effect on redevelopment during the next gestation. Results from an earlier study did indicate a likely effect of teat use or non-use in one parity on its productivity in the subsequent lactation, yet, there was a confounding effect of treatment with teat location (Fraser *et al.*, 1992). Recent data permitted to clearly establish that non-suckling of a teat in first parity impairs its development in second parity (Farmer *et al.*, 2012c). Either the same teats or different teats were blinded during the first and second lactation and teats which were not suckled in first parity had less parenchymal tissue and less parenchymal DNA and RNA at the end of the second lactation than teats which were previously suckled. This indicates the occurrence of both hyperplasia and increased metabolic activity of parenchymal cells from glands that were previously used. Furthermore, piglets suckling previously-used teats weighed 1.12 kg more at 56 days of age than piglets suckling previously unused teats (Farmer *et al.*, 2012c).

4.8 Conclusions

Mammary development can be altered by many factors including nutrition and endocrine status of the gilt or sow, but much still remains to be learned in order to develop optimal management strategies for replacement gilts, gestating gilts and lactating sows that will maximize their milk production. Table 4.1 and Table 4.2 summarize the various treatments that can significantly affect mammogenesis in growing gilts and

Table 4.1. Effects of various treatments imposed to growing gilts on their mammary development at puberty or at the end of gestation.

Treatment[1]	Significant effects on	Reference
Lack of estrogen and relaxin post-puberty	↓ parenchymal cross-section area	Winn *et al.* (1994)
Prolactin injections for 29 days as of 75 kg BW	↑ parenchymal mass and DNA	Farmer and Palin (2005)
Feeding genistein from 90 to 183 days of age	↑ parenchymal DNA	Farmer *et al.* (2010a)
Feeding 10% flaxseed from 88 to 212 days of age	-	Farmer *et al.* (2007)
14.4 vs. 18.7% dietary CP from 90 to 202 days of age	-	Farmer *et al.* (2004)
34% feed restriction from 28 to 90 days of age	-	Sorensen *et al.* (2006)
20% feed restriction from 90 to 202 days of age	↓ parenchymal mass	Farmer *et al.* (2004)
26% feed restriction from 90 to 170 days of age	↓ parenchymal mass	Sorensen *et al.* (2006)
Restricting growth from 9 to 12 and 15 to 20 weeks of age: effect in gestation	↓ parenchymal mass	Lyvers-Peffer and Rozeboom (2001)
Feed restriction then over-feeding inprepuberty: effect at puberty	↓ parenchymal mass	Farmer *et al.* (2012a)
Feed restriction then over-feeding in prepuberty: effect in gestation	Tendency to ↓ % protein in parenchyma	Farmer *et al.* (2012b)

[1] Effects on glands collected on the last day of treatment unless mentioned otherwise.

Table 4.2. Effects of various treatments imposed to gestating gilts on their mammary development or that of their offspring.

Treatment[1]	Significant effects on	Reference
Inhibition of relaxin from days 80 to 110	↓ parenchymal cross-section area	Hurley *et al.* (1991)
GRF injections from day 100 of gestation to 29 of lactation	↓ parenchymal mass, ↑ parenchymal DNA	Farmer *et al.* (1997)
Inhibition of prolactin from days 70 to 110	↓ parenchymal mass, DNA, RNA and % protein	Farmer *et al.* (2000)
Inhibition of prolactin from days 90 to 110	↓ parenchymal mass and % protein	Farmer and Petitclerc (2003)
Feeding 4 g twice daily of the plant extract silymarin from days 90 to 110	-	Farmer *et al.* (2014)
Feeding 10% flaxseed from day 63 of gestation to end of lactation: effect on offspring at puberty	↑ parenchymal mass	Farmer and Palin (2008)
Feeding 10.5 vs. 5.76 Mcal ME/d from days 75 to 105	↓ parenchymal mass and DNA	Weldon *et al.* (1991)
Feeding 330 vs. 216 g crude protein/d from days 75 to 105	-	Weldon *et al.* (1991)
Feeding 16 vs. 4 g/d of lysine from days 25 to 105	-	Kusina *et al.* (1999)
Feed restriction then over-feeding: effect in gestation	↓ parenchymal mass	Farmer *et al.* (2014)
Feed restriction then over-feeding: effect in lactation	-	Farmer *et al.* (2014b)

