

Review: Maternal programming of development in the pig and the lactocrine hypothesis

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Maternal effects on development are profound. Together, genetic and epigenetic maternal effects define the developmental trajectory of progeny and, ultimately, offspring phenotype. Maternally provisioned environmental conditions and signals affect conceptus, fetoplacental and postnatal development from the time of conception until weaning. In the pig, reproductive tract development is completed postnatally. Porcine uterine growth and uterine endometrial development occur in an ovary-independent manner between birth (postnatal day = PND 0) and PND 60. Milk-borne bioactive factors (MbFs), exemplified by relaxin, communicated from lactating dam to nursing offspring via a lactocrine mechanism, represent an important source of extraovarian uterotrophic support in the neonatal pig. Lactocrine deficiency from birth affects both the neonatal porcine uterine developmental program and trajectory of uterine development, with lasting consequences for endometrial function and uterine capacity in adult female pigs. The potential lactocrine signaling window extends from birth until the time of weaning. However, it is likely that the maternal lactocrine programming window – that period when MbFs communicated to nursing offspring have the greatest potential to affect critical organizational events in the neonate – encompasses a comparatively short period of time within 48 h of birth. Lactocrine deficiency from birth was associated with altered patterns of endometrial gene expression in neonatally lactocrine-deficient adult gilts during a critical period for conceptus–endometrial interaction on pregnancy day 13, and with reduced litter size, estimated at 1.4 pigs per litter, with no effect of parity. Data were interpreted to indicate that reproductive performance of female pigs that do not receive sufficient colostrum from birth is permanently impaired. Observations to date suggest that lactocrine-dependent maternal effects program postnatal development of the porcine uterus, endometrial functionality and uterine capacity. In this context, reproductive management strategies and husbandry guidelines should be refined to ensure that such practices promote environmental conditions that will optimize uterine capacity and fecundity. This will entail careful consideration of factors affecting lactation, the quality and abundance of colostrum/milk, and practices that will afford neonatal pigs with the opportunity to nurse and consume adequate amounts of colostrum.

Keywords: colostrum, nursing, neonate, uterus, postnatal development

Implications

This review focuses on maternal lactocrine programming of postnatal reproductive tract development in pigs by way of mother's milk. The importance of nursing from birth on reproductive development and performance is emphasized, with data on both short-term effects in the neonate and long-term effects in adults. Data support the lactocrine hypothesis and milk as a conduit for delivery of maternally derived bioactive factors driving postnatal development. Results reinforce the

importance of optimizing conditions that ensure adequate consumption of first milk (colostrum) by nursing young through effective reproductive management in swine production systems. Lactocrine programming has broad implications for human health.

Introduction

Maternal effects on development and reproductive efficiency include environmental conditions and signals provided by the dam that affect developmental trajectory and offspring

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phenotype (Bagnell and Bartol, 2019). For economically important domestic animals such as the pig (*Sus scrofa domestica*), an optimal developmental trajectory is defined by a series of organizational events effecting a developmental program that results in an adult phenotype with potential for maximal fertility and fecundity. Because phenotype is not fixed genetically, but is defined dynamically through the course of development (Bartol *et al.*, 2017), a single genotype can produce more than one phenotype. Practically, these observations reinforce the importance of husbandry in management of animal production environments throughout the life cycle.

In the pig, as in other litter-bearing species, prolificacy – the number of offspring per litter – is a function of ovulation rate, fertilization rate and embryo survival (Kemp *et al.*, 2018). The latter is determined to a significant extent by functional uterine capacity, defined as the maximum number of fetuses that can be carried to term (Bennett and Leymaster, 1989; Vallet *et al.*, 2013a). Clearly, litter size can be no greater than either maternal ovulation rate or uterine capacity (Bennett and Leymaster, 1989). In a mathematical model, changing either ovulation rate or uterine capacity independently did not predict large changes in litter size (Bennett and Leymaster, 1989), indicating that both must change coordinately to achieve a positive effect on prolificacy. To complicate this picture, effective selection for larger litters is associated with lower and more variable piglet birth weights, and increased pre-weaning mortality (Kemp *et al.*, 2018). However, positive selection for uterine capacity did improve fetal survival and lifetime sow productivity (Freking *et al.*, 2016). Thus, conditions that optimize functional uterine capacity should optimize fecundity.

Large litter sizes characteristic of modern swine production challenge the capacity of highly prolific dams to support offspring postnatally (Foxcroft, 2012; Kraeling and Webel, 2015). In such circumstances, marked pre-weaning piglet mortality often reflects insufficient colostrum (first milk) consumption by piglets that, on a within litter basis, typically vary significantly in size and must compete for access to the udder and teat position (Wu *et al.*, 2010; Vallet *et al.*, 2015). Because they are immunologically incompetent at birth, colostrum consumption is essential for piglet survival (Vallet *et al.*, 2013b; Vallet *et al.*, 2015; Poonsuk and Zimmerman, 2018). Transmission of immunoglobulins and other milk-borne bioactive factors (**MbFs**) to nursing piglets in colostrum provides protection against infectious diseases and supports maturation of the gastrointestinal tract as they gain immunological competence during the first month of neonatal life (Poonsuk and Zimmerman, 2018). These observations alone encourage management strategies designed to improve colostrum availability and quality in order to minimize pre-weaning losses and optimize fecundity (Rohrer *et al.*, 2014; Vallet *et al.*, 2015; Farmer, 2018). However, beyond neonatal survival, evidence indicating that colostrum consumption on the day of birth (postnatal day = **PND 0**) has lasting effects on fecundity in adult female pigs (Bartol *et al.*, 2013; Vallet *et al.*, 2015) elevates the importance of

colostrum (Vallet *et al.*, 2015) as a maternal factor affecting reproductive development and performance.

