SPLASH!® milk science update
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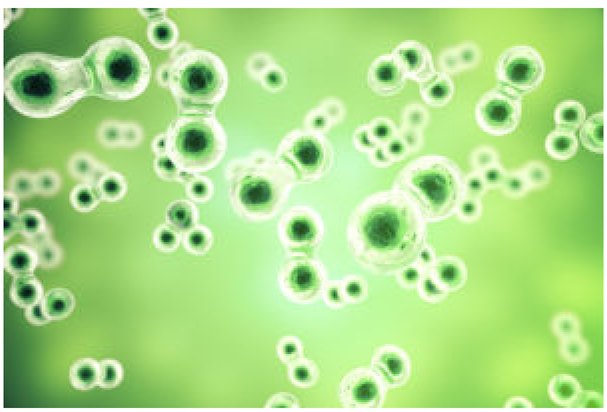
INTERNATIONAL MILK GENOMICS CONSORTIUM

This month's issue features breastmilk stem cells, spider milk, and ancient dairy pastoralism.

Even to the Brain: Yes, Breastmilk Stem Cells Do Transfer to Organs of Offspring

- **Mother's milk contains stem cells, which are able to cross the gut and migrate into the blood of the nursed offspring.**
- **From the blood, they travel to various organs including the brain, where they turn into functioning cells.**
- **This breastmilk stem cell transfer from mother to offspring appears to be more than just a random event, potentially contributing important developmental attributes.**
- **Future research must concentrate on how this phenomenon can be salvaged medically, for example to help preterm infants survive and develop optimally.**

What would you think if you were told that your baby contains part of your body? Literally. Or that you actually have inside you part of your baby's body? Yes, literally. As much as it sounds more of an emotional expression of love between the mother and her baby, some of the cells that contain all our genetic information (not half) are indeed exchanged between the mother and her baby, remaining alive and active in each other's bodies for at least…decades. And although this reciprocally occurs during pregnancy via the placenta, it also continues to a large extent during breastfeeding. After all, as Barinaga beautifully exclaimed in 2002, mother's love is enduring [1].



It's one thing when a scientist provides the first evidence on a phenomenon, and quite a fascination when someone else backs it up independently. Previous research is confirmed and strengthened. Those precious, and until recently, unknown little gems of breastmilk, its stem cells, have been further studied for their migration and potential functions in the nursed offspring. And, the outcome? Yes, they do somehow make it through the delicate gastrointestinal tract into the blood of the young; and from there, to various organs, including the brain! Let's follow their journey together.

I haven't written for *SPLASH!* in a while now. What broke my silence was one of the most promising pieces of research I have come across in the lactation field. It's about those neglected cells for which so little is known, yet they keep fascinating us: the breastmilk stem cells.

Aydin and colleagues [2] of Istanbul Medipol University, Turkey, took the courageous decision to dig further into a field that has left the scientific community with feelings of awe and excitement, but also a number of questions. Their study, published in *Scientific Reports* in September 2018, independently repeated previous mouse milk transfer studies, confirming the migration and homing of stem cells from mother's milk into the brain of suckling pups [2,3]. And when these cells get there, they do something amazing…they integrate in the brain, turning into what is needed most there, functioning neurons and glia [2]!

Cells had been known to be a part of mother's milk for a few decades, but the fact that amongst them there would also be stem cells was unheard of; until some revolutionary reports were published between 2007 and 2012 by members of Professor Peter Hartmann's group at the University of Western Australia, of which I also had the privilege to be part. First, Dr. Mark Cregan of this group then reported the presence of progenitor cells in breastmilk [4], those cells that are a bit more flexible in terms of their ability to turn into various cell types than your usual skin cell or heart cell, for example.

Inspired by Cregan's work, in a turning point in the field a few years later, we found that breastmilk actually contains stem cells, which are not only undifferentiated (i.e., not committed to turn into any specific cell type), but also are capable of becoming any cell of the human body [5]. And this could happen spontaneously to a small extent in the culture dish, being further reinforced when these cells were exposed to the right chemical microenvironment [5].

By definition, this is a property of those stem cells that are found in the very early stages of an embryo. But it looks like similar properties are shared by an elite group of cells in breastmilk [5-8]. These cells not only behave similarly to embryonic stem cells in terms of their abilities to turn into any cell of the body, but they also express the same proteins that are known to be specific to embryonic stem cells such as OCT4, SOX2, NANOG, TRA-1-60, and others [5].

