



### SPLASH! milk science update

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This month's issue features articles about sex-biased milk production, milk as brain food, lactation and cancer research, and milk's valuable fats. Enjoy!

## Fetal Daughters Influence Milk Production in Cows

- **Holstein cows produce significantly more milk after gestating a daughter.**
- **The percentage of milk fat, protein, and lactose are the same after gestating a daughter or a son.**
- **"Daughter-biased" milk production may be an evolutionary adaption or a physiological constraint.**
- **Universal adoption of sex-semen conception for nulliparous heifers alone could generate up to ~\$200 million.**

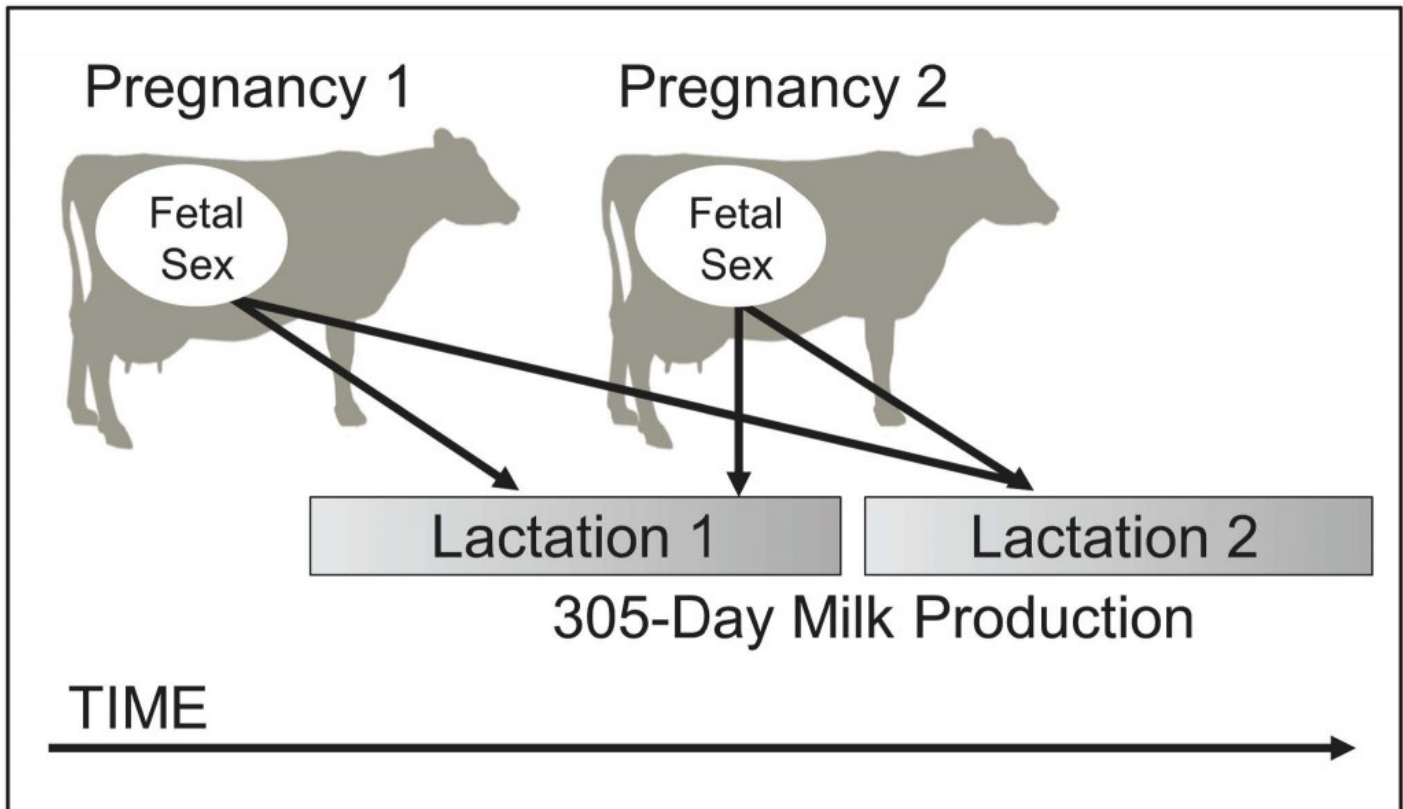
Functional development of the mammary gland occurs during pregnancy. When dairy cows and goats are gestating twins, mammary gland development is amplified due to hormonal signals from the much larger fetal-placental unit. Carrying twins seemingly programs higher milk production to meet the needs of "double the trouble" (Nielen et al., 1989, Hayden et al., 1979). But what if fetal-placental signals aren't just about the number of offspring, what if other features are signaled that influence milk production, features like infant gender (sidebar: dairy scientists use the term "gender," evolutionary biologists use the term "sex")? Sex-biased lactation has received substantial research effort in the last few years, has been documented in humans, monkeys, rodents, deer, and marsupials, and was featured in a [2012 SPLASH column](#). But how does the mammary gland "know" that milk is being produced for a son or a daughter?