[1] Effects on glands collected on the last day of treatment unless mentioned otherwise.

gestating animals, respectively. In summary, feed restriction as of 90 days of age in growing gilts hinders mammary development and there are indications that supplying the phytoestrogen genistein or increasing concentrations of prolactin stimulate mammogenesis. In gestation, the essential role of relaxin and prolactin for mammary development was demonstrated, however, it is not known if exogenous administration of these hormones would stimulate mammogenesis. Nutritional studies in gestation mainly showed negative effects, whereby over-feeding energy or using feed restriction followed by over-feeding reduced parenchymal mass. Management during lactation, such as altering the number of glands suckled and the duration of suckling of these glands, also affects mammary development at the end of lactation and teat use in first-parity will increase its productivity and development in the subsequent lactation. It is obvious from our current knowledge that the ideal feeding regimes to optimize mammary development of growing, pregnant and lactating gilts or sows have yet to be developed.

References

Adlercreutz, H., Höckerstedt, K., Bannwart, C., Bloigu, S., Hämäläinen, E., Fotsis, T. and Ollus, A., 1987. Effect of dietary components, including lignans and phytoestrogens, on enterohepatic circulation and liver metabolism of estrogens and on sex hormone binding globulin (SHBG). Journal of Steroid Biochemistry 27: 1135-1144.

Algers, B., Madej, A., Rojanasthien, S. and Uvnas-Moberg, K., 1991. Quantitative relationships between suckling-induced teat stimulation and the release of prolactin, gastrin, somatostatin, insulin, glucagon and vasoactive intestinal polypeptide in sows. Veterinary Research Communications 15: 395-407.

Atwood, C.S. and Hartmann, P.E., 1995. Assessment of mammary gland metabolism in the sow. III. Cellular metabolites in the mammary secretion and plasma following weaning. Journal of Dairy Research 62: 221-236.

Bevers, M.M., Willemse, A.H. and Kruip, T.A.M., 1978. Plasma prolactin levels in the sow during lactation and the postweaning period as measured by radioimmunoassay. Biology of Reproduction 19: 628-634.

Beyer, M., Jentsch, W., Hoffmann, L., Schiemann, R. and Klein, M., 1994. Studies on energy and nitrogen metabolism of pregnant and lactating sows and sucking piglets. 4. Chemical composition and energy content of the conception products, the reproductive organs as well as liveweight gains or losses of pregnant and lactating sows. Archives Animal Nutrition 46: 7-36.

Borena, B.M., Bussche, L., Burvenich, C., Duchateau, L. and Van de Walle, G.R., 2013. Mammary stem cell research in veterinary science: An update. Stems Cells and Development 22: 1743-1751.

Boutinaud, M., Guinard-Flament, J. and Jammes, H., 2004. The number and activity of mammary epithelia cells, determining factors for milk production. Reproduction, Nutrition, Development 44: 499-508.

Capasso, R., Aviello, G., Capasso, F., Savino, F., Izzo, A.A., Lembo, F. and Borrelli, F., 2009. Silymarin BIO-C®, an extract from *Silybum marianum* fruits, induces hyperprolactinemia in intact female rats. Phytomedicine 16: 839-844.

Chang, K., Kurtz, H.J. and Mirocha, C.J., 1979. Effects of the mycotoxin zearalenone on swine reproduction. American Journal of Veterinary Research 40: 1260-1267.

Cromwell, G.L., Stahly, T.S., Edgerton, L.A., Monegue, H.J., Burnell, T.W., Schenck, B.C. and Schricker, B.R., 1992. Recombinant porcine somatoptropin for sows during late gestation and throughout lactation. Journal of Animal Science 70: 1404-1416.

Cross, B.A., Goodwin, R.F.W. and Silver, I.A., 1958. A histological and functional study of the mammary gland in normal and agalactic sows. Journal of Endocrinology 17: 63-74.