Studies of uterine development in the pig and other domestic ungulate species (Bartol *et al.*, 1993; Spencer *et al.*, 2019) established that female reproductive tract tissues, including the uterus, remain organizationally plastic during early neonatal life. Data for the pig (Bartol *et al.*, 1993), indicating that uterine growth and uterine wall development proceed normally prior to PND 60 in gilts ovariectomized at birth, suggested that extraovarian factors support porcine uterine development in the postnatal period. Colostrum was proposed as a potential source of such uterotrophic support (Yan *et al.*, 2006b; Bartol *et al.*, 2008). The term 'lactocrine' was coined to describe a mechanism by which MbFs are communicated from mother to offspring in colostrum/milk by consequence of nursing (Bartol *et al.*, 2008). The 'lactocrine hypothesis' for maternal programming of postnatal development posits that disruption of lactocrine signaling shortly after birth will alter the program and trajectory of development with short-term organizational effects and long-term consequences for adult phenotype. Studies designed to test the lactocrine hypothesis for maternal programming of uterine and reproductive development in the pig are summarized in several recent reviews (Bagnell *et al.*, 2017; Bartol *et al.*, 2017; Bagnell and Bartol, 2019). Here, objectives are to provide an overview of this work from a production point of view in the context of maternal programming of postnatal reproductive development and performance.

Milk as a delivery system for bioactive factors

Milk-borne bioactive factors of environmental origin

Maternal effects on development begin at conception and can be influenced *in utero* during pregnancy, as well as postnatally by environmental exposures that can alter the trajectory of offspring development. These effects can be either positive or negative and ultimately can influence the adult phenotype. There is a large literature in support of environmental effects (i.e., nutrition, climate and chemical or hormonal perturbations) during critical periods affecting the programming in early development of the fetus that led to the concept of developmental origins of health and disease (Barker, 1998). In addition, since development continues postnatally, nursing provides a means of extending maternal influence by delivery of MbFs, including environmental agents that can affect neonatal outcomes.

Maternal exposure to environmentally derived endocrine disrupting agents can have lasting consequences on offspring development into adulthood (Bartol and Bagnell, 2012). For example, bisphenol A (**BPA**), an estrogenic endocrine disrupting agent, was detected in both cow and human milk (Mendonca *et al.*, 2014). Relatively high BPA in maternal serum–breast milk dyad samples suggested high BPA exposure by consequence of nursing. Postnatal BPA exposure was linked to delays in development of the

pituitary–neuroendocrine axis and onset of puberty (Franssen *et al.*, 2016). Toxic environmental agents can also be concentrated in milk. Lipophilic xenobiotics can pass from maternal adipose stores into the maternal circulation and can be concentrated in milk (Lehmann *et al.*, 2014). Thus, in comparison to maternal exposure to environmental toxins, neonatal exposure to milk-borne toxicants may occur at higher levels, over shorter time periods when postnatal development of multiple organ systems is occurring. Since milk intake in neonatal pigs is estimated to be up to 30% of body weight (Coalson and Lecce, 1973), maternal exposure to environmental endocrine disrupting chemicals and/or toxicants could pose a significant exposure risk to nursing young (Bartol and Bagnell, 2012).

Milk-borne bioactive factors of maternal origin

In addition to providing nutritional and immunological support for developing offspring, colostrum/milk provides a means for delivery of a wide variety of maternally derived MbFs in support of neonatal growth and development. These include growth factors as well as steroid and peptide hormones found in higher concentrations in milk than in the maternal circulation. Metabolic hormones including leptin, ghrelin, adiponectin and glucocorticoids are transferred from mother to offspring in milk (Power and Schulkin, 2013) and can affect metabolism, growth and development. Glucocorticoids in milk were linked to more nervous and less confident temperament in both human and non-human primate offspring (Hinde *et al.*, 2015).

The value of maternally derived MbFs in support of postnatal development is evident from loss of function studies showing that the absence of specific MbFs in milk has deleterious effects on development. Increased adiposity and altered hypothalamic gene expression were found in wild-type mice fostered to interleukin-6-null dams, in which milk leptin content was twofold higher than in wild-type dams, suggesting that milk composition has programming effects on adiposity (Lager *et al.*, 2011). This supported earlier studies indicating that neonatal rats cross-fostered to enable nursing of diabetic dams showed hypothalamic changes and altered expression of genes involved in body weight regulation (Fahrenkrog *et al.*, 2004). Likewise, maternal tumor necrosis factor- α (TNF α) deficiency led to reduced milk chemokine levels and improved adult spatial memory, suggesting a TNF α -regulated lactocrine pathway programming brain development and memory (Liu *et al.*, 2014). Similarly, peroxisome proliferator-activated receptor- γ (PPAR γ)-null mice produced a toxic milk, high in inflammatory lipids resulting in hair loss and growth retardation in nursing young, illustrating the importance of lactocrine-active PPAR γ in protecting nursing offspring (Wan *et al.*, 2007).

Milk also contains small, non-coding microRNAs (miRNAs) that regulate gene expression by blocking translation and/or promoting messenger RNA (mRNA) degradation. These miRNAs are enclosed as cargo in milk-borne exosomes that also carry mRNA, protein and lipids and provide another

means for lactocrine transmission of information. Exosomes, which protect miRNAs from degradation by heat and acidic conditions, are found in milk of several species (Bartol *et al.*, 2017) including the pig (Gu *et al.*, 2012). These milk-borne miRNAs pass the intestinal barrier and enter the bloodstream to target organ systems in a lactocrine manner. Data for the pig indicate that these milk-borne miRNAs are functionally important for development of the neonatal immune system (Gu *et al.*, 2012).