In collaboration with Barry Bradford and Abigail Kennedy, Kansas State University and John Clay, Director of the Dairy Records Management Systems, we used 2.39 million lactation records from 1.49 million Holstein dairy cows from 1995 to 1999 to investigate whether the sex of the fetus influences the capacity of the mammary gland to synthesize milk during lactation. Standardized husbandry practices, systematic milking procedures, detailed record keeping, and large sample sizes make the dairy cow a powerful model for the exploration of milk synthesis from both mechanistic and evolutionary perspectives. Notably, calves are removed from the dam shortly after birth, allowing us to specifically investigate prenatal mechanisms of sex-biased milk synthesis independent of postnatal maternal care and suckling behavior.

Holsteins bias milk production in favor of daughters, not sons. Across a standard 305-day lactation, total milk production was significantly higher after gestating a daughter, ranging between ~100-150 kg more milk across lactation (220-310 lb). This effect was strongest in cows during their first lactation, likely because substantial changes occur in the mammary gland the first time it gears up for milk synthesis. The percentage of fat and protein in milk did not differ between cows that gestated a son or daughter, so the "quality" of milk was the same. But because the "quantity" was higher after gestating a daughter, the *total* kilograms of milk fat and protein after gestating a daughter were higher than after gestating a son. This sex-biased milk did not occur when farmers used bST (recombinant bovine somatotropin) on multiparous cows, indicating that bST exerts a stronger effect on the mammary gland of a cow that gestated a son. But bST use for cows on the first lactation did not "overcome" the effect of fetal daughters. These data suggest that cows on their first pregnancy are particularly sensitive to fetal sex. How does the fetus mechanistically influence milk synthesis? It's likely that hormones from the fetus and placenta may differ between fetal sons and daughters, subsequently enter the maternal bloodstream, and affect the milk producing cells in the mammary gland.

But fetal sons and daughters don't just affect the current lactation. Dairy cows are often concurrently pregnant and lactating, typically 200+ days of the 305-day lactation. We restricted a new analysis to a smaller conservative, longitudinal dataset of the first and second lactations for individual cows (N=113,750 cows) with no cases of dystocia (calving score 1 or 2 indicating no or little difficulty during parturition), and no reported administration of bSt. Fetal sex interacted dynamically across lactations, in part because the second pregnancy overlapped with the first lactation. Cows that gestated a son and then another son synthesized significantly less milk during the standard 305-day first lactation. Cows that first gestated a son and then a daughter on the second pregnancy could partially "rescue" 305-day milk production, but total milk production remained substantially lower compared to cows that had a daughter on their first pregnancy. Additionally, cows that had a son on their first pregnancy were handicapped in their milk production, even on their second

lactation, and *especially* if they also had a son on their second pregnancy. Specifically, cows with two daughters back-to-back produced ~445 kg (~980 lb) more milk across the first two lactations than did cows with back-to-back sons. Yeah, it's super complicated so we made a "conceptual model."



**Conceptual Model Figure.** Milk production is influenced by fetal sex across lactations. Fetal sex in pregnancy 1 affected milk production in lactation 1 and lactation 2 because of the critical steps in mammary development that occur during pregnancy 1. In the cow, pregnancy 2 typically overlaps with lactation 1, so calf sex in pregnancy 2 also impacted milk production in lactation 1. (Figure credit: Hinde et al., 2014)