DeHoff, M.H., Stoner, C.S., Bazer, F.W., Collier, R.J., Kraeling, R.R. and Buonomo, F.C., 1986. Temporal changes in steriods, prolactin and growth hormone in pregnant and pseudopregnant gilts during mammogenesis and lactogenesis. Domestic Animal Endocrinology 3: 95-105.

Di Pierro, F., Callegari, A., Carotenuto, D. and Tapia, M.M., 2008. Clinical efficacy, safety and tolerability of BIO-C® (micronized Silymarin) as a galactagogue. Acta Biomedica 79: 205-210.

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.

Farmer, C., Knight, C. and Flint, D., 2007a. Mammary gland involution and endocrine status in sows: effects of weaning age and lactation heat stress. Canadian Journal of Animal Science 87: 35-43.

Farmer, C., Lapointe, J. and Palin, M.F. , 2014a. 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. and Palin, M.F., 2008. Feeding flaxseed to sows during late-gestation affects mammary development but not mammary expression of selected genes in their offspring. Canadian Journal of Animal Science 88: 585-590.

Farmer, C., Palin, M.F., Gilani, G.S., Weiler, H., Vignola, M., Choudhary, R.K. and Capuco, A.V., 2010a. Dietary genistein stimulates mammary hyperplasia in gilts. Animal 4: 454-465.

Farmer, C., Palin, M.F. and Hovey, R., 2010b. Greater milk yield is related to increased DNA and RNA content but not to mRNA abundance of select genes in sow mammary tissue. Canadian Journal of Animal Science 90: 379-388.

Farmer, C., Palin, M.F. and Martel-Kennes, Y., 2012a. Impact of diet deprivation and subsequent over-allowance during prepuberty. Part 1. Effects on growth performance, metabolite status, and mammary gland development in gilts. Journal of Animal Science 90: 863-871.

Farmer, C., Palin, M.F. and Martel-Kennes, Y., 2012b. Impact of diet deprivation and subsequent over-allowance during prepuberty. Part 2. Effects on mammary gland development and lactation performance of sows. Journal of Animal Science 90: 872-880.

Farmer, C., Palin, M.F. and Martel-Kennes, Y., 2014b. Impact of diet deprivation and subsequent over-allowance during gestation on mammary gland development and lactation performance. Journal of Animal Science 92: 141-151.

Farmer, C., Palin, M.F., Theil, P.K., Sorensen, M.T. and Devillers, N., 2012c. Milk production in sows from a teat in second parity is influenced by whether it was suckled in first parity. Journal of Animal Science 90: 3743-3751.

Farmer, C., Pelletier, G., Brazeau, P. and Petitclerc, D., 1997. Mammary gland development of sows injected with growth hormone-releasing factor during gestation and(or) lactation. Canadian Journal of Animal Science 77: 335-338.

Farmer, C., Petit, H.V., Weiler, H. and Capuco, A.V., 2007b. Effects of dietary supplementation with flax during prepuberty on fatty acid profile, mammogenesis, and bone resorption in gilts. Journal of Animal Science 85: 1675-1686.

Farmer, C. and Petitclerc, D., 2003. Specific window of prolactin inhibition in late gestation decreases mammary parenchymal tissue development in gilts. Journal of Animal Science 81: 1823-1829.

Farmer, C., Petitclerc, D., Sorensen, M.T., Vignola, M. and Dourmad, J.Y., 2004. Impacts of dietary protein level and feed restriction during prepuberty on mammogenesis in gilts. Journal of Animal Science 82: 2343-2351.

Farmer, C., Sorensen, M.T. and Petitclerc, D., 2000. Inhibition of prolactin in the last trimester of gestation decreases mammary gland development in gilts. Journal of Animal Science 78: 1303-1309.

Farmer, C., Sorensen, M.T., 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.

Ford, Jr., J.A., Kim, S.W., Rodriguez-Zas, S.L. and Hurley, W.L., 2003. Quantification of mammary gland tissue size and composition changes after weaning in sows. Journal of Animal Science 81: 2583-2589.

Fraser, D., Thompson, B.K. and Rushen, J., 1992. Teat productivity in second lactation sows: influence of use or non-use of teats during the first lactation. Animal Production 55: 419-424.