Maternal somatic cell transfer by way of milk is documented in several species as another means of communication with nursing offspring. Porcine milk contains maternal immune cells (Scharek-Tedin *et al.*, 2015) that, when ingested during nursing, can cross the neonatal intestine, enter the bloodstream and populate neonatal organs (Jain *et al.*, 1989). In humans, breast milk contains mammary stem cells that were reported to colonize neonatal tissues with potential to alter postnatal development by way of microchimerism (Barinaga, 2002). Collectively, these observations establish that milk is more than food (Hinde and German, 2012) and lactocrine transmission evolved as a means of delivering a plethora of non-nutritive, MbFs to nursing offspring (Bartol and Bagnell, 2012).

Relaxin: a prototypical lactocrine-active factor

A series of studies in neonatal gilts established relaxin as a prototypical lactocrine-active factor. Relaxin is a 6-kDa peptide hormone and member of a family of neohormones that evolved to support viviparity and lactation (Ivell and Anand-Ivell, 2017). Well known as a hormone of pregnancy, actions of relaxin also include effects on cervical connective tissue remodeling and growth-promoting effects on the uterus. Evidence for trophic effects of relaxin on the neonatal porcine uterus (Yan *et al.*, 2006a) led to studies designed to identify a source of relaxin in the neonatal pig. Porcine colostrum was identified as this source (Yan *et al.*, 2006b), an observation consistent with detection of relaxin in the milk of other species (Bagnell and Bartol, 2019). Detection of a bioactive factor in milk does not, by itself, constitute evidence for action of that MbF in the neonate. Therefore, criteria for determining the physiological relevance of MbFs in the neonate were defined (Peaker and Neville, 1991). Observations indicating that relaxin meets the criteria for a lactocrine-active MbF in the pig include (1) detection of biologically active prorelaxin in colostrum (Frankshun *et al.*, 2011); (2) immunoreactive relaxin detected in the neonatal circulation only in pigs allowed to nurse (Yan *et al.*, 2006b); (3) relaxin receptor (RXFP1) expression in porcine uterine (Yan *et al.*, 2006b) and cervical (Yan *et al.*, 2008) tissues from birth; (4) growth-promoting effects of relaxin administered for 2 days from birth on the neonatal uterus (Yan *et al.*, 2006a) and cervix (Yan *et al.*, 2008). Taken together, these studies established relaxin as a prototypical lactocrine-acting factor and supported the idea that milk is an important conduit for communication of MbFs to nursing offspring (Bagnell and Bartol, 2019).

Lactocrine effects on neonatal reproductive development

Uterus

To test the lactocrine hypothesis, a lactocrine-null condition was imposed during the first 48 h of life, by feeding a porcine milk replacer in lieu of nursing, and effects on uterine development were evaluated on PND 2 and PND 14. There was no effect of replacer feeding from birth on uterine weight (Chen *et al.*, 2011) or endometrial histoarchitecture (Miller *et al.*, 2013) by PND 2. However, in the absence of nursing, uterine glandular and luminal epithelial cell proliferation were reduced and there was a decrease in endometrial stromal estrogen receptor- α (ESR1) localization by PND 2 (Miller *et al.*, 2013). Effects of replacer feeding for 2 days from birth were evident by PND 14, when imposition of the lactocrine-null condition reduced both endometrial thickness and uterine gland development (Miller *et al.*, 2013). Antiadenogenic effects, including reduced gland penetration depth, observed at PND 14 in response to replacer feeding for 48 h from birth were similar to those observed in gilts treated daily from birth with the anti-estrogen ICI 182 780 (Tarleton *et al.*, 1999). Notably, returning gilts deprived of colostrum for the first 48 h of life to nursing at the end of PND 2 failed to rescue the uterine phenotype of reduced endometrial thickness and glandular development observed in replacer-fed gilts at PND 14 (Miller *et al.*, 2013). These studies reinforced the importance of lactocrine signaling from birth on uterine endometrial development in the pig.

Colostrum composition fluctuates over the course of porcine lactation. Consumption of colostrum can be delayed if sows fail to initiate lactation, fail to produce enough colostrum for the litter or if access to colostrum consumption is compromised by within litter competition for access to the udder (Vallet *et al.*, 2013b). In pigs, timing of colostrum intake coincides with a period of gut permeability to colostrum macromolecules, which are typically present in high concentrations at birth and decline over the next 24 to 48 h in association with the loss of gut permeability, termed gut closure (Poonsuk and Zimmerman, 2018). In the tammar wallaby, timing of milk intake, milk composition and rate of milk production influence growth of pouch young and offspring phenotype dramatically (Trott *et al.*, 2003). Therefore, it was of interest to determine whether timing of colostrum consumption or duration of nursing in pigs affected lactocrine-mediated development of uterine and cervical tissues.

Studies of the neonatal porcine uterine transcriptome indicated that matrix metalloproteinases (MMPs) and tissue inhibitors of the MMPs (TIMPs) were affected by both age and lactocrine signaling between birth and PND 2 (Rahman *et al.*, 2016). The MMP2 and MMP9 gelatinases remodel the extra cellular matrix and are co-expressed with TIMPs, which regulate MMP activity. Uterine (Chen *et al.*, 2011; Ho *et al.*, 2017) and cervical (Frankshun *et al.*, 2012) proMMP9 (latent) and MMP9 (active) as well as uterine TIMP protein abundance was greater in nursed gilts when compared to replacer-fed animals. However, none of these

proteins were detectable in porcine reproductive tissues when nursing was delayed by 12 h (Ho *et al.*, 2017). In addition, duration of nursing is important since extending nursing from 30 min to 12 h from birth, increased active and latent MMP9 proteins in reproductive tissues to levels comparable to those observed for gilts nursed for 2 days from birth (Ho *et al.*, 2017). By contrast, uterine MMP2 levels were detected but unchanged by age at first nursing or duration of nursing, indicating that not all uterine protein production is lactocrine-sensitive. Uterine MMP2 and MMP9 activities, detected by zymography, mirrored immunoblotting data. In other studies, a single feeding of colostrum was effective in supporting cervical (Camp *et al.*, 2014) and endometrial cell proliferation at 12 h postnatal (George *et al.*, 2018). In addition, there was no effect of method of delivery of a single dose of colostrum, either by nursing, bottle feeding or orogastric gavage, on uterine developmental markers at 12 h postnatal (George *et al.*, 2018). Collectively, these data indicate that both age at first nursing and duration of nursing are important in neonatal porcine female reproductive tract development, and that lactocrine effects can be detected in nursing piglets within 12 h of birth (Bagnell and Bartol, 2019).