### Real World Implications

Dairy herd management decisions can be informed by the effects we report here, particularly improving the targeted selection of cows artificially inseminated with sex-selected semen. Artificial insemination is standard practice in dairying, and sex-selected semen is widely used. The use of sexed semen with a virgin heifer increases her long-term milk production. Rough, "back of the napkin" calculations, taking into account the current wholesale value of milk, the number of two-year-old heifers added to U.S. dairy herds annually, the production advantage across the first two lactations of conceiving a daughter on the first pregnancy, and the increased probability of conceiving a daughter from sex-selected semen suggests a gross value in the neighborhood of ~\$200 million in milk production across the first two lactations in the United States alone. In cows whose first pregnancy yields a bull calf, the use of sexed semen for the next pregnancy can partially recover milk production during the first lactation and increase it during the second lactation. In cows whose first pregnancy yielded a heifer calf, the use of sexed semen for the second pregnancy does *not* seem to create additional economic benefit in terms of milk production. Moreover, there may be other differences, such as the concentration of micronutrients in milk after gestating a son or daughter. Such information may enhance the formulation of milk replacer for calves.

But can we extrapolate from cows to humans? In the last few years, scientists have reported differences in milk composition produced for sons and daughters among different human populations (Powe et al., 2010, Thakkar et al., 2013, but see Quinn et al., 2013 for no difference), although no info yet on difference in milk volume. Fetal signals may play a role in generating these differences between breastmilk produced for daughters and milk produced for sons. Humans have a very invasive placenta (Abrams and Rutherford, 2011) that would allow fetal hormones to pass into maternal circulation and possibly influence mammary gland development. Sex-differentiated milk may provide important nutrients for the developmental priorities of young, such as more calcium for daughters whose skeletons develop faster than sons, as we found in rhesus monkeys last year (Hinde et al., 2013). Further investigations of milk synthesis specific to whether a mother has a son or a daughter could have far-reaching implications for nutritional approaches in the NICU

via improved selection and matching of donor milks. Moreover, for women who do not breastfeed or supplement breastmilk, the recipe for infant formulas could be better tailored to the differing physiological needs of sons and daughters. Hopefully, collaborations among animal scientists, clinical practitioners, and evolutionary biologists will continue to yield new insights from already existing data and shape future research directions.

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## Brain Building Blocks in Milk

- Milk contains fat-sugar molecules called gangliosides, which are building blocks of brain and other tissues.
- Gangliosides have been isolated in human and cow's milk.
- Gangliosides act as bait for pathogens in order to prevent infection.
- Gangliosides' consumption is associated with higher cognitive performance.

The combination of fat and sugar may be off limits for South Beach dieters, but a fat-sugar molecule could be just what human infants need to help their brains develop and to fight off pathogens. These molecules, called gangliosides, have been identified in human, bovine, and other mammalian milks. Although it has long been known that milk gangliosides are involved in infant immunity and neural development, researchers are only now beginning to elucidate the specific, and critical, roles they play in each process. And what scientists have uncovered just might make even the most careful dieter think twice about fat and sugar.

### Ganglioside biology – a quick primer

Gangliosides are glycolipids (molecules with a carbohydrate attached to a lipid) and get their name from the ganglion or nerve cells where they were first discovered. In addition to brain tissue, gangliosides have been identified in other animal tissues, such as smooth and skeletal muscle, lymphocytes, plasma, the placenta, mammary glands, and milk (McJarrow et al., 2009).



What sets gangliosides apart from other glycolipids is their unique structure. First, the lipid component is a group of fatty acids linked to a specific amino alcohol called a sphingosine. Also called sphingolipids, these types of fats are important structural components of brain tissue. Second, the carbohydrate of a ganglioside is an oligosaccharide. Human milk oligosaccharides manage to avoid digestion by stomach acids, allowing them to bind to pathogens in the digestive

tract and prevent infection. In turn, 80% of milk gangliosides survive passage through the stomach and are instead absorbed in the intestines (Rueda, 2007). Finally, the carbohydrate is linked to one or more sialic acids. Sialic acids have been referred to as “brain food” and are an essential nutrient for fetal and neonatal brain growth (Ryan et al., 2013; Wang, 2009).

Gangliosides are considered the most complex of the glycolipids because there are so many varieties or “species” (McJarrow et al., 2009). Species names all start with “G” (for ganglioside), followed by a letter representing the number of sialic acids (M for mono, or 1; D for di, or 2, T for tri, or 3, etc.), and finally the number of non-sialic acid sugars. For example, GD3 is a ganglioside with 2 sialic acids and 3 non-sialic acid sugars. Variation in the number and types of carbohydrates may equate to high diversity in ganglioside function. Thus, when discussing gangliosides in milk, it isn't just about how much of them are there but which species are present as well.