Hacker, R.R. and Hill, D.L., 1972. Nucleic acid content of mammary glands of virgin and pregnant gilts. Journal of Dairy Science 55: 1295-1299.

Harkins, M., Boyd, R.D. and Bauman, D.E., 1989. Effects of recombinant porcine somatotropin on lactational performance and metabolite patterns in sows and growth of nursing pigs. Journal of Animal Science 67: 1997-2008.

Harrell, R.J., Thomas, M.J. and Boyd, R.D., 1993. Limitations of sow milk yield on baby pig growth. In: Cornell University (ed.) Proceedings of the Cornell Nutrition Conference for Feed Manufacturers. October 19-21, 1993. Rochester, NY, USA, pp. 156-164.

Head, R.H. and Williams, I.H., 1991. Mammogenesis is influenced by pregnancy nutrition. In: Batterham, E.S. (ed.) Manipulating pig production III. Australasian Pig Science Association, Atwood, Australia, 33 pp.

Horigan, K.C., Trott, J.F., Barndollar, A.S., Scudder, J.M., Blauwiekel, R.M. and Hovey, R.C., 2009. Hormone interactions confer specific proliferative and histomorphogenic responses in the porcine mammary gland. Domestic Animal Endocrinology 37: 124-138.

Hovey, R.C. and Aimo, L., 2010. Diverse and active roles of adipocytes during mammary gland growth and function. Journal of Mammary Gland Biology and Neoplasia 15: 279-290.

Hughes, P.E. and Varley, M.A., 1980. Lactation. In: Hughes, P.E. and Varley, M.A. (eds.) Reproduction in the pig. Butterworth & Co., London, UK, pp. 136-158.

Hurley, W.L., Doane, R.M., O'Day-Bowman, M.B., Winn, R.J., Mojonnier, L.E. and Sherwood, O.D., 1991. Effect of relaxin on mammary development in ovariectomized pregnant gilts. Endocrinology 128: 1285-1290.

Ji, F., Hurley, W.L. and Kim, S.W., 2006. Characterization of mammary gland development in pregnant gilts. Journal of Animal Science 84: 579-587.

Kensinger, R.S., Collier, R.J. and Bazer, F.W., 1986a. Ultrastructural changes in porcine mammary tissue during lactogenesis. Journal of Anatomy 145: 49-59.

Kensinger, R.S., Collier, R.J. and Bazer, F.W., 1986b. Effect of number of conceptuses on maternal mammary development during pregnancy in the pig. Domestic Animal Endocrinology 3: 237-245.

Kensinger, R.S., Collier, R.J., Bazer, F.W. and Kraeling, R.R., 1986c. Effect of number of conceptuses on maternal hormone concentrations in the pig. Journal of Animal Science 62: 1666-1674.

Kensinger, R.S., Collier, R.J., Bazer, F.W., Ducsay, C.A. and Becker, H.N., 1982. Nucleic acid, metabolic and histological changes in gilt mammary tissue during pregnancy and lactogenesis. Journal of Animal Science 54: 1297-1308.

Kim, S.W., Easter, R.A. and Hurley, W.L., 2001. The regression of unsuckled mammary glands during lactation in sows: the influence of lactation stage, dietary nutrients, and litter size. Journal of Animal Science 79: 2659-2668.

Kim, S.W., Hurley, W.L., Han, I.K. and Easter, R.A., 1999a. Changes in tissue composition associated with mammary gland growth during lactation in sows. Journal of Animal Science 77: 2510-2516.

Kim, S.W., Hurley, W.L., Han, I.K. and Easter, R.A., 2000. Growth of nursing pigs related to the characteristics of nursed mammary glands. Journal of Animal Science 78: 1313-1318.

Kim, S.W., Hurley, W.L., Han, I.K., Stein, H.H. and Easter, R.A., 1999b. Effect of nutrient intake on mammary gland growth in lactating sows. Journal of Animal Science 77: 3304-3315.

Kim, S.W., Osaka, I., Hurley, W.L. and Easter, R.A., 1999c. Mammary gland growth as influenced by litter size in lactating sows: impact on lysine requirement. Journal of Animal Science 77: 3316-3321.