Nursing and the neonatal uterine transcriptome

Global analysis of neonatal porcine gene expression in response to age and lactocrine signaling from birth to PND 2 was investigated by RNA sequencing (RNAseq) (Rahman *et al.*, 2016). With respect to age, more than 3200 uterine genes in nursed gilts and over 4500 genes in lactocrine-null gilts were differentially expressed on PND 2 when compared to uterine gene expression in uterine tissues obtained from gilts at birth. With respect to lactocrine effects, more than 890 differentially expressed genes were identified on PND 2 when nursed and milk replacer-fed gilts were compared. Bioinformatic analyses of biological processes revealed age-sensitive pathways that included ESR1 and hedgehog signaling cascades. Lactocrine-sensitive pathways in the neonatal porcine uterus identified on PND 2 included those involved in response to wounding, cell adhesion, the plasminogen activator network and coagulation (Rahman *et al.*, 2016).

Post-transcriptional regulation of gene expression by miRNAs is a mechanism that could be responsible, in part, for global uterine gene expression differences observed in response to age and nursing. Small non-coding miRNAs target mRNAs and can decrease mRNA stability and block translation. Consequently, effects of age and nursing from birth on the porcine uterine miRNA transcriptome were evaluated in tissues obtained on PND 2 (George *et al.*, 2017) using the same neonatal uterine tissues on which RNAseq analyses were performed (Rahman *et al.*, 2016). Integration of miRNAseq and mRNAseq data enabled target prediction analyses designed to identify potential miRNA–mRNA interactions. Results showed that about 10% of age- and lactocrine-sensitive differences in uterine gene expression could be explained by differential uterine miRNA expression

(George *et al.*, 2017). Biological processes predicted to be affected by age and nursing in uterine tissues on PND 2 as defined by the miRNA–mRNA interactome included cell-to-cell signaling, cell and tissue morphology, and cell growth and proliferation (George *et al.*, 2017). Observations were consistent with morphogenetic activities associated with uterine growth and endometrial development in the early neonatal period (Bartol *et al.*, 1993).

Cervix

Cervical histology of gilts nursed from birth to PND 2 was similar to that of replacer-fed gilts. However, by PND 14, imposition of the lactocrine-null state from birth reduced cervical crypt depth and luminal epithelial height when compared to gilts nursed over the same period (Camp *et al.*, 2014). Cervices from replacer-fed, PND 14 gilts were histologically similar to cervices from newborn pigs. Similar to observations for the uterus (Miller *et al.*, 2013), returning replacer-fed gilts to nursing on PND 2 failed to rescue the PND 14 cervical phenotype in that both cervical crypt and stromal cell proliferation at PND 14 were reduced to levels comparable to those reported for gilts fed milk replacer from birth (Camp *et al.*, 2014).

In other studies designed to evaluate short-term effects of nursing in the cervix and to develop a more efficient bioassay protocol for assessment of lactocrine effects in the neonate, a single feeding of hour 0 colostrum or milk replacer was given at birth, followed by milk replacer feeding through 12 h postnatal. The single feeding of colostrum, but not replacer, increased cervical cell proliferation by 12 h postnatal (Camp *et al.*, 2014). In addition, when delivered orally, IGF1 found naturally in relatively high concentrations in pig milk (Simmen *et al.*, 1988), increased cervical cell proliferation and markers of IGF1 action, including phosphorylated AKT and anti-apoptotic B-cell lymphoma 2 (BCL2), by 12 h postnatal in both colostrum and replacer-fed gilts. Taken together, these data showed that nursing supports cervical development in neonatal pigs, and that IGF1 is a potential lactocrine-active factor for reproductive tract development as illustrated in a 12-h bioassay system to identify lactocrine active MbFs.

Testis

Evidence for lactocrine effects on development of the male reproductive system comes from studies on neonatal testicular development in boars (Rahman *et al.*, 2014). Development and proliferation of two major cell types in the pig testis, Sertoli (McCoard *et al.*, 2001) and Leydig cells (Franca *et al.*, 2000), occurs within the first month of neonatal life. In addition, Sertoli cell number, determined before puberty (Franca *et al.*, 2000), influences testicular size and sperm production (McCoard *et al.*, 2001), indicating that the neonatal period is critical for porcine testicular development.

Nursing for 2 days from birth increased Sertoli cell proliferation when compared to boars fed a commercial pig milk replacer over the same period (Rahman *et al.*, 2014). In a similar manner, Sertoli cell number and GATA4 protein abundance

were greater in nursed boars on PND 2. There was no effect of age or nursing on Leydig cell-associated testicular protein levels, including the steroidogenic enzyme P450scc or insulin-like factor 3. However, testicular *RXFP1* expression increased from birth to PND 2 in replacer-fed, but not in nursed, boars. This was thought to be due, in part, to the absence of milk-borne relaxin in replacer-fed animals (Yan *et al.*, 2006b). Relaxin is detectable in colostrum and in the circulation of nursed pigs (Yan *et al.*, 2006b) and administration of exogenous relaxin decreased *RXFP1* expression in the neonatal porcine uterus and cervix (Yan *et al.*, 2008). Given that relaxin increased Sertoli cell proliferation *in vitro* (Cardoso *et al.*, 2010), the absence of milk-borne relaxin in replacer-fed boars could remove inhibition of testicular *RXFP1* expression and alter testicular development. Potential for maternal lactocrine programming of testicular function remains to be explored.