### **Taking the bait**

Perhaps the best example to illustrate the importance of variation in ganglioside structure is their role in preventing infection. Quite simply, gangliosides are decoys for pathogens. Pathogens that enter the infant's stomach are looking to bind to receptors. Like a key in a lock, each pathogen has a particular receptor to which they are able to attach. Like any good decoy, gangliosides look the part. In fact, their carbohydrate chains are so structurally similar to those on pathogen receptors, pathogens bind to them thinking they've found their target (Rueda, 2007). Importantly, if the pathogen is bound to a ganglioside, it is no longer a threat to the infant.

Having only one type of ganglioside would mean having just one lock. But human infants are vulnerable to numerous pathogens, a veritable janitor's key ring. Variation in ganglioside structure provides locks for all of these keys. For example, cholera toxin and *E. coli* bind to different configurations of the ganglioside GM1 while GT1 binds the botulism neurotoxin (Iwamori et al., 2008; Rueda, 2007).

### **Passive and aggressive**

Acting as a doppelganger for pathogen receptors allows gangliosides to prevent infection by becoming unintended receptors, but they also take on a more active role in infant immunity. Supplementation studies in mice (reviewed in Rueda, 2007) demonstrate that milk gangliosides increase the number of immune cells in the infant's intestine that secrete immunoglobulin A (IgA), the primary antibody directed against enteric pathogens. Supplemented mice also show increased production of cytokines, chemical messengers involved in stimulating both humoral (antibiotic) and cellular (T cell) immune activity. Finally, fecal samples from human preterm infants supplemented with gangliosides have lower *E. coli* counts and higher Bifidobacteria counts than controls, suggesting gangliosides even have a prebiotic function (Rueda, 2007). Taken together, these studies demonstrate that dietary gangliosides are key molecules in preventing infection in infants from enteric pathogens.

### **This is your brain on gangliosides**

Getting their name from brain ganglion cells, it is no surprise that gangliosides are implicated in fetal and infant brain development. Although their precise functions in the brain are still poorly understood, they are believed to be key players in myelination, nerve cell communication, memory formation, and cognitive performance (McJarrow et al., 2009; Ryan et al., 2013).

After passing intact through the infant's stomach, milk-derived gangliosides still have much work to do. In fact, their most important tasks may still be to come. Once they are absorbed by the intestine, they are transferred to different cell membranes throughout the body, with an emphasis on neural cells (McJarrow et al., 2009).

Numerous lines of evidence suggest that increased consumption of gangliosides leads to an increase in brain ganglioside concentration. Perhaps the best illustration of this comes from a study that compares ganglioside concentration in brains of breast-fed and formula-fed infants (Wang et al., 2003). Human milk has a higher concentration of gangliosides compared to bovine milk and infant formulas derived from bovine milk. As predicted, infants that received only formula had lower levels of gangliosides in their brains (specifically their frontal cortex) than those that were breastfed (Wang et al., 2003).

More isn't always better, but in the case of brain gangliosides, it most certainly is. Studies in rats find decreased learning ability with lower brain ganglioside levels, attributed to decreased formation of neural synapses and other key differences in neurodevelopment (McJarrow et al., 2009). And in the only supplementation study conducted to date in human infants, Gurnida et al. (2012) demonstrate higher performance scores on various cognitive tasks in six-month-olds that received ganglioside-supplemented formula.

## Gangliosides: A formula for success?

Gangliosides have a pretty impressive resume. Indeed, if out on an interview, the hiring committee would be hard pressed to come up with a weakness in gangliosides. So it would seem the next logical step should be to ensure all infants, both breast and formula fed, get more of them in their diet. But how much? And how do we increase milk ganglioside concentration?

Presently there have only been a handful of studies that have investigated the effects of ganglioside supplementation in human infants, and maternal supplementation experiments have been limited to animal models. In all studies, subjects were supplemented with bovine-derived gangliosides. Although lower in total ganglioside concentration than human milk, bovine milk does have GD3 and GM3 (Lee et al., 2013), the predominant gangliosides in human milk.

Larger studies are required to establish the safety and efficacy of ganglioside supplementation and to determine what concentrations may be considered optimal. Gurnida et al. (2012) and Ryan et al. (2013) both suggest using levels in human milk as a model for formula supplementation. While a reasonable suggestion, it ignores what could be very important variation in ganglioside concentration and composition across and within human populations. As research on milk ganglioside function moves forward, it is imperative to establish the magnitude and potential sources of variation in milk gangliosides across human populations.

