ADSA Foundation Scholar Award: A role for serotonin in lactation physiology—Where do we go from here?

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ABSTRACT

Lactation is a physiological event that is exclusive to mammals. Lactation evolved as a strategy to improve the survival of the young by providing them with the complete nutrition that is required for survival upon birth as well as maternal–offspring bonding. Typically, milk production by the dam matches the demand of the young. The dairy cow is a unique exception in which the discoveries and genetic selection related to lactation physiology have been applied and resulted in a dramatic increase in milk yield of dairy cows. Studies on the role of mammary-derived serotonin and the coordination of various aspects of milk production and maternal metabolism have revealed novel mechanisms by which milk production and maternal metabolism can be improved. Furthermore, the investigation into molecular and cellular mechanisms regulating mammary gland function has revealed the importance of epigenetics on mammary gland function. Understanding mammary gland function at the cellular and physiological levels will be important for improving mammary gland control of maternal metabolism during early lactation. The early lactation period is a critical time for a dairy cow as that is when she is most susceptible to disease and metabolic disorders that can lead to negative effects on her productive capacity and overall health. Our research in the area of serotonin physiology has illustrated the importance of serotonin on the regulation of lactation and maternal homeostasis. Future research in the area of lactation physiology should be targeted at improving maternal health and longevity in the herd through manipulation of the signals the mammary gland sends to coordinate maternal metabolism and synthesize milk. Specifically, we believe that serotonin will play a central role in understanding the communication between the mammary gland and the maternal physiology during lactation.

Key words: lactation, mammary gland, serotonin

INTRODUCTION

As recently reviewed by Collier and Bauman (2017), the latter half of the 20th century and early portion of the 21st century represents a “golden age” of lactation biology. Numerous recent studies have focused on a variety of new biological influences, such as serotonin and epigenetic effects of heat stress, on the mammary gland, and subsequent effects on milk yield and maternal health. The mammary gland produces bioactive factors critical for autocrine-paracrine regulation of milk synthesis (Wall and McFadden, 2012; Weaver and Hernandez, 2016). Beyond understanding genetic influences on mammary gland physiology, delineation of the endocrine and autocrine-paracrine regulation of mammary gland development and function is critical. This is of particular importance because milk synthesis and secretion varies between species, particularly in relation to their reproductive strategy (e.g., tammar wallaby, fur seal, bovine). It is widely accepted that the amount of milk produced by a mammary gland is affected by the nursing frequency of the gland, and this is largely governed by autocrine or paracrine regulation (Tucker, 1966; Neville et al., 1988; Wall et al., 2012). Nursing frequency and increased milking frequency are strategies that are widely applied in both humans and dairy cattle to increase milk synthesis and secretion during established lactation (Hill et al., 2001; Wall et al., 2012). Furthermore, it has been clearly established that nutrients are partitioned during lactation to support milk synthesis due to various homeostatic mechanisms (Bauman and Currie, 1980). Therefore, research on factors produced by the mammary gland that control maternal metabolism (i.e., energy homeostasis, calcium homeostasis, endocrine status) to regulate milk production is of great interest. Although milk production is an important focus, research on factors that improve maternal health and metabolism during lactation will be critical to the success of the lactation. The rate-limiting enzyme required for peripheral serotonin synthesis, tryptophan hydroxylase 1, was initially discovered to be expressed in the mammary gland when milk filled the mammary gland (Matsuda et al., 2004). This discovery

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was instrumental in the investigation of how serotonin affects the mammary gland and lactation. My career thus far has focused on understanding how the monoamine serotonin plays a role in the regulation of maternal and mammary gland metabolism during lactation.

THE TRANSITION PERIOD

The greatest risk for cows leaving the herd (dead or sold) occurs in early lactation (Fetrow et al., 2006; De Vries et al., 2010), particularly around calving (Thomsen et al., 2004; Dechow and Goodling, 2008; McConnell et al., 2009). Approximately 45% of mortality in herds occurs in the first 60 d postpartum, and up to 52% occurs from 21 d prepartum to 60 d postpartum (Hadley et al., 2006; McConnell et al., 2009). Production diseases such as milk fever or hypocalcemia, ketosis, fatty liver, metritis, retained placenta, displaced abomasum, and mastitis have all been shown to have a negative effect on potential production and to increase the risk of involuntary culling. Chamberlin et al. (2013) found that hypocalcemic cows had significantly higher mean plasma nonesterified fatty acid concentrations on the day of calving as well as a higher liver lipid percentage on d 7 postcalving. Several studies have indicated that cows with subclinical hypocalcemia (**SCH**) do in fact have a greater risk of developing a displaced abomasum and consequently being culled within the first 60 DIM (Seifi et al., 2011; Chapinal et al., 2012).