These findings were reported in the journal *Stem Cells* in 2012 [5]. Shortly after, we embarked into a journey to examine the fate of these cells in the offspring. Why would any such special cell be in breastmilk to start with? Aside from the fact that we showed that these cells were hiding inside the lactating breast [5-8], everyone's burning question was why are they there, in the milk?

Using a TdTomato mouse model, we were able to demonstrate for the first time that milk stem cells as well as immune cells survive the neonatal gut, migrate into the blood, and from there travel and integrate into various organs of the suckling pups, including the thymus, liver, pancreas, spleen, kidneys and the brain [3]. There, they actually seem to turn into specialized cells of each specific organ [3].

The TdTomato mouse model was based on mouse mothers that ubiquitously expressed this red fluorescent gene in every cell of their body, including in their milk cells. These mothers fed pups that did not express it at all. So, any red fluorescing cell in the pups' body had to come from the milk [3,7,8].

The new study by Aydin and colleagues used the same principal but in a different mouse model, in which mouse mothers ubiquitously expressed the green fluorescent protein GFP, whereas the pups they fostered did not express it at all [2]. Their study now confirms and strengthens our previous findings, demonstrating that milk stem cells indeed survive inside the young's gastrointestinal tract, and from there they are transferred into the blood and the brain of the young. There, they are coaxed by specific brain microenvironmental cues to become specialized brain cells of two types: neuronal and glial, the two main brain cell types.

What makes this finding all the more exciting is the presence of the blood brain barrier. We all have it. Its purpose is to allow selective trafficking to and from the brain, for the obvious need to protect this important organ. Very few cells are capable of passing through it. However, in the neonate this barrier is leaky, allowing more trafficking than the normal we see in adults [9,10]. And it looks like the milk stem cells are of the lucky few that can make it through!

This phenomenon of transfer and integration of foreign cells into an organism is called microchimerism, and is more common than we think. In fact, it has been previously demonstrated to occur reciprocally between the mother and the embryo during pregnancy, with embryonic cells found alive and integrated within the mother's brain and other organs many years after the birth of her child [11,12].

In turn, maternal microchimerism, the transfer of maternal cells to the offspring, can happen not only *in utero* but also during lactation [11-15]. This had been previously shown for either immune-like cells of milk or indeterminate cells of milk [13-15]. Now, for the first time two independent groups have shown this for stem cells of breastmilk in two mouse models [2,3,7,8]. What is fascinating is that all evidence thus far supports the notion that these stem cells become active and functioning parts of the body of the young [2,3,16]!

And of course, in addition to stem cells, the breastfed infant receives immune cells from mother's milk [6,8]. Both these two milk cell types have been shown to home also in the infant thymus, amongst other organs [3,8]. The thymus is responsible for the maturation of our immune cells. Because of this, it is thought that through their homing inside the thymus, the maternally-derived milk cells facilitate both the cellular tolerance between the mother and the infant, and the maturation of the infant's immune system [7,8,17].

The above, together with evidence that chimeric cells persist in the offspring long term [16] suggest that maternal microchimerism in the offspring is not a random event, but rather, a well-designed and specifically orchestrated integral characteristic of breastfeeding, aimed at boosting and multilaterally supporting the optimal development of the infant, and at protecting the infant against infectious diseases [7,8].

Indeed, it has been long known that breastfeeding offers immune protection to the infant. What was less known is that this protection is likely facilitated not only by immunoprotective molecules of breastmilk (such as immunoglobulins, cytokines, etc.) but also by immune cells and stem cells of breastmilk [8]!

Now, I must raise the question what happens to infants who are not breastfed or who are fed breastmilk deprived in live cell properties (such as frozen or refrigerated breastmilk)? What do these infants miss at? What are the long-term consequences for an infant who did not receive fresh mother's own milk? And does prolonged breastfeeding offer additional cellular benefits compared with shorter breastfeeding periods?