Long-term lactocrine effects in the adult

Lactocrine-null conditions, imposed experimentally by feeding milk replacer in lieu of nursing from birth, altered the developmental program in neonatal porcine reproductive tract tissues (Bagnell *et al.*, 2017; Bartol *et al.*, 2017). However, lactocrine deficiency can also occur naturally through maternal (e.g., mastitis and agalactia) as well as neonatal factors (e.g., within litter competition for teat position, birth rank and low birth weight) (Wu *et al.*, 2010). An immunoglobulin immunocrit assay, developed to monitor immunoglobulin transfer from mother to offspring during nursing (Vallet *et al.*, 2013b), was established as an indirect measure of colostrum intake in nursing pigs (Vallet *et al.*, 2015). The lactocrine hypothesis predicts that minimal colostrum consumption on PND 0, indicated by low serum immunocrit and lactocrine deficiency in nursing gilts, will be associated, ultimately, with reduced adult uterine capacity. A retrospective study of 381 gilts showed that low serum immunocrit on the day of birth was linked to reduced lifetime fecundity and live litter size across four parities (Bartol *et al.*, 2013). Subsequently, in a large prospective study, PND 0 immunocrit was obtained from 16 762 piglets and subsets of these gilts were assigned to study a variety of reproductive parameters (Vallet *et al.*, 2015). Results showed that low PND 0 immunocrit was associated with reduced growth and increased age at puberty. In addition, in a group of 799 females, low immunocrit on the day of birth was associated with reduced number of piglets born alive, consistent with the initial report (Bartol *et al.*, 2013). Litter size differences for adult females with low *v.* high PND 0 immunocrit were approximately 1.4 piglets per litter (Vallet *et al.*, 2015). In addition, high PND 0 immunocrit in neonatal gilts was linked to improved lactational performance when these females reached adulthood, suggesting lactocrine effects on programming of mammary gland function.

Disruption of uterine receptivity to implantation during the periattachment period of pregnancy can lead to reduced

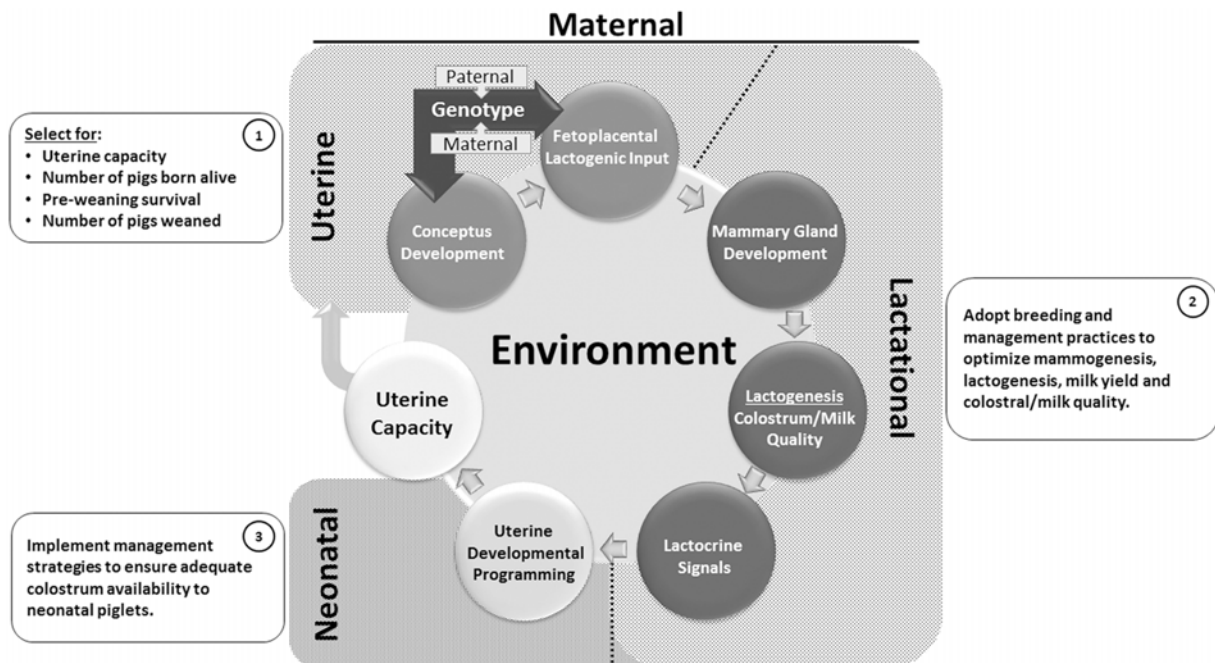


Figure 1. Programming porcine uterine capacity. Uterine capacity is determined by an interaction of genotype with maternally provisioned environmental conditions affecting mammogenesis, lactogenesis and lactocrine programming of postnatal uterine development. With conceptus genotype established, interactions between developing conceptuses and the intrauterine environment determine patterns of conceptus development, survival and fetoplacental lactogenic potential. In turn, endocrine conditions of pregnancy define patterns of mammogenesis and lactogenesis. Nursing ensures lactocrine transmission of MbFs. Lactocrine signaling affects the neonatal uterine developmental program, the trajectory of uterine development and uterine capacity. Reproductive performance of female piglets that do not receive sufficient colostrum is permanently impaired. Therefore, management strategies designed to improve colostrum quality and availability are important for optimization of uterine capacity. Practical actions to optimize uterine capacity and maternal lactocrine programming of postnatal development (boxes 1 to 3) include (1) selection for uterine capacity, number of piglets born alive, pre-weaning survival rate and number of pigs weaned; (2) adoption of breeding and management practices designed to optimize mammogenesis, lactogenesis, milk yield and colostrum/milk quality; and (3) implementation of management strategies designed to ensure adequate colostrum availability to neonatal piglets. Adapted with permission from Bartol and Bagnell (2012). MbF = milk-borne bioactive factor.