In addition to the effects of hypocalcemia on energy metabolism, Ca has a critical role in muscle function. Hypocalcemia leads to a reduction in all muscle contractions, which can decrease rumen and abomasal motility, therefore further increasing the risk of a displaced abomasum. Likewise, insufficient contraction of the teat sphincter muscle consequently increases the risk of mastitis (Goff, 2008). Martinez et al. (2012) showed a compromised innate immune system in SCH cows compared with normocalcemic cows. Neutrophils in the blood of SCH cows were less capable of phagocytizing and killing pathogenic bacteria *in vitro*. The SCH cows were further compromised by an overall smaller leukocyte population, and they had a 3.2-fold increase in the incidence of metritis. Although it is recognized that cows experience immunosuppression during the periparturient period, the study of Martinez et al. (2012) suggests that immunosuppression is further exacerbated by low blood Ca levels. Kimura et al. (2006) offered data suggesting that periparturient immunosuppression in dairy cows is due, in part, to the depletion of intracellular Ca stores. A recent study using an EGTA challenge model demonstrated that cows with SCH had fewer neutrophils undergoing phagocytosis and a lower oxidative burst response after incubation

with pathogenic bacteria (Martinez et al., 2014). This further supports the idea that a reduction in Ca could lead to immunosuppression in a periparturient cow.

Although dietary changes have reduced clinical hypocalcemia in the United States dairy herd from as high as 50% to approximately 5% (Block, 1984; USDA, 2010), the impact of SCH is still of great concern. In 2011, the incidence of SCH in cows of second lactation or greater was estimated to be approximately 47%, with 25% of all first-lactation cows being affected (Reinhardt et al., 2011). It is estimated that each case of hypocalcemia results in a loss of at least \$300 (Guard, 1996), whereas every case of SCH costs, on average, \$125 (Oetzel, 2013). In an effort to reduce SCH in periparturient cows, several groups have focused on novel ways to utilize prepartum DCAD diets to improve postpartum calcium status, including use of DCAD diets in combination with vitamin D supplementation, varying levels of DCAD in combination with low energy diets, or feeding DCAD in combination with 5-hydroxy-L-tryptophan supplementation (Leno et al., 2017; Martinez et al., 2018; Rodney et al., 2018; Slater et al., 2018). Research targeted at improving Ca homeostasis during the periparturient period will be critical for improving cow health and longevity. Our research on how serotonin coordinates Ca metabolism during lactation is a novel area in which we have demonstrated the presence of a physiological axis during lactation that can be manipulated to improve cow health and productivity.

SEROTONIN

Serotonin is a biogenic amine that has been preserved over the last 2 billion years of evolution from protozoa to mammals and has been demonstrated to influence development and plasticity of various tissues in different species (Turlejski, 1996; Raymond et al., 2001). Serotonin is one of the most ancient signaling molecules, and it is found in many animals, plants, and unicellular organisms, with the first receptor subtype appearing 750 million years ago (Turlejski, 1996; Raymond et al., 2001). The early appearance of serotonin in the evolutionary record has resulted in the regulation of a variety of physiological functions, including fundamental mechanisms such as homeostasis, feeding, immunity, glucose regulation, cardiovascular function, behavior, gut motility, and reproduction (Gershon and Tack, 2007; Horsemann and Collier, 2014; Wyler et al., 2017). Additionally, serotonin receptors are thought to be one of the oldest rhodopsin-like receptors in existence (Garattini and Valzelli, 1965; Nichols and Nichols, 2008). Serotonin is synthesized from the amino acid L-tryptophan. L-Tryptophan is converted to 5-hydroxy-L-tryptophan (**5-HTP**) via tryptophan