And of course, whilst the plot thickens even further when wet nursing comes into play, the main issue in my mind concentrates on what we could do better for preterm infants in the neonatal intensive care unit (NICU). Is the frozen mother's own milk or the pasteurized donor milk they are usually given ideal for them? Cells do not survive in breastmilk after freezing, refrigeration for a few hours, or pasteurization [8]. Therefore, we currently choose to deprive these vulnerable infants of breastmilk stem cells and immune cells, components that can potentially offer them a multitude of benefits, both developmentally and for their very own survival [8].

Moles and colleagues very rightly called breastmilk the postnatal maternal blood, through which a multitude of active soluble and cellular factors are delivered to the offspring, in continuation from the gestational period [17]. Do we place the right emphasis on the medical importance of this phenomenon? Clearly, more basic and applied research is urgently needed in this field to shed light into all these questions and open the possibility for enhanced survival potential for our most vulnerable. An exciting piece of work on this subject will be discussed soon in *SPLASH!* Tune in to find out more.

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A Jumping Spider That Provides Milk and Maternal Care

- **Females of a species of jumping spider have been reported to produce a milk-like substance from the part of the body from which eggs emerge.**
- **This substance contains some fat, and is particularly high in protein.**
- **Through a series of experiments, researchers have shown that the secretion is essential to young spiderling survival, and that maternal care matters in this sense.**

High school science classes the world over teach that mammals are unique because females furnish their young with milk. The reality is somewhat more nuanced and depends on your definition of milk. Indeed, there are various specialized food sources that a few non-mammals provide their offspring, among these the antibody-containing epidermal mucus found in some fish and trophic eggs in amphibians (as well as other classes of animal). In a recent issue of the journal *Science*, a group of researchers associated mainly with the Chinese Academy of Sciences report on the curious life of an ant-mimicking jumping spider, *Toxeus magnus*. They find that females of this species produce protein-rich droplets for their offspring to consume that are essential for the youngest spiders' survival [1]. Curiously, female offspring continue to drink this milk even after they have become sexually mature.

Jumping Spider. Image by CHEN Zhanqi

Jumping spiders make up about 13% of all spiders. As their name suggests, they jump to pounce upon their prey and to escape predators. Because there are so many species, various specialists have commented that other closely related species might also demonstrate the kind of maternal care behavior recently discovered in *T. magnus.* This care consists not merely of providing a nutritive liquid, but also of extensive cleaning and repairing of the nest, which is thought to keep the spiderling's parasite loads as bay.

The milk that *T. magnus* produces dribbles out of the mother's epigastric furrow, the part of her body from which her eggs emerge. The paper's authors call the substance "milk" on the basis of two facts. First, its fatty and protein-rich composition: it contains 2.0 mg/ml sugar, 5.3 mg/ml fat, and a whopping 123.9 mg/ml protein—which is quadruple the concentration of protein found in cow's milk. More detailed information about the types of sugars, fats, and proteins is not reported in the *Science* paper. The second reason for thinking of this substance as milk-like is its apparently central role in the extended spiderling-mother relationship.

Experiments reported in the paper show that very young spiders are entirely dependent on the substance for their survival. The authors demonstrated this by gumming up the mother's epigastric furrow with correction fluid (which they separately checked to ensure did not in itself affect spiderling survival). Blocking the source of milk in this way led to the death of all hatchling spiders at less than 10-and-a-half days old.

When the supply was blocked later in development, when spiderlings were 20 to 40 days old, for example, survival was affected, but spiderling body weight was not. The reason was that by that age the spiderlings were able to leave the nest to forage—an inherently dangerous activity—and make up the shortfall in their energy intake. These spiderlings still tried to suckle from their mother's epigastric flow. The role of maternal care was laid plain by another manipulation: the 20–40-day-old spiderlings without milk and with their mother in the nest were significantly more likely to survive than those of the same age for which the mother was removed, presumably because the latter grew in unkept nests and were consequently more burdened by parasites. At 60 days of age, *T. magnus* becomes sexually mature. At that point, the experiments showed that some female offspring remain in the nest and may continue to consume mother's milk. (Males, however, were kept out, perhaps to avoid inbreeding.)

There are other non-mammals that are said to produce a sort of milk. More milk-like than epidermal mucus and trophic eggs is the crop milk produced by both male and female pigeons [2] and the brood-sac secretions of the cockroach, *Diploptera punctata* [3]. The authors speculate that *T. magnus*'s epigastric furrow secretions may have evolved from trophic eggs, under the strong selection pressures of high predation risk and uncertain access to food. That story of origins, related to the question of whether this kind of maternal care is found in other species of *Toxeus* and evolutionary proximate genera, is just one area primed for new research. Rarely does a paper about spiders shake zoology so intensely.