reproductive performance in pigs (Bazer *et al.*, 2011). On pregnancy day (PxD) 13, elongated porcine conceptuses initiate attachment to uterine luminal epithelium. Documented negative effects of neonatal lactocrine deficiency on live litter size in adult female pigs (Bartol *et al.*, 2013; Vallet *et al.*, 2015) prompted study of the impact of lactocrine deficiency on the endometrial transcriptome during the periattachment period of early pregnancy on PxD 13. Global transcriptomic analysis revealed more than 1100 differentially expressed endometrial mRNAs at PxD 13 in high *v.* low immunocrit gilts (George *et al.*, 2019). In addition, in terms of miRNA–mRNA interactions, target prediction analysis revealed 5 differentially expressed miRNAs predicted to target over 60 differentially expressed mRNAs in the endometrium of high *v.* low immunocrit gilts on PxD 13. These endometrial mRNAs and related miRNA–mRNA interactions were associated with lactocrine-sensitive gene families for which predicted functions included solute transport, endometrial receptivity and immune response (George *et al.*, 2019). Taken together, these observations showed that impairment of reproductive performance in lactocrine-deficient, adult female pigs is reflected by alterations in endometrial gene expression in the periattachment period of early pregnancy.


Conclusions


Studies designed to test the lactocrine hypothesis for maternal programming of reproductive development and uterine capacity in the pig indicate that, beyond ensuring postnatal survival through the passive transmission of immune competence from mother to nursing offspring (Poonsuk and Zimmerman, 2018), lactocrine communication via colostrum affects uterine developmental trajectory and, ultimately, determines functional uterine capacity in adults. Genetically, selection for uterine capacity over 11 generations increased live litter size by approximately 1.6 piglets (Freking *et al.*, 2016). This kind of genetic advantage could be effectively negated by failure to ensure adequate colostrum consumption by nursing piglets at birth. Reduction in live litter size for neonatally lactocrine-deficient gilts was estimated at 1.4 piglets per litter with no effect of parity (Vallet *et al.*, 2015). Such permanent impairment of reproductive performance in adult, neonatally lactocrine-deficient female pigs is significant. Observations emphasize the importance of developmentally critical interactions between genotype and the maternally provisioned lactocrine environment in programming uterine capacity and reproductive efficiency. While MbFs responsible for lactocrine programming of

reproductive development remain to be defined, husbandry guidelines aimed at optimization of genetic and environmental conditions affecting porcine uterine capacity can be proposed, as summarized in Figure 1. Evidence for lactocrine programming of uterine development demands studies designed to identify MbFs responsible for lactocrine signaling and related mechanisms regulating organizational processes and events that ultimately determine adult uterine capacity and fecundity in the pig.

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Declaration of interest

Authors declare no conflict of interest.

Ethics statement

None.

Software and data repository resources

None.

References

Bagnell CA and Bartol FF 2019. Relaxin and the 'milky way': the lactocrine hypothesis and maternal programming of development. *Molecular and Cellular Endocrinology* 487, 18–23.

Bagnell CA, Ho TY, George AF, Wiley AA, Miller DJ and Bartol FF 2017. Maternal lactocrine programming of porcine reproductive tract development. *Molecular Reproduction and Development* 84, 957–968.

Barinaga M 2002. Cells exchanged during pregnancy live on. *Science* 296, 2169–2172.

Barker DJ 1998. In utero programming of chronic disease. *Clinical Science* 95, 115–128.

Bartol FF and Bagnell CA 2012. Lactocrine programming of female reproductive tract development: environmental connections to the reproductive continuum. *Molecular and Cellular Endocrinology* 354, 16–21.

Bartol FF, Wiley AA and Bagnell CA 2008. Epigenetic programming of porcine endometrial function and the lactocrine hypothesis. *Reproduction in Domestic Animals* 43, 273–279.

Bartol FF, Wiley AA, George AF, Miller DJ and Bagnell CA 2017. Physiology and endocrinology symposium: postnatal reproductive development and the lactocrine hypothesis. *Journal of Animal Science* 95, 2200–2210.

Bartol FF, Wiley AA, Miller DJ, Silva AJ, Roberts KE, Davolt ML, Chen JC, Frankshun AL, Camp ME, Rahman KM, Vallet JL and Bagnell CA 2013. Lactation biology symposium: lactocrine signaling and developmental programming. *Journal of Animal Science* 91, 696–705.

Bartol FF, Wiley AA, Spencer TE, Vallet JL and Christenson RK 1993. Early uterine development in pigs. *Journal of Reproduction and Fertility* 48, 99–116.

Bazer FW, Spencer TE, Johnson GA and Burghardt RC 2011. Uterine receptivity to implantation of blastocysts in mammals. *Frontiers in Bioscience* 3, 745–767.

Bennett GL and Leymaster KA 1989. Integration of ovulation rate, potential embryonic viability and uterine capacity into a model of litter size in swine. *Journal of Animal Science* 67, 1230–1241.

Camp ME, Wiley AA, Boulos MB, Rahman KM, Bartol FF and Bagnell CA 2014. Effects of age, nursing, and oral IGF1 supplementation on neonatal porcine cervical development. *Reproduction* 148, 441–451.

Cardoso LC, Nascimento AR, Royer C, Porto CS and Lazari MF 2010. Locally produced relaxin may affect testis and vas deferens function in rats. *Reproduction* 139, 185–196.

Chen JC, Frankshun AL, Wiley AA, Miller DJ, Welch KA, Ho TY, Bartol FF and Bagnell CA 2011. Milk-borne lactocrine-acting factors affect gene expression patterns in the developing neonatal porcine uterus. *Reproduction* 141, 675–683.