hydroxylase (**TPH**), which is the rate-limiting step in serotonin production. The intermediate 5-HTP is then converted to serotonin (**5-HT**) by aromatic amino acid hydroxylase. Serotonin is produced from tryptophan by enzymes commonly used in anaerobic organisms before oxygen was formed inside cells, making plants the most efficient producer of serotonin (Azmitia, 2007). Two isoforms of TPH exist: the non-neuronal isoform TPH1 is expressed in the pineal gland and other non-neuronal tissues, and TPH2 is expressed in the dorsal raphe nucleus of the brain and the intestine (Côté et al., 2003). The non-neuronal serotonergic system was the first to appear in evolutionary history; however, a lot of the focus of serotonin research is on the neuronal effects (Turlejski, 1996). Centrally, serotonin functions as a neurotransmitter produced in the hindbrain raphe neurons, which innervate almost all regions of the brain (Jacobs and Azmitia, 1992). Peripheral serotonin is synthesized in a variety of tissues including the intestine, pancreatic cells, adipose tissue, and the mammary gland (Matsuda et al., 2004; Berger et al., 2009; Wyler et al., 2017). Serotonin does not cross the blood-brain barrier; therefore, the neuronal and peripheral systems represent 2 distinct pools of serotonin (Berger et al., 2009).

Serotonergic control over a multitude of physiological processes has resulted in the evolution of many serotonin receptor subtypes (Peroutka and Howell, 1994). The complexity of the serotonin system can be attributed to the large number of receptor genes, alternative splicing of receptor transcripts, RNA editing, varied combinations of receptor subunits, and heterodimerization with non-serotonin receptors (Hoyer et al., 2002; Wyler et al., 2017). Serotonin has been implicated in regulating proliferation, differentiation, and migration of cells in various tissue types and is involved in the regulation of a multitude of physiological processes (Turlejski, 1996).

Matsuda et al. (2004) demonstrated that filling of the mouse mammary gland increased the expression of the rate-limiting enzyme for serotonin production, TPH1. Furthermore, researchers demonstrated a significant effect of serotonin on expression of milk protein genes. It has also been illustrated that the serotonin produced by the mammary gland during lactation contributes to approximately 50% of the circulating serotonin concentrations in mice (Weaver et al., 2017). These results led to the initial experiment we conducted in dairy cows to demonstrate whether the serotonergic system was present in bovine mammary tissues. We characterized TPH1 and serotonin receptor subtype expression in the bovine mammary gland. Additionally, intramammary infusions of serotonin resulted in a 10% decrease in milk production (Hernandez et al., 2008). A recent RNA sequencing analysis of lactating mammary glands

collected from wild type and TPH1 knockout mice revealed gene ontology pathways affected by serotonin in the mammary gland, including calcium homeostasis, lipid metabolism, and hypoxia (Laporta et al., 2015). These initial findings demonstrate the importance of serotonin for mammary gland function during lactation.

SEROTONIN SIGNALING

Due to the physiological functions of serotonin throughout the body, it signals through a variety of different receptors. A diversity of receptor subtypes provides an organism with flexibility in its response to 5-HT and facilitates adaptability during physiological and environmental challenges (Uphouse, 1997). Fifteen different receptor subtypes of serotonin have been identified thus far (Noda et al., 2004). The serotonin 1, 2, 4, 5, 6, and 7 receptor subtypes are coupled to G-protein receptors, and the serotonin 3 receptor subtype is linked to gated ion channels. Some receptor subtypes are more highly involved and expressed in the central nervous system than in peripheral parts of the body (variants of the serotonin 1 receptor and the serotonin 5 receptor subtype), whereas others are more highly expressed in the periphery but are still present in the central nervous system (serotonin 2, 3, 4, 6, and 7 receptor subtypes; Raymond et al., 2001). The diversity and number of serotonin receptor subtypes allows serotonin to regulate numerous functions of tissues throughout the body.

Gene expression studies in mouse, human, and bovine mammary glands have determined the receptor patterns present within these species. Some serotonin receptor subtypes are conserved in the mammary gland; however, there are some unique receptor subtypes present in different species (Pai et al., 2009). We initially described the presence of at least 5 serotonin receptor subtypes in the dairy cow mammary gland (Hernandez et al., 2009). Furthermore, various breast cancer cell lines have diverse serotonin receptor subtypes (Pai et al., 2009). Much of the early work on serotonin and mammary gland function revealed that serotonin receptor subtype 7 was important for the formation and disruption of tight junctions between epithelial cells (Stull et al., 2007; Pai and Horseman, 2008). Interestingly, the effects on tight junctions by serotonin are biphasic, with intermediate concentrations of serotonin potentiating closure of tight junctions and high concentrations causing disassembly of tight junctions (Pai and Horseman, 2008). Additionally, lactating mice lacking the serotonin receptor subtype 7 had dysregulated epithelial cell structure, reduced capability to shed cells during mammary gland involution, and an impaired capacity to feed their pups (Pai et al., 2015). In addition