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Dairy Pastoralism in Mongolia Began at Least 3,300 Years Ago

- **Remains of milk proteins have been discovered on teeth of individuals from Late Bronze Age burial mounds in northern Mongolia.**
- **Dairy pastoralism was practiced in Mongolia at least 3,300 years ago.**
- **Dairy pastoralism was transferred to an ancient Mongolian population by cultural exchange from its neighbor and not by population replacement.**

People have been migrating since the dawn of human existence. It's in our nature to survive and that drove generations of ancient humans to walk to nearly all corners of the world. The history of human migration is inscribed in detail within the DNA code of modern-day people. It is a fantastic book to read, full of drama and intrigue. One chapter contains descriptions of an ancient population migration into Europe that resulted in major cultural changes. Scientists recently concluded that the Eurasian steppe was the ancient cradle for today's European populations and it was also one of the primary origins of dairy pastoralism [1-5]. How and why ancient Eurasian populations migrated into Europe are being revealed by scientists using new technologies that trace massive ancient population migrations, changes in diets, and the movement of dairy pastoralism beginning about 4,500 years ago. The scientists along the way have answered one of the most debated questions of history. How did new ideas, especially knowledge of dairying, spread in ancient populations? Was it due to population migration and then replacement of indigenous populations or the adoption of new ideas taken from neighbors? The answer is both, but in different places.

Ancient Dairy Goes International

Huge is the only word to describe the Eurasian steppe, which is a vast low rainfall grassland on rolling plains connecting present-day Hungary with Manchuria in north-eastern China. The steppe is swept by weather extremes. To the north, the steppe is bounded by winter's cold and to the south lie semi-arid deserts. The steppe is divided into two massive regions by the Altai mountains lying at the junction of five modern-day countries; Russia, Kazakhstan, Mongolia, and China. The western steppe is about 4,000 km west to east and about 300–1,000 km north to south. Big! The eastern steppe is a mere 2,500 km west to east and 600–800 km north to south, but it includes all of Mongolia. The eastern steppe is higher, colder and more barren than the western steppe. About 7,000 years ago, nomadic human populations on the Eurasian steppe first began to herd sheep, goats, cows, yaks, and horses [6, 7]. This was the beginnings of subsistence dairy pastoralism. For reasons still not understood, about 4,500 years ago the western steppe herders (WSH) started to migrate westwards toward central Asia and northern Europe, taking their dairy animals with them [2-5]. The journey was slow and took many generations. Scientists conducting genetic analyses of samples from WSH graves concluded that during this massive migration, the WSH largely replaced indigenous populations and spread WSH culture into the heartlands of Europe. It was a profound and far-reaching transformation. Indeed, Haak and colleagues [3] concluded that the precursors of many modern Indo-European languages have their origins in this tidal wave of human migration. Today, the genetic footprints of the WSH remain in many northern European populations [2]. Several groups of scientists attributed the success of the WSH migration to mobile dairy pastoralism, which gave the WSH a distinct advantage over indigenous populations and eventually led to the latter's replacement [1, 7, 8].

While the westward migration of the WSH into central Asia and northern Europe is well documented [1-6, 8], much less is known about the origins of the eastern steppe populations, the timing and extent of eastern expansion of the WSH, and the development of dairy pastoralism in Mongolia, where it is still a dominant feature of today's population. Recently, Jeong and 25 colleagues from five countries published a scientific paper [1] describing ancient human population movements and "…*the rise of dairy pastoralism on the eastern Eurasian steppe*." The research was published in the prestigious scientific journal Proceedings of the National Academy of Science (USA). Their study generated a few surprises leading to a new concept about how societies change. The investigators provided evidence that cultural transfer between populations rather than population replacement by migration can be a powerful means of societal evolution. Their example was the adoption of dairy pastoralism by an early Bronze Age population living in what is now northern Mongolia.