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## Breastmilk as a Tool to Shed Light on Breast Cancer

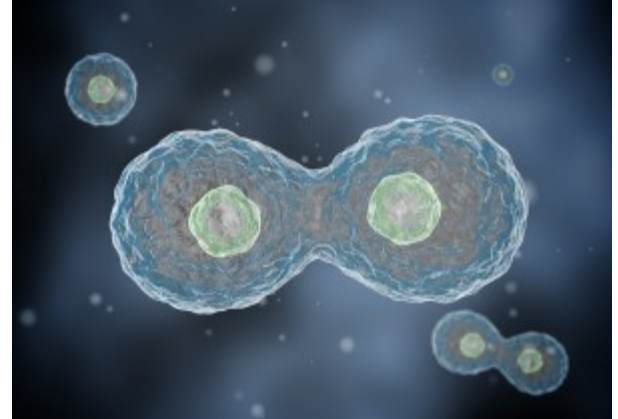
- The breast undergoes significant expansion during pregnancy and lactation.
- Some of the mechanisms of breast epithelial expansion occurring in pregnancy and lactation are also observed in breast cancer.
- Comparison of these mechanisms between the normal lactating breast and breast tumours may give insight into molecular events that lead to cancer.
- Breastmilk provides a direct and non-invasive source of all the types of epithelial cells found in the lactating breast. Thus, it could help delineate mechanisms leading to cancer and potentially, cancer risk.

When we discuss breastmilk we usually think of the baby. And rightly so, because this golden liquid contains all the nutrition, protection, and developmental signals the baby needs to grow healthily and appropriately. Research, however, is now starting to also consider the mother and ask what breastmilk can tell us about her health, the function of her breasts, her predisposition to developing breast cancer, and ultimately, the mechanisms that can lead to cancer. What is in the milk that can answer these questions?

## The breast fully matures only in lactation

Lactation is the only time during the life of a woman when her breasts become fully mature and functional organs. This process begins when a woman becomes pregnant, it progressively continues during different stages of pregnancy, and culminates after delivery of her child. This amazing transformation of the breast from an esthetic part of the body to a milk secretion factory that nourishes, protects, and programs human development occurs because of the mother's changing hormonal environment that is mediating alterations in the breast at the cellular and molecular levels<sup>1</sup>. Interestingly, some, but not all, of these alterations are also observed in breast cancer<sup>2, 3</sup>.

From birth until the first pregnancy of a woman, the breast is a quiescent and underdeveloped organ. During each pregnancy and upon the progressive effect of a changing hormonal circuit, the breast undergoes a massive transformation<sup>1</sup>. From few small epithelial ducts at the beginning of pregnancy, it grows to contain numerous longer ducts that have primary, secondary, and tertiary branches leading to spherical structures called alveoli<sup>4</sup>. Alveoli are specifically formed during this period and contain cells that are programmed to synthesize and secrete copious amounts of milk, which typically happens after delivery of the baby<sup>1, 4</sup>. Milk production continues throughout lactation. During weaning, special mechanisms are activated to regress the mammary gland back to a near-resting state, similar to the pre-pregnancy ductal epithelial tree that does not synthesize milk<sup>4</sup>.



### Mechanisms controlling normal breast expansion may also be implicated in oncogenesis

This remarkable transformation of the breast during pregnancy and lactation, although in existence for millions of years, since the first mammals, is still poorly understood. What is well established is that hormonal signals act on populations of breast cells, which in turn signal to other mammary cells. These cell-cell interactions are tightly regulated to allow for repeated occurrence during the life of a woman and are primarily restricted to pregnancy and lactation under normal conditions<sup>2, 5</sup>.

The hormonal and signaling effects result in activation of cell division in breast stem cells. These cells are rare when the breast is in a resting state but actively divide, and thus become more common, during pregnancy. After these stem cells create many daughter cells, the daughter cells start to gradually change towards functional cells that are primed to either synthesize milk or assist its secretion both shortly after a woman gives birth and throughout lactation<sup>4</sup>.

This fully functional cellular picture of the breast is only evident during lactation. However, some of the mechanisms involved in the expansion of breast tissue are similar, although not identical, to specific characteristics of breast cancer<sup>2, 3, 6</sup>. In this pathological situation, these mechanisms have been aberrantly activated, resulting in uncontrolled cell division. Because of these similarities between the lactating breast and breast cancer, we and others suggest that it is more logical to use the lactating breast, and not its resting counterpart, as a point of comparison when trying to understand breast cancer<sup>3, 5, 7</sup>.