to the 7 receptor subtype, we have investigated the role of the serotonin 2B receptor subtype in the mammary gland. Initial results in rodents suggested a role for this receptor in mammary epithelial cell proliferation through the extracellularly related kinase pathway (Laporta et al., 2014a). Further exploration of mammary gland serotonin receptor subtypes is necessary to fully understand serotonergic regulation of lactation.

SEROTONIN AND EPIGENETIC CONTROL OF LACTATION

Epigenetics has been an evolving research area over the last several years. Epigenetics is defined as heritable changes across the genome that regulate gene transcription without modifying the DNA sequence associated with the gene (Dupont et al., 2009). Numerous factors, including serotonin, influence epigenetics (Holloway and Gonzalez-Maesó, 2015). Global DNA methylation is associated with mammary cell differentiation, for example, in regulation of milk protein genes. Specifically, DNA methylation occurs at promoters of the mouse casein gene cluster, as well as in potential distal regulatory elements (Rijnkels et al., 2010). Additionally, the α_{S1} -casein gene is methylated during involution and mastitis in the bovine mammary gland (Singh et al., 2009, 2012). Analysis of promoter methylation in exfoliated epithelial cells isolated from breast milk in healthy women revealed a significant relationship between methylation of 6 tumor suppressor genes and increased breast cancer risk (Wong et al., 2010). A recent study reported that milk production was reduced in the offspring of heat-stressed cows that were not provided cooling during their dry period (Monteiro et al., 2016). There was no difference in genetic potential between the sires of the heifers used in the study, suggesting epigenetic regulation. Mammary tissue collected from that study had a significant amount of differential methylation of CpG islands across coding and noncoding regions of the genome in a group of approximately 50 genes, as measured by bisulfite sequencing (Dahl et al., 2017). These results suggest that epigenetic modifications play an important role in the regulation of mammary gland functions.

Serotonin signaling also has epigenetic effects on a variety of behavioral and physiological responses. A recent review covered the epigenetic contributions of serotonin signaling largely on psychiatric disorders (Holloway and Gonzalez-Maesó, 2015). Comparison of methylation of 20 CpG sites closest to the transcriptional start site of the serotonin reuptake transporter, the target of selective serotonin reuptake inhibitors, predicted amygdala reactivity in adolescents and young adults and suggested this was related to epigenetic effects of

serotonin (Nikolova et al., 2014). Another study found that epigenetic changes resulted in increased expression of the serotonin 2A receptor subtype in the frontal cortex of offspring born to dams exposed to mouse-adapted influenza A (Moreno et al., 2011). Maternal stress in rats resulted in decreased mRNA expression of the serotonin 1A receptor subtype in the hippocampus with a concordant increase in DNA methylation at the promoter region of the gene (Van den Hove et al., 2006). Independent associations between epigenetic regulation of serotonin and lactation prompted our research into the role of serotonin on epigenetic control of the mammary gland. Using a mouse deficient in production of peripheral serotonin, we demonstrated that serotonin increases transcription of parathyroid hormone-related protein (*Pthrp*) by methylation of the promoter for the upstream sonic hedgehog gene (Laporta et al., 2014b). We are currently exploring this phenomenon in the dairy cow and how it may control calcium metabolism in the mammary gland. Transition cow nutrition, environmental exposures, and administration of hormones or monoamines may result in epigenetic alterations of dam and offspring mammary glands and their future lactation potential. Therefore, exploring molecular mechanisms of mammary gland control are critical for the lactation outcomes of the dairy cow.