Huge Advantages of Dairy Pastoralism for Ancient Humans

One of the first steps at the dawn of agriculture was the herding of ruminant animals, which were used as a reliable and mobile food source [7]. Many areas of the world, including most of the Eurasian steppes, were unsuitable for growing crops to provide food for human populations. However, ruminant animals were used by ancient populations to efficiently convert low-quality pasture into energy-dense meat and milk for human consumption. Ancient nomadic human populations quickly learned that the biological equation for human benefit from consuming milk or foods derived from milk over the lifetime of an animal far outweighed the human benefit from the slaughter of the animal solely for meat [9]. Thus, dairy pastoralism generated big nutritional advantages for ancient human populations at a time when acquiring adequate food was an unrelenting daily challenge. The dairy animals additionally provided clothing, ultimately meat, and tools derived from horn and bone [6-8]. Nomadic ancient humans also efficiently adapted to changing weather and seasonal variations by moving their animals to fresh pastures. They could use different animal types to suit the climate and vegetation, while the number of animals was efficiently aligned with the size of the human population it supported. Thus, dairy pastoralism was efficient, highly mobile, and it had enormous benefits to ancient human populations, especially migratory populations [1, 3, 7].

Dairy Pastoralism in Mongolia Was Adopted from Neighbors

Jeong and colleagues [1] focused their scientific attention on the origins of dairy pastoralism in the eastern Eurasian steppes, i.e., present-day Mongolia. The investigators used biological samples from individuals present in 22 Late Bronze Age burial mounds at six sites in northern Mongolia (the Deer Stone-Khirigsuur Complex; DSKC). The sites are characterized by engraved upright stones, stone mounds, and the absence of pottery [1, 10]. Scientists who excavated related ancient sites found the bones of sheep, goats, cattle, and horses, but it was unclear whether these animals were used for milk production [1, 10]. Jeong and colleagues dated the human individuals present in the DSKC mounds by radiocarbon analysis to between 3,000–3,300 years ago. The investigators initially used variations in DNA sequence to genetically profile the individuals. This powerful technique allowed the identification of related groups of people, their likely ancestral populations, and thereby helped map ancient human migrations across the continental landscape and over long periods of time. Unexpectedly, the investigators discovered that the 22 DSKC individuals (except for one person) were genetically distinct from the neighboring WSH. Jeong and colleagues concluded that the DSKC individuals were descended from an early Bronze Age hunter-gatherer population located immediately to the north of the Mongolian burial mounds, i.e., in the Lake Baikal region of southern Siberia. This is the largest fresh water lake (by volume) in the world and presumably, it was a natural ancestral home for the hunter-gatherer population.

Jeong and colleagues next turned their attention to the teeth of nine individuals from the DSKC burial mounds [1]. The investigators explained that dental calculus or tartar, which is a form of hardened plaque on teeth, contains traces of what is eaten by a human. They predicted that if the DSKC population was practicing dairy pastoralism then there should be detectable milk proteins on the teeth of people from the Mongolian burial mounds. The investigators used a highly sensitive and precise technology, mass spectrometry, for analying the tartar. They discovered fragments of two milk proteins, casein and lactoglobulin, on the teeth from seven of nine of the individuals. Additionally, the investigators determined that these milk proteins came from several ruminant species, including sheep, goat, and cow. They concluded that these results provided direct evidence that the DSKC population practiced dairy pastoralism. There was also good evidence that the WSH adopted dairying much earlier than the DSKC population [7]. This information and the absence of WSH genetics in the DSKC population strongly suggested to the investigators that DSKC dairy pastoralism was culturally adopted from the WSH. Jeong and colleagues' conclusion sharply contrasted with what happened when the WSH migrated into northern Europe, where there was a wholesale population replacement that caused a major cultural transition and the introduction of dairying into Europe.

The investigators also highlighted another intriguing feature of the WSH and DSKC populations—some WSH acquired a new genetic mutation, lactase persistence, about 7,500 years ago [11, 12]. The WSH then carried the mutation during their massive western migration into Europe starting about 4,500 years ago [9, 11]. Jeong and colleagues explain that the mutation enabled human adults to drink milk and efficiently metabolize milk lactose to generate energy. People who did not carry the mutation were unable to efficiently extract this energy because they were intolerant to lactose, which made them avoid milk [9, 13]. The inference from multiple scientific groups is that the mutation gave the migrating WSH a big advantage over western indigenous populations with the result that the WSH prospered, reproduced more efficiently, and more rapidly spread their genetics, thereby eventually replacing the original populations [9, 11, 13]. Strikingly, the mutation was absent in all 22 of the DSKC individuals. Clearly, dairy pastoralism was advantageous to the DSKC population over thousands of years even though they did not carry the lactase persistence mutation. Perhaps the DSKC population developed food preparation processes that reduced the level of lactose in milk so that they could maximally benefit from its many additional nutritional advantages such as high levels of protein, fat, and calcium.

