



SPLASH! milk science update SEPTEMBER 2013 issue

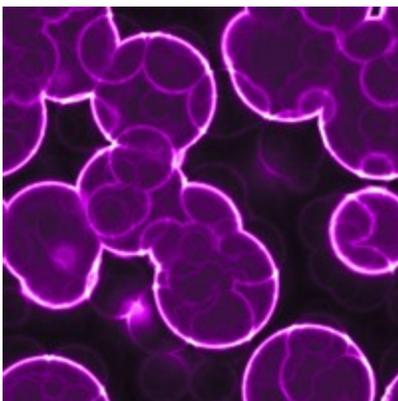
This month's issue covers a breakthrough study on the [expression of milk-making genes in humans](#), the [genetic selection of the best dairy cows](#), an archeological discovery of [ancient cheesemaking in Europe](#), and future opportunities in [milk metabolomics](#).

Enjoy!

Visions of Human Milk Production

- **Study reveals which genes are expressed to make colostrum, and transitional and mature milk.**
- **Surprisingly, genes that produce ferritin are highly expressed during production of colostrum.**
- **Disruptions in insulin signaling may be a physiological cause of insufficient milk supply.**

One cell, all by itself, can make milk. A single cell makes a very tiny amount, however, while more of them working together make a copious supply (or so we hope). That some mothers are not able to make enough milk is exactly what motivated scientists at Cincinnati Children's Hospital Medical Center and University of California, Davis, to determine what genes are turned "on" to make milk.¹



If a tree falls in a forest and nobody is there to hear it, does it still make a sound? Human cells have been making milk for as long as there have been humans, but scientists are just now developing techniques to take a snapshot of which genes are turned "on" in those cells while they make milk. In the late 1980s, Patton and Huston discovered that some globules of milk fat carry tiny samples of the cell that produced them.² When these tiny samples are captured and extracted, it is possible to "see" what genes are being expressed. We (Lemay at UC Davis and Nommsen-Rivers at Cincinnati Children's) combined this technique with next generation sequencing technologies to take snapshots of gene expression in milk-making cells.¹

We captured such snapshots while mothers produced colostrum (the very first milk after birth), transitional milk (not really colostrum, not yet mature), and mature milk. Traditionally, milk was usually classified by day of lactation (day postpartum), but one of the interesting findings of this study was that on day two, some mothers were still producing colostrum, while others were already producing transitional milk. The groups of genes turned "on" or "off" were distinctly different between colostrum, transitional, and mature milk stages.

It turns out that milk-making cells do a lot of what we might expect. A few proteins are copiously abundant in milk and the genes turned "on" are those needed to transcribe, translate, and package those milk proteins for delivery outside the cell. During the colostrum phase, immune-related genes are uniquely expressed. This makes sense given that colostrum is the very first food to which a new baby's gut is exposed.

Surprisingly, two of the most abundantly expressed genes during colostrum production were those for producing ferritin, a molecule involved in iron transport. Ferritin has a lot of slots to hold iron, but the ferritin that is in colostrum does not hold much iron,³ so we know it is not there to deliver iron to the baby, nor does iron absorption seem to be a vital function in the first days of life. So why is ferritin so highly expressed?

Usable iron is a scarce resource among life forms. We all need it to survive and we go to battle over it. Bacteria steal it from each other, animals sequester it from bacteria, and plants suck it out of the soil. And although we need it, it is not without harmful effects. Iron can cause oxidative damage to our cells. So perhaps a mother passes ferritin to her baby via colostrum so that it has a way to store iron safely and to sequester it from pathogens. It's also possible this colostrum ferritin has nothing to do with iron and everything to do with the sugars that hang off of it, like the [lactoferrin story](#).

Perhaps the most important snapshots captured during this study were those of “insulin signaling,” observed as cells transitioned from making colostrum to mature milk. Insulin is the hormone that tells cells, “Hey, fuel is present! Use it!” In older mothers, cells are either not getting enough of this signal (not enough insulin) or they are ignoring it (insulin: oh, that old thing again). As more women delay child-bearing and more women get diabetes at a younger age, more and more mothers are having trouble making enough milk. Disruptions in insulin signaling may well be the cause. Look for future work by Nommsen-Rivers et al. to lead the way.

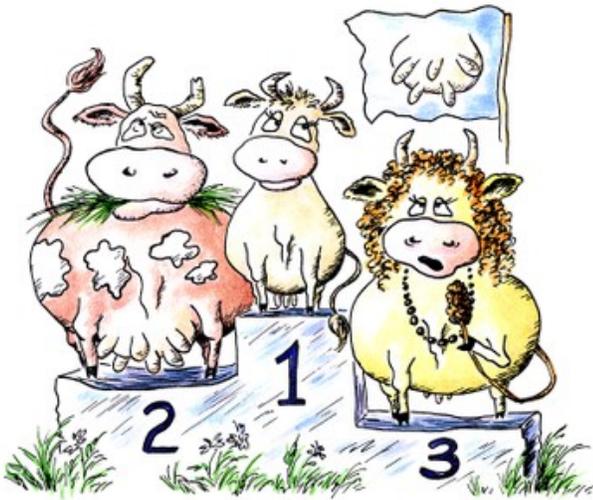
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What Makes a Good Dairy Cow?

- Dairy cattle have been selected for thousands of years
- The selection process is written in their DNA
- A small number of DNA regions have tell-tale signs in Brown Swiss cattle
- These signs provide hints about the genes that make a good dairy cow



Cattle have been domesticated since the Neolithic Age, but did Stone Age farmers select their cattle? And what about the thousands of years between first domestication and the modern era, how much selection has taken place? Animal geneticists have been interested in estimating the impact of selection on cattle for many years, and since the cattle genome sequencing project was completed, they have developed new and powerful tools that have heightened this interest. In a newly published study, Utsunomiya and colleagues (1) brought together cattle genetic data from around the world to reveal those regions of the genome that light up with the hallmarks of selection.

The process of domestication probably took place more than 10,000 years ago from a founding population of fewer than 100 cattle in modern-day Turkey. We recognize two major cattle sub-species, *Bos taurus* and *Bos indicus*,

suggesting that cattle were domesticated in two geographical regions. We know that cattle were then adapted for different uses, including as a source of meat and milk, and for pulling wagons and plows, and we can imagine that this gave rise to early breeding practices with some form of farmer-assisted selection. Fast forward to the modern era; breed formation has favored a few breeds of dairy cow that have been progressively selected for being the best performers, especially over the past 50 years. This process of selective breeding is written into the DNA of dairy cows.

Darwinian selection, or “survival of the fittest,” is of course a process that occurs in nature, and is the driving force of evolution. Selective breeding is similar to natural selection in genetic terms, but accelerates the process on the basis of productivity gains for dairy farmers. Just