We are interested in determining whether serotonin's effects on the promoters of sonic hedgehog and parathyroid hormone-related protein (**PTHrP**) are driven by epigenetic changes or the process of serotonylation. Serotonin itself does not have a methyl group; therefore, we are interested in the mechanism whereby serotonin can contribute to epigenetic changes on the DNA. One of our current hypotheses regarding serotonin's regulation of calcium metabolism in the mammary gland is through the process of serotonylation. In this process, the enzyme transglutaminase catalyzes the transamidation of serotonin to a protein-bound glutamine residue, resulting in the amino group of the serotonin being covalently bound to the gamma carboxamide of glutamine (Muma and Mi, 2015). Serotonin can also serve as a transamidation substrate to neurotransmitters. Targets for serotonylation include small G proteins, heterotrimeric G α proteins (Go1 and Gq), fibronectin, and cytoskeleton proteins. Serotonylation regulates numerous physiological processes, but it was first described in platelets where serotonin was transamidated to fibrinogen and von Willebrand factor on the cell surface after platelet activation (Dai et al., 2011). Since that discovery, serotonylation has been implicated in the regulation of the serotonin reuptake transporter, insulin release, smooth muscle contraction, pulmonary hypertension, bone extracellular matrix, extracellular and glial membrane proteins, and neuron cells. The

ability of serotonin to transamidate and covalently bind to glutamine results in an additional mechanism whereby serotonin epigenetically regulates a variety of physiological processes beyond interaction with its classical receptor subtypes. The phenomenon of serotonylation could be a key underlying event how serotonin effects mammary gland function.

The serotonylation of small G proteins downstream of one of the serotonin receptors could be responsible for the induction of PTHrP in the mammary gland. We are currently performing experiments to test this hypothesis in both mouse and cow models. This information could inform how serotonin regulates future potential lactation outcomes in lactating cows and its effect on the offspring.

SEROTONIN AND CALCIUM HOMEOSTASIS DURING LACTATION

Total Ca is maintained between 2.1 and 2.5 mmol/L in a healthy cow. Approximately 50% of this Ca is bound to proteins and is not biologically active. In contrast, 42 to 48% Ca is ionized (iCa) and is biologically active. During a normal physiological state, Ca homeostasis functions via a traditional negative-feedback system. When iCa concentrations drop below the threshold required by the cow, the parathyroid responds by secreting parathyroid hormone (PTH). Circulating PTH then travels through the blood and binds to receptors on the osteoblasts in bone as well as to its receptor in the kidneys. In the kidneys, PTH enhances renal reabsorption of Ca as well as increasing production of 1,25-dihydroxyvitamin D, the active form of vitamin D. Vitamin D then binds to and stimulates intestinal epithelial cells resulting in increased active transport of Ca (Goff, 2014). If dietary Ca is insufficient to restore Ca homeostasis, Ca resorption from the bone tissue begins. Although osteoblasts contain PTH receptors, it is their communication with osteoclasts via various signaling factors that actually break down the bone to liberate Ca (Goff, 2014). Dairy cows, unlike most mammals, initiate Ca resorption from bone more slowly than the accelerating demand for Ca by the mammary gland, potentially resulting in clinical hypocalcemia or SCH.

Until about 30 yr ago, the parathyroid was thought to be the primary regulatory gland for blood Ca, regardless of age or physiological state of the animal. However, in 1987, Moseley et al. (1987) released their findings describing a protein—PTHrP—that was described to have biological activities similar to PTH. This protein was initially isolated from cells of a lung tumor of a patient suffering from hypercalcemia. It was named because of its similarity to PTH in both structure and function (Moseley et al., 1987). Since the

initial discovery of PTHrP, it has been found in a wide variety of cell types and organs, including the mammary gland, skeleton, placenta, smooth muscle, pancreas, and a variety of tumors (Wysolmerski, 2012). Unlike PTH, PTHrP functions as a local paracrine regulator and is detected in the circulation only during instances of lactation or humoral hypercalcemia (Wysolmerski, 2010). Much work has focused on the role of PTHrP in the mammary gland during lactation (Wysolmerski, 2010). Recent work in our laboratory has shown that serotonin stimulates PTHrP in the mammary gland during lactation.