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

King, R.H., Mullan, B.P., Dunshea, F.R. and Dove, H., 1997. The influence of piglet body weight on milk production in sows. Livestock Production Science 47: 169-174.

King, R.H., Pettigrew, J.E., McNamara, J.P., McMurty, J.P., Henderson, T.L., Hathaway, M.R. and Sower, A.F., 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.

Knight, C.H. and Peaker, M., 1984. Mammary development and regression during lactation in goats in relation to milk secretion. Quarterly Journal of Experimental Physiology 69: 331-338.

Kopinski, J.S., Blaney, B.J., Downing, J.A., McVeigh, J.F. and Murray, S.A., 2007. Feeding sorghum ergot (*Claviceps africana*) to sows before farrowing inhibits milk production. Australian Veterinary Journal 85: 169-176.

Kusina, J., Pettigrew, J.E., Sower, A.F., Hathaway, M.R., White, M.E. and Crooker, B.A., 1999. Effect of protein intake during gestation on mammary development of primiparous sows. Journal of Animal Science 77: 925-930.

Lyvers-Peffer, P.A. and Rozeboom, D.W., 2001. The effects of a growth-altering pre-pubertal feeding regimen on mammary development and parity-one lactation potential in swine. Livestock Production Science 70: 167-173.

Manjarin, R., Trottier, N.L., Weber, P.S., Liesman, J.S., Taylor, N.P. and Steibel, J.P., 2011. A simple analytical and experimental procedure for selection of reference genes for reverse-transcription quantitative PCR normalization data. Journal of Dairy Science 94: 4950-4961.

Marshall, K.M., Hurley, W.L., Shanks, R.D. and Wheeler, M.B., 2006. Effects of suckling intensity on milk yield and piglet growth from lactation-enhanced gilts. Journal of Animal Science 84: 2346-2351.

McLaughlin, C.L., Byatt, J.C., Curran, D.F., Veenhuizen, J.J., McGrath, M.F., Buonomo, F.C., Hintz, R.L. and Baile, C.A., 1997. Growth performance, endocrine, and metabolite responses of finishing hogs to porcine prolactin. Journal of Animal Science 75: 959-967.

Miller, Y.J., Collins, A.M., Smits, R.J., Thomson, P.C. and Holyoake, P.K., 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.

Motyl, T., Gajkowska, B., Wojewodzka, U., Wareski, P., Rekiel, A. and Ploszaj, T., 2001. Expression of apoptosis-related proteins in mammary gland of sow. Comparative Biochemistry and Physiology Part B 128: 635-646.

Nielsen, O.L., Pederson, A.R. and Sorensen, M.T., 2001. Relationships between piglet growth rate and mammary gland size of the sow. Livestock Production Science 67: 273-279.

Nordskog, A.W. and Clark, R.T., 1945. Ergotism in pregnant sows, female rats and guinea pigs. American Journal of Veterinary Research 6: 107-116.

O'Day, M.B., Winn, R.J., Easter, R.A., Dziuk, P.J. and Sherwood, O.D., 1989. Hormonal control of the cervix in pregnant gilts. II. Relaxin promotes changes in the physical properties of the cervix in ovariectomized hormone-treated pregnant gilts. Endocrinology 125: 3004-3010.

Peel, C.J. and Bauman, D. E., 1987. Somatotropin and lactation. Journal of Dairy Science 70: 474-486.

Porter, D.G., Friendship, R.M., Ryan, P.L. and Wasnidge, C., 1992. Relaxin is not associated with poor milk yield in the postpartum sow. Canadian Journal of Veterinary Research 56: 204-207.

Quesnel, H., Farmer, C. and Theil, P.K., 2015. Colostrum and milk production. Chapter 8. In: Farmer, C. (ed.) The gestating and lactating sow. Wageningen Academic Publishers, Wageningen, the Netherlands, pp. 173-192.

Renaudeau, D., Lebreton, Y., Noblet, J. and Dourmad, J.Y., 2002. Measurement of blood flow through the mammary gland in lactating sows: methodological aspects. Journal of Animal Science 80: 196-201.