Implications

Dairy pastoralism was strongly advantageous to ancient human populations [7, 8]. Jeong and colleagues concluded that this type of food production system was culturally transferred between populations on the Eastern Eurasian steppe and not by migration and population replacement. In contrast, dairy pastoralism was introduced into northern Europe and central Asia by the replacement of the indigenous populations by migrating WSH [9, 11, 13]. The success of the WSH migration westwards into Europe may have been greatly strengthened by presence of the lactase persistence mutation in their genome. Thus, ancient cultural practices and ideas were transferred between populations using different mechanisms in different places. The recent and surprising identification of cheese in the 3,200-year-old Egyptian tomb of Ptahmes [14] suggests that the extent of cultural transmission of dairy pastoralism between populations may have been underestimated. Cheese production greatly reduces lactose content and allows consumption of dairy products even when adult populations do not carry the lactase persistence mutation. Dairy products, like cheese, are an example of know-how and ideas that were transferred between populations just as fast or even faster than ancient population migrations. The Egyptian cheese also contained bacteria that cause a highly contagious disease in humans and livestock, brucellosis. Cultural transfers between populations may have passed on more than just ideas.

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Infants Gain More Weight When Bottle Fed Mom's Milk

- **Rapid weight gain during infancy is linked to obesity in childhood and adulthood.**
- **A new study from 2,500 Canadian mother-infant pairs reports that breastfeeding was inversely associated with body mass index, weight gain velocity, and risk of overweight at 12 months of age.**
- **Infants that received some breast milk from the bottle gained more weight than infants fed directly at the breast but were still leaner than those fed formula.**
- **Although direct breastfeeding had optimal growth outcomes, pumping is an integral part of breastfeeding for the majority of women and allows them to continue breastfeeding rather than switch to formula.**

If you want to understand how an infant's diet influences its health, you might ask, "What did the infant eat?" But the results of a new study [1] on infant diet and weight gain suggest that this simple question is no longer sufficient; in addition to asking what, we need to be asking how, and for how long. Newly published results from over 2,500 mother-infant pairs demonstrate that the longer a mother is able to *directly* provide breast milk, the more closely the infant's rate of weight gain over the first 12 months of life matches the World Health Organization's (WHO) standards [1]. Considering the prevalence of pumping among many breastfeeding mothers, these novel findings shouldn't be reported without consideration of the many positive outcomes associated with feeding expressed breast milk.

Baby Fat

During the first year of an infant's life they produce a lot of data. Length, weight, and head circumference are measured in the U.S. at least every three months, along with other important developmental milestones such as rolling over, following an object with the eyes, and controlling the head. Rolling over ahead of schedule may be celebrated by parents and their physician as a sign of good coordination and muscle strength in an infant, but getting ahead of the growth charts can be cause for concern. Rapid weight gain during infancy is an established risk factor for obesity later in life; infants that gain weight too quickly and end up with excess body weight at the end of infancy are more likely to carry that weight with them into childhood and adulthood [1].

What an infant eats obviously plays a role in how quickly it gains weight, and breastfeeding has been associated with a reduced risk for developing obesity in childhood and later in life [1, 2]. However, despite being used by the WHO as the feeding standard for optimal growth outcomes [3], the mechanism connecting breastfeeding to obesity is still unclear [1, 2].

"The link between breastfeeding and lower obesity risk might be because of differences in bioactives altering metabolic development in bottle-fed babies, leading them to grow faster, or store more fat" explains Dr. Melanie A. Martin, an Assistant Professor of Anthropology at the University of Washington who studies biocultural influences on growth, development, and reproduction but was not involved with this study. "But a second possibility, supported by a good amount of research, is that infants don't self-regulate as well when they are nursing from a bottle. Caregiver feeding can also play a role in this, in that adults may be conditioned to giving infants a set amount of liquid and thinking they need to finish it rather than looking for infant cues of satiety."