Indeed, research has now started to utilize the physiological cascade of events occurring in the breast during lactation to model what goes wrong when tumors form. The idea is that the breast outside of the pregnancy and lactation cycle is at rest and therefore does not contain the complete cellular hierarchy that we observe in the same organ during lactation<sup>3, 5</sup>. Thus, comparisons of the resting breast with the pathological events occurring in breast cancer would not be as direct and relevant as those of the lactating breast.

But how can we ever conduct such research on the large scale required if we have to use breast tissue biopsied from lactating women? The invasiveness of the procedure would deter most women from participating. An easy and arguably superior alternative is breastmilk.

### Breastmilk as a model that can teach us how cancer occurs

Breastmilk contains nutritional factors, such as lipids, carbohydrates, and proteins. It is also rich in bioactive factors that boost the infant's immunity and direct its normal development. Among these milk components are cells. We have recently shown that the cells in mother's milk directly reflect the epithelium of the lactating breast, are highly heterogeneous, and include the complete cellular hierarchy that initiates and maintains the expansion and milk-secretory features of the gland<sup>5, 8</sup>. This hierarchy includes the epithelial stem cells, progenitor cells (cells that are more specific than stem cells but not fully differentiated), and the functional, differentiated epithelial cells of the breast.

What is more, via isolated breastmilk epithelial cells, we can access the genetic makeup, including the gene and signaling pathways that control the normal expansion of breast epithelial cells. More recently, it has been suggested that in addition to cells themselves, RNA isolated from milk fat is also representative of the breast epithelial cells<sup>9</sup>, albeit specifically of the milk-secretory cells and not their precursors.

In our laboratory, we have pioneered characterization of the breastmilk cellular hierarchy and heterogeneity, and, having access to a rare and precious archive of human resting and lactating breast tissues, we are able to compare this cellular hierarchy in milk with that in the breast tissue the milk originated from as well as its resting counterpart. In a recent study, we used these tools in addition to biopsies from women with various breast tumours<sup>3</sup>. What was unique about this data set of breast tumours was that they all contained cells that produced milk proteins; milk could even be readily secreted from the affected breast of some of these patients. This is a phenomenon that is sometimes seen in breast malignancies, but it is still poorly understood.

Therefore, there was something common between our breastmilk cells and the breast tumours we had in hand: they both had lactating features. This provided us with the perfect opportunity to compare the normal with the abnormal to see what differed between them. In theory, that would lead us to pathways that are deregulated in cancer.

We found that a gene network controlling cell self-renewal, which is typically expressed in embryonic stem cells, is activated normally during pregnancy and lactation<sup>3, 8</sup>. At any other time during a woman's life, only a rare subset of stem cells expresses these genes<sup>8</sup>. Intriguingly, increase in expression of these genes was seen both during pregnancy/lactation and in the tumours with lactating features, with the highest expression in the tumours<sup>3</sup>. This agrees with the fact that both the normal lactating breast and the tumours are characterized by increased cell proliferation, but in the tumours this process is out of control.

Based on these results, we reasoned that deregulation of this gene network at any time during the life of a woman can potentially lead to aberrant cell proliferation and malignant transformation and may be at the basis of some aggressive breast cancers<sup>3</sup>. This now opens the door for further use of breastmilk cells as models in breast cancer research as well as in improving our understanding of the normal biology of the breast.

### **Breastmilk may also be used to indicate breast cancer risk**

Furthermore, breastmilk may have the potential to be used prognostically, to indicate cancer risk, and diagnostically, to detect early stages of cancer<sup>7</sup>. We still do not know the normal range of expression of the various self-renewal genes in the lactating breast. As soon as this is elucidated, we may be able to detect outliers and examine associations with breast cancer risk. In addition to self-renewal genes, other genes such as those associated with tumour suppression have been examined in breastmilk epithelial cells.

Arcaro and colleagues examine DNA methylation in the promoter regions of various genes in breastmilk cells as a tool to develop markers for breast cancer risk. DNA methylation is an epigenetic event, i.e., an event that is not controlled by the DNA but which influences the function of genes in the DNA. When the promoter of a gene is methylated in specific areas, the gene does not function. In other words, it is silenced. In breast cancer, epigenetic changes can result in the silencing of certain genes that have important functions in suppressing cell proliferation, in controlling the cell cycle, in repairing the DNA, or metabolizing toxicants. When a normal cell undergoes some of these epigenetic changes, its chances of becoming cancerous are greatly increased.