Coalson JA and Lecce JG 1973. Influence of nursing intervals on changes in serum proteins (immunoglobulins) in neonatal pigs. *Journal of Animal Science* 36, 381–385.

Fahrenkrog S, Harder T, Stolaczyk E, Melchior K, Franke K, Dudenhausen JW and Plagemann A 2004. Cross-fostering to diabetic rat dams affects early development of mediobasal hypothalamic nuclei regulating food intake, body weight, and metabolism. *Journal of Nutrition* 134, 648–654.

Farmer C 2018. Nutritional impact on mammary development in pigs: a review. *Journal of Animal Science*, doi: 10.1093/jas/sky243, published online 15 June 2018.

Foxcroft GR 2012. Reproduction in farm animals in an era of rapid genetic change: will genetic change outpace our knowledge of physiology? *Reproduction in Domestic Animals* 47, 313–319.

Franca LR, Silva VA, Jr., Chiarini-Garcia H, Garcia SK and Debeljuk L 2000. Cell proliferation and hormonal changes during postnatal development of the testis in the pig. *Biology of Reproduction* 63, 1629–1636.

Frankshun AL, Chen J, Barron LA, Ho TY, Miller DJ, Rahman KM, Bartol FF and Bagnell CA 2012. Nursing during the first two days of life is essential for the expression of proteins important for growth and remodeling of the neonatal porcine cervix. *Endocrinology* 153, 4511–4521.

Frankshun AL, Ho TY, Reimer DC, Chen J, Lasano S, Steinetz BG, Bartol FF and Bagnell CA 2011. Characterization and biological activity of relaxin in porcine milk. *Reproduction* 141, 373–380.

Franssen D, Gerard A, Hennuy B, Donneau AF, Bourguignon JP and Parent AS 2016. Delayed neuroendocrine sexual maturation in female rats after a very low dose of bisphenol A through altered GABAergic neurotransmission and opposing effects of a high dose. *Endocrinology* 157, 1740–1750.

Freking BA, Lents CA and Vallet JL 2016. Selection for uterine capacity improves lifetime productivity of sows. *Animal Reproduction Science* 167, 16–21.

George AF, Ho TY, Prasad N, Keel BN, Miles JR, Vallet JL, Bartol FF and Bagnell CA 2019. Neonatal lactocrine deficiency affects the adult porcine endometrial transcriptome at pregnancy day 13. *Biology of Reproduction* 100, 71–85.

George AF, Rahman KM, Miller DJ, Wiley AA, Camp ME, Bartol FF and Bagnell CA 2018. Effects of colostrum, feeding method and oral IGF1 on porcine uterine development. *Reproduction* 155, 259–271.

George AF, Rahman KM, Camp ME, Prasad N, Bartol FF and Bagnell CA 2017. Defining age- and lactocrine-sensitive elements of the neonatal porcine uterine microRNA-mRNA interactome. *Biology of Reproduction* 96, 327–340.

Gu Y, Li M, Wang T, Liang Y, Zhong Z, Wang X, Zhou Q, Chen L, Lang Q, He Z, Chen X, Gong J, Gao X, Li X and Lv X 2012. Lactation-related microRNA expression profiles of porcine breast milk exosomes. *PLoS One* 7, e43691. doi: 10.1371/journal.pone.0043691.

Hinde K and German JB 2012. Food in an evolutionary context: insights from mother's milk. *Journal of the Science of Food and Agriculture* 92, 2219–2223.

Hinde K, Skibieli AL, Foster AB, Del Rosso L, Mendoza SP and Capitano JP 2015. Cortisol in mother's milk across lactation reflects maternal life history and predicts infant temperament. *Behavioral Ecology* 26, 269–281.

Ho TY, Rahman KM, Camp ME, Wiley AA, Bartol FF and Bagnell CA 2017. Timing and duration of nursing from birth affect neonatal porcine uterine matrix metalloproteinase 9 and tissue inhibitor of metalloproteinase 1. *Domestic Animal Endocrinology* 59, 1–10.

Ivell R and Anand-Ivell R 2017. Neohormones in milk. *Best Practice and Research: Clinical Endocrinology and Metabolism* 31, 419–425.

Jain L, Vidyasagar D, Xanthou M, Ghai V, Shimada S and Blend M 1989. In vivo distribution of human milk leucocytes after ingestion by newborn baboons. *Archives of Disease in Childhood* 64, 930–933.