Smith, B.B. and Wagner, W.C., 1984. Suppression of prolactin in pigs by *Escheriscia coli* endotoxin. Science 224: 605-607.

Sorensen, M.T., Farmer, C., Vestergaard, M., Purup, S. and Sejrsen, K., 2006. Mammary development in prepubertal gilts fed restrictively or *ad libitum* in two sub-periods between weaning and puberty. Livestock Science 99: 249-255.

Sorensen, M.T., Sejrsen, K. and Purup, S., 2002. Mammary gland development in gilts. Livestock Production Science 75: 143-148.

Spinka, M., Illmann, G., Stetkova, Z., Krejc, P., Tomanek, M., Sedlak, L. and Lidicky, J., 1999. Prolactin and insulin levels in lactation sows in relation to nursing frequency. Domestic Animal Endocrinology 17: 53-64.

Stefanon, B., Colitti, M., Gabai, G., Knight, C.H. and Wilde, C.J., 2002. Mammary apoptosis and lactational persistency in dairy animals. Journal of Dairy Research 69: 37-52.

Tedesco, D., Tava, A., Galletti, S., Tameni, M., Varisco, G., Costa, A. and Steidler, S., 2004. Effects of silymarin, a natural hepatoprotector, in periparturient dairy cows. Journal of Dairy Science 87: 2239-2247.

Theil, P.K., Labouriau, R., Sejrsen, K., Thomsen, B. and Sorensen, M.T., 2005. Expression of genes involved in regulation of cell turnover during milk stasis and lactation rescue in sow mammary tissue. Journal of Animal Science 83: 2349-2356.

Theil, P.K., Sejrsen, K., Hurley, W.L., Labouriau, R., Thomsen, B. and Sorensen, M.T., 2006. Role of suckling in regulating cell turnover and onset and maintenance of lactation in individual mammary glands of sows. Journal of Animal Science 84: 1691-1698.

Thodberg, K. and Sorensen, M.T., 2006. Mammary development and milk production in the sow: Effects of udder massage, genotype and feeding in late gestation. Livestock Science 101: 116-125.

Thorup, F., 1998. Kuldudjaevningens betydning for fravaenningsvaegten. Erfaring fra Landsudvalget for svin Copenhagen, Denmark.

Turner, C.W., 1952. The anatomy of the mammary gland of swine. In: Turner, C.W. (ed.) The mammary gland. I. The anatomy of the udder of cattle and domestic animals. Lucas Brothers, Columbia, MO, USA, pp. 279-314.

VanKlompenbeg, M.K., Manjarin, R., Trot, J.F., McMicking, H.F. and Hovey, R.C., 2013. Late-gestational hyperprolactinemia accelerates mammary epithelial cell differentiation that leads to increased milk yield. Journal of Animal Science 91: 1102-1111.

Weldon, W.C., Thulin, A.J., MacDougald, O.A., Johnston, L.J., Miller, E.R. and Tucker, H.A., 1991. Effects of increased dietary energy and protein during late gestation on mammary development in gilts. Journal of Animal Science 69: 194-200.

Wilde, C.J., Addey, C.V.P., Boddy, L.M. and Peaker, M., 1995. Autocrine regulation of milk secretion by a protein in milk. Biochemistry Journal 305: 51-58.

Winn, R.J., Baker, M.D., Merle, C.A. and Sherwood, O.D., 1994. Individual and combined effects of relaxin, estrogen, and progesterone in ovariectomized gilts. II. Effects on mammary development. Endocrinology 135: 1250-1255.

Zaleski, H.M., Winn, R.J., Jennings, R.L. and Sherwood, O.D., 1996. Effects of relaxin on lactational performance in ovariectomized gilts. Biology of Reproduction 55: 671-675.

Zhao, W., Shahzad, K., Jiang, M., Graugnard, D.E., Rodriguez-Zas, S.L., Luo, J., Loor, J.J. and Hurley, W.L., 2013. Bioinformatics and gene network analyses of the swine mammary gland transcriptome during late gestation. Bioinformatics and Biology Insights 7: 193-216.