Thus, by analyzing the epigenetic profile of breastmilk cells, one could theoretically detect early pre-cancer events and potentially help determine a patient's breast cancer risk. This can only be achieved if first the normal range of the milk epigenome associated with lactation is characterized. Arcaro and colleagues have reported increased DNA methylation in some tumor suppressor genes in breastmilk epithelial cells from healthy women with family history of breast cancer or with a previous benign biopsy, suggesting they may be at increased risk of cancer<sup>10, 11</sup>.

### **Next challenge: Optimization and standardization of methodology**

Although research on milk as a model and indicator of breast cancer is increasing, unfortunately important methodological differences exist among studies, which may influence the findings and interpretation of results. There is therefore an urgent need to optimize and standardize the procedures of milk collection and processing to advance the use of milk as a biospecimen<sup>12</sup>.

Ideally, collection times should consider the dynamic nature of breastmilk such as the significant effects of breast fullness<sup>13</sup>, the stage of lactation, milk volume expressed, the health status of the mother-infant dyad<sup>14</sup>, all of which change the biochemical and cellular composition of the milk<sup>5</sup>. Importantly, the health statuses of the mother and infant have rarely

been taken into account in studies of breastmilk composition. We now know that even a mild cold virus in the infant or a urinary infection in the mother can significantly change breastmilk composition, again in both bioactives and cells<sup>14</sup>.

Optimization of milk storage and processing, particularly for new and emerging techniques, are yet to be conducted. For example, freezing whole milk results in cell lysis (breakdown of the cell) and therefore prevents appropriate milk cell analysis<sup>5</sup>. Similar effects, albeit at a slower rate, together with activation of cellular apoptosis occur when whole milk is refrigerated for more than a few hours, also suggesting suboptimal storage conditions for cellular analyses, particularly at the molecular level.

Processing of the milk and its fractionation has been standardized in our and other laboratories over the years for various cellular and biochemical analyses and is critical to reducing variability of published results and improving interpretation of findings<sup>5, 12</sup>.

### The future

As more research is being done in this area, we faster come to the realization that gene networks and signaling pathways playing crucial roles in the normal biology of the breast are also key contributors to breast carcinogenesis. Breastmilk cell biology has a lot to offer in illuminating these interactions. In addition, its unfolding potential prognostic and diagnostic value could warrant its routine use in screening lactating women, particularly those belonging to cancer-susceptible groups or with a family history of breast cancer. Before such advances can be achieved, a great challenge we face is to distinguish between changes in milk proteome, transcriptome, and epigenome that are related to lactation and characteristics that may predict cancer risk. There is certainly a lot of work ahead.

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## Getting More (Phospholipids) Out of Milk

- **Milk is rich in phospholipids, phosphate-containing fats that are used to build cell membranes.**
- **The types of fats found in milk tend to vary with the size of milk fat globules, which varies over the course of lactation.**
- **While fats have a major role in fuelling a growing baby mammal, they may have other roles in facilitating healthy development.**
- **Over time, the variation in milk fats with lactational stage could be put to use by the food and medical industries to create products tailored to particular health problems.**

In recent years, each solid fraction of milk has been revealed to contain functionally relevant complexity that had previously gone unappreciated. The protein portion of breast milk, for example, is broken down by milk enzymes into many smaller peptides, of which at least [41 fight bacteria](#). The oligosaccharide portion has a [long list of roles](#), from nourishing 'good' gut bacteria to encouraging proper immune system development. And now there is also some evidence that different fats appear in milk in different proportions at different times in a young mammal's life and in patterns that may help the young mammal to grow healthily. Moreover, researchers are asking whether the fats in question also influence the health of older humans, a line of investigation that could one day lead to fat-specific dairy products for the purpose of addressing certain health issues.