- Kemp B, Da Silva CLA and Soede NM 2018. Recent advances in pig reproduction: focus on impact of genetic selection for female fertility. *Reproduction in Domestic Animals* 53, 28–36.
- Kraeling RR and Weibel SK 2015. Current strategies for reproductive management of gilts and sows in North America. *Journal of Animal Science and Biotechnology* 6, 3. doi: [10.1186/2049-1891-6-3](https://doi.org/10.1186/2049-1891-6-3).
- Lager S, Asterholm IW, Schele E, Jansson N, Nilsson S, Jansson JO, Lonn M and Holmang A 2011. Perinatal lack of maternal IL-6 promotes increased adiposity during adulthood in mice. *Endocrinology* 152, 1336–1346.
- Lehmann GM, Verner MA, Luukinen B, Henning C, Assimon SA, LaKind JS, McLanahan ED, Phillips LJ, Davis MH, Powers CM, Hines EP, Haddad S, Longnecker MP, Poulsen MT, Farrer DG, Marchitti SA, Tan YM, Swartout JC, Sagiv SK, Welsh C, Campbell JL, Jr., Foster WG, Yang RS, Fenton SE, Tornero-Velez R, Francis BM, Barnett JB, El-Masri HA and Simmons JE 2014. Improving the risk assessment of lipophilic persistent environmental chemicals in breast milk. *Critical Reviews in Toxicology* 44, 600–617.
- Liu B, Zupan B, Laird E, Klein S, Gleason G, Bozinoski M, Gal Toth J and Toth M 2014. Maternal hematopoietic TNF, via milk chemokines, programs hippocampal development and memory. *Nature Neuroscience* 17, 97–105.
- McCoard SA, Lunstra DD, Wise TH and Ford JJ 2001. Specific staining of sertoli cell nuclei and evaluation of sertoli cell number and proliferative activity in meishan and white composite boars during the neonatal period. *Biology of Reproduction* 64, 689–695.
- Mendonca K, Hauser R, Calafat AM, Arbuckle TE and Duty SM 2014. Bisphenol A concentrations in maternal breast milk and infant urine. *International Archives of Occupational and Environmental Health* 87, 13–20.
- Miller DJ, Wiley AA, Chen JC, Bagnell CA and Bartol FF 2013. Nursing for 48 hours from birth supports porcine uterine gland development and endometrial cell compartment-specific gene expression. *Biology of Reproduction* 88, 4. doi: [10.1095/biolreprod.112.105056](https://doi.org/10.1095/biolreprod.112.105056).
- Peaker M and Neville MC 1991. Hormones in milk: chemical signals to the offspring? *Journal of Endocrinology* 131, 1–3.
- Poonsuk K and Zimmerman J 2018. Historical and contemporary aspects of maternal immunity in swine. *Animal Health Research Reviews* 19, 31–45.
- Power ML and Schulkin J 2013. Maternal regulation of offspring development in mammals is an ancient adaptation tied to lactation. *Applied & Translational Genomics* 2, 55–63.
- Rahman KM, Camp ME, Prasad N, McNeel AK, Levy SE, Bartol FF and Bagnell CA 2016. Age and nursing affect the neonatal porcine uterine transcriptome. *Biology of Reproduction* 94, 46. doi: [10.1095/biolreprod.115.136150](https://doi.org/10.1095/biolreprod.115.136150).
- Rahman KM, Lovich JE, Lam C, Camp ME, Wiley AA, Bartol FF and Bagnell CA 2014. Nursing supports neonatal porcine testicular development. *Domestic Animal Endocrinology* 48, 84–92.
- Rohrer GA, Rempel LA, Miles JR, Keele JW, Wiedmann RT and Vallet JL 2014. Identifying genetic loci controlling neonatal passive transfer of immunity using a hybrid genotyping strategy. *Animal Genetics* 45, 340–349.
- Scharek-Tedin L, Kreuzer-Redmer S, Twardziok SO, Siepert B, Klopffleisch R, Tedin K, Zentek J and Pieper R 2015. Probiotic treatment decreases the number of CD14-expressing cells in porcine milk which correlates with several intestinal immune parameters in the piglets. *Frontiers in Immunology* 6, 108. doi: [10.3389/fimmu.2015.00108](https://doi.org/10.3389/fimmu.2015.00108).
- Simmen FA, Simmen RC and Reinhart G 1988. Maternal and neonatal somatomedin C/insulin-like growth factor-I (IGF-I) and IGF binding proteins during early lactation in the pig. *Developmental Biology* 130, 16–27.
- Spencer TE, Kelleher AM and Bartol FF 2019. Development and function of uterine glands in domestic animals. *Annual Review of Animal Biosciences* 7, 125–147.
- Tarleton BJ, Wiley AA and Bartol FF 1999. Endometrial development and adenogenesis in the neonatal pig: effects of estradiol valerate and the antiestrogen ICI 162 780. *Biology of Reproduction* 61, 253–263.
- Trott JF, Simpson KJ, Moyle RL, Hearn CM, Shaw G, Nicholas KR and Renfree MB 2003. Maternal regulation of milk composition, milk production, and pouch young development during lactation in the tammar wallaby (*Macropus eugenii*). *Biology of Reproduction* 68, 929–936.
- Vallet JL, McNeel AK, Johnson G and Bazer FW 2013a. Triennial reproduction symposium: limitations in uterine and conceptus physiology that lead to fetal losses. *Journal of Animal Science* 91, 3030–3040.
- Vallet JL, Miles JR and Rempel LA 2013b. A simple novel measure of passive transfer of maternal immunoglobulin is predictive of preweaning mortality in piglets. *Veterinary Journal* 195, 91–97.
- Vallet JL, Miles JR, Rempel LA, Nonneman DJ and Lents CA 2015. Relationships between day one piglet serum immunoglobulin immunocrit and subsequent growth, puberty attainment, litter size, and lactation performance. *Journal of Animal Science* 93, 2722–2729.
- Wan Y, Saghatelian A, Chong LW, Zhang CL, Cravatt BF and Evans RM 2007. Maternal PPAR gamma protects nursing neonates by suppressing the production of inflammatory milk. *Genes & Development* 21, 1895–1908.
- Wu WZ, Wang XQ, Wu GY, Kim SW, Chen F and Wang JJ 2010. Differential composition of proteomes in sow colostrum and milk from anterior and posterior mammary glands. *Journal of Animal Science* 88, 2657–2664.
- Yan W, Chen J, Wiley AA, Crean-Harris BD, Bartol FF and Bagnell CA 2008. Relaxin (RLX) and estrogen affect estrogen receptor alpha, vascular endothelial growth factor, and RLX receptor expression in the neonatal porcine uterus and cervix. *Reproduction* 135, 705–712.
- Yan W, Ryan PL, Bartol FF and Bagnell CA 2006a. Uterotrophic effects of relaxin related to age and estrogen receptor activation in neonatal pigs. *Reproduction* 131, 943–950.
- Yan W, Wiley AA, Bathgate RAD, Frankshun AL, Lasano S, Crean BD, Steinetz BG, Bagnell CA and Bartol FF 2006b. Expression of LGR7 and LGR8 by neonatal porcine uterine tissues and transmission of milk-borne relaxin into the circulation by suckling. *Endocrinology* 147, 4303–4310.