Like PTH, PTHrP acts as a hormonal regulator of Ca and is the critical regulatory hormone responsible for the mobilization of bone Ca during lactation. Bone Ca is needed during lactation to synthesize milk, while still maintaining maternal physiology (Sowers et al., 1996; Mather et al., 1999). Sowers and colleagues (1996) demonstrated that elevated circulating PTHrP concentrations in women were positively correlated with their breastfeeding status (i.e., the frequency and exclusivity of their particular lactation). Furthermore, elevated concentrations of PTHrP were correlated with a decrease in bone mineral density in these women. Mather et al. (1999) outlined the case study of a woman with hypoparathyroidism who required supplemental Ca before pregnancy. As she reached the end of her pregnancy, she became hypercalcemic and exhibited increased PTHrP concentrations. It is believed that the increased PTHrP during the periparturient period was actually responsible for the sudden increase in blood calcium, despite her previous diagnosis of hypoparathyroidism. Circulating PTHrP has been detected in humans, rats, mice, goats, and cows (Rakopoulos et al., 1992; Ratcliffe et al., 1992; Wysolmerski, 2010; Moore et al., 2015). Across various species, PTHrP is critical for regulating Ca homeostasis during the periparturient period and lactation. Indeed, lactation appears to be the only normal physiological time during an animal's life when PTHrP enters the systemic circulation (VanHouten, 2005). Mouse mammary epithelial cells are a primary source of circulating PTHrP during lactation. Deletion of PTHrP in the mammary gland during lactation results in decreased plasma PTHrP concentrations, and preservation of bone mass (VanHouten, 2005). In addition, the lactating mammary gland functions as a calcium-sensing organ, which in turn responds to changes in extracellular Ca concentrations, primarily through the calcium-sensing receptor (CaSR). The basolateral CaSR detects blood concentrations of Ca and cooperates with PTHrP in response to changing Ca concentrations (VanHouten, 2005). Milk concentrations of PTHrP are 100 to 1,000 times higher than those detected in the circulation; however, the function that

PTHrP serves in the offspring is unknown (Thiede, 1994; Onda et al., 2006; Filipović et al., 2008). Very little research on PTHrP has been performed in the dairy cow model, and this should be further explored based on the rodent and human literature.

Recent research has focused on serotonin's role in Ca regulation, particularly during lactation. We demonstrated that mice deficient in TPH1 have decreased gene and protein expression of PTHrP in their mammary glands. Furthermore, we were unable to detect concentrations of PTHrP in plasma of dams during lactation (Hernandez et al., 2012). Through the administration of a single i.p. injection of 100 mg/kg of 5-HTP, circulating PTHrP concentrations were restored within 1 h, and PTHrP gene and protein expression was restored in the mammary gland. These data suggest that serotonin is critical for the regulation of PTHrP production in the mammary gland during lactation. As a follow-up, rats were fed a diet containing 0.2% 5-HTP from d 13 of pregnancy through d 9 of lactation, resulting in increased circulating PTHrP concentrations and increased total Ca (tCa) concentrations on d 1 of lactation (Laporta et al., 2013). As such, administration of 5-HTP during the periparturient period may increase circulating tCa concentrations during the onset of lactation.

Our recent research in dairy cows has been reviewed in Hernandez (2017). Briefly, we have shown that administration of 5-HTP to periparturient cows increased postpartum tCa concentrations (Weaver et al., 2016; Hernández-Castellano et al., 2017). Furthermore, administration of 5-HTP prepartum increased postpartum tCa concentrations but did not change circulating PTH concentrations (Hernández-Castellano et al., 2017). Cows receiving the saline control infusion had increased circulating PTH concentrations and decreased tCa concentrations compared with the 5-HTP-treated cows. Given our data in rodents and cows, we hypothesize that serotonin, PTHrP, and Ca during lactation are working independently of the classic PTH–vitamin D feedback loop that regulates circulating Ca concentrations.

CONCLUSIONS AND FUTURE DIRECTIONS

Over the last 13 yr, much has been learned regarding the role of serotonin in the mammary gland. However, much remains unknown about the contribution of serotonin to the interface of the mammary gland and maternal metabolism during lactation. Specifically, there have been critical findings regarding the regulation of PTHrP production by the mammary gland during lactation and potential direct roles on Ca metabolism. Our future research will focus on the roles of epigenetics

and serotonylation in regulating mammary gland function during lactation. We are particularly interested in the interaction of serotonin and Ca; however, our RNA sequencing data suggest the need to further investigate lipid metabolism and hypoxia, among other pathways in the mammary gland. Additionally, we are working on various aspects of how serotonin may affect mammary gland function and maternal health in lactating women. In particular, we are interested in how serotonin-dependent antidepressants during pregnancy and lactation affect maternal bone health. Understanding the mammary gland's contribution to the regulation of maternal metabolism is critical and applicable to a variety of mammalian species.

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