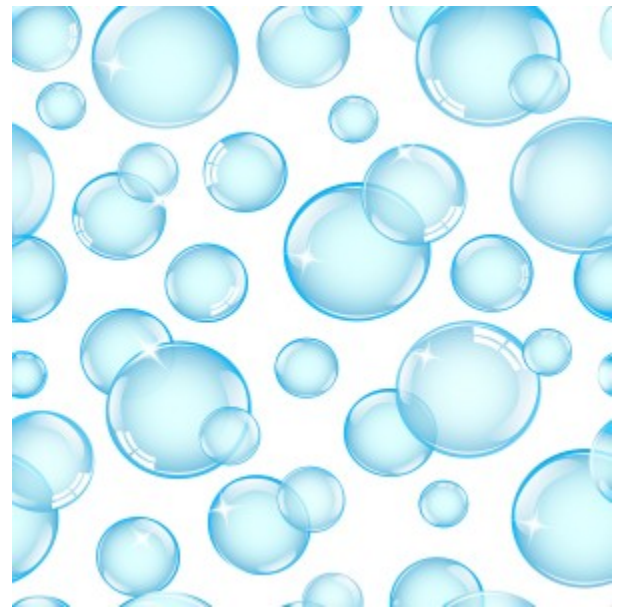
Much of the work in this field is recent. One of the main tasks is to build a really detailed picture of the kinds of fats that appear in milk, such as phospholipids. Looking at breast milk from women with four-month-old infants, Francesca Giuffrida and colleagues recently employed liquid chromatography combined with an evaporative light scattering detector to determine the amounts and types of phospholipids present. Of the various phospholipid classes, sphingomyelin occurred at the highest concentrations. The researchers also calculated how much a typical, exclusively breast fed four-month-old consumes: 140 mg of phospholipids per day.

This number confirms that babies get about half of their daily calories from fat. But the idea that the fats in milk are purely there to be metabolized seems improbable given what research has turned up about the many unforeseen roles of milk proteins and carbohydrates.

Nurit Argov-Argaman, a professor and lipid metabolism expert at the Hebrew University of Jerusalem in Israel, has published several papers on lipids in cow milk. She has found that globules get smaller on average as lactation progresses and larger volumes of milk are produced. This happens until about six months in, when the globule size starts to rise again and milk production falls. And smaller average globule size tends to mean a higher proportion of saturated fatty acids. In milk produced two months after giving birth, the small fat globules in cow's milk have 10% more saturated fatty acids than the larger fat globules do.

Argov-Argaman thinks the answer to the question of why the fats in milk vary over time lies partly in the practical functioning of the mammary gland. "The reason for the largest globules [appearing] at the very beginning of lactation is probably attributed to the fact that the mammary epithelial cells acquire fat synthesis capacity days and even weeks before parturition...and only very close to parturition acquire the secretion capacity," she says. However, the needs of the infant are probably also relevant. "But let's not forget that the changes in milk fat globule mean diameter occur simultaneously with developmental changes in newborn physiology. The same can be said for different fatty acids, of which some induce hepatic metabolism by increasing beta oxidation and energy production while others induce triglyceride secretion into the blood stream for utilization by peripheral tissues like adipose." To really understand what might be going on, more data on the correlation between newborn developmental stages and milk fat compositions is needed.

That will take a while. Still, the knowledge that smaller globules means more saturated fat, and that globule size changes over the course of lactation, could be useful for the food industry. Furthermore, the proportion of phospholipids in milk could prove



Like soap bubbles, milk fat globules are produced in different sizes.

particularly interesting. Since phospholipids compose fat globule membranes, the smaller the average globule (in other words, the higher its surface area to volume ratio), the greater the proportion of phospholipids.

Some researchers are already probing how that knowledge might be made practically useful. Andrew Scholey of Swinburne University of Technology, Melbourne, and his colleagues are investigating whether a concentrate of phospholipid-rich milk protein might help elderly people struggling with age-associated memory impairment. They are running a trial where over 55s will either receive a supplement of phospholipids from milk or one of two placebos. Their hopes are founded on various findings that have linked phospholipids to cognitive performance. One phospholipid, phosphatidylcholine, has for example been demonstrated to enhance the activities of membrane-bound enzymes involved in signal transduction. Phosphatidylcholine also restores age-related reductions in nerve cells called choline-acetyltransferase-positive neurons.

If this trial and others like it in the future prove successful, elderly people everywhere and the dairy industry will have much to celebrate. But even if it doesn't, there is a sense that the field of milk lipids is encouraging a shift in perspective. "We just need to realize that each individual cow produces milk which is different from the average milk, according to her metabolic state... lactation stage, genetics and probably many other facts that influence milk fat globule diameter and hence milk fat composition," says Argov-Argaman. "If we look at the cow in a slightly different manner, we will be able to produce milk which can be customized better to the public needs."

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