It is important to notice that Martin distinguishes between breastfeeding and bottle-feeding, rather than breast milk and formula. Bottles are not simply for formula anymore. “Studies consistently show that pumping is part of breastfeeding for American mothers, but few researchers are actually studying women’s experiences with pumping,” says human biologist Dr. EA Quinn, an Associate Professor of Anthropology at Washington University in St. Louis who studies human milk composition and breastfeeding, but was not involved with this study. Considering the prevalence of pumping, it may seem surprising that studies examining infant diet and weight gain rarely distinguish between direct breastfeeding (at the breast) and bottled breast milk feeding.

WHO Knew

A new study by Azad et al. [1] uniquely considered feeding methods in their examination of breastfeeding, infant weight gain, and body composition during the first 12 months of life. Mother-infant dyads (n = 2,553) were recruited as part of the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort, which collected data on infant body size (weight and length), infant diet (breastfeeding initiation and cessation, feeding of expressed breast milk, use of formula, and introduction of solid foods), and other factors known to influence infant body size (e.g., infant sex, birth weight, maternal age, maternal diet quality, maternal body size, and ethnicity).

Their primary outcome measure was body mass index (BMI) Z score, a known predictor of childhood obesity [1]. BMI Z-scores compare an infant’s weight, adjusted for their age and sex, relative to a reference population; scores can be positive (higher BMI than the reference population), negative (lower BMI than the reference population), and zero (BMI is the same as the mean for the reference population).

Across the entire CHILD birth cohort, breastfeeding was inversely associated with BMI, weight gain velocity, and risk of overweight at 12 months of age [1]. However, the longer an infant was directly breastfed, the closer their BMI Z-score matched WHO infant growth standards (i.e., the closer it was to zero).

Infants that followed the WHO’s recommendation of at least 6 months of exclusive breastfeeding had a mean BMI Z-score of −0.4. Over half (54.6% to be exact) of the infants in the group of exclusively breastfed at 6 months occasionally received some expressed breast milk, and when analyzed separately, their BMI Z-scores were significantly higher (mean BMI Z-score of +0.14) than those that only nursed directly (−0.02) [1]. Importantly, they were still leaner than infants that were only partially breastfed (breast milk and formula, (+0.28) or not breastfed at all (+0.45).

Rather than thinking of breast milk vs. formula, these findings highlight the need to consider the heterogeneity that exists in infant feeding strategies. But when reporting these results, we must also be mindful of *why* there is variation in infant feeding strategies.

“We need to collectively recognize that every mom and baby are different, have different needs and challenges, and have different pathways to achieving what is optimal for them,” says Martin. “Some mothers are always going to have working conflicts or other obstacles that don’t align with optimal practice. So we need to dually emphasize practical solutions and realities.”

The Message to Mothers

In order to offer practical solutions, we first need to identify the problem. Why would pumped breast milk alter infant BMI and rate of weight gain? “Very, very few studies have looked at the effects of long-term storage under home conditions on human milk,” explains Quinn. “My guess, however, would be that the modification of hormones or other bioactives by freezing is not the concern.”

Instead, both Quinn and Martin believe the issue is the bottle rather than the composition of the breast milk in the bottle. And this is good news, because this can be modified. For example, when infants were fed from bottles that were weighted and opaque, caregivers fed less milk than when using clear plastic bottles [4]. Presumably, they stopped feeding based on infant cues of satiety as opposed to how much milk they could see or feel was left in the bottle. Although it is not identical to infant self-regulation at the breast, these types of changes in bottle-feeding behavior may prevent overfeeding, which in turn may play a role in programming satiety [1].

When working on messaging to mothers, it is also important to look at the positive outcomes associated with feeding expressed breast milk. The WHO advocates for exclusive breastfeeding for 6 months and continued breastfeeding alongside solid foods for 12−24 months. Pumping may actually help more women meet these optimal infant-feeding standards.

“I think research, public messaging, and conversations can better support the idea that bottle-feeding can help moms achieve prolonged breastfeeding,” says Martin. Quinn agrees. “Pumping allows women to keep breastfeeding and providing their infants with human milk after they return to work when otherwise they would have to use formula. And it should be celebrated for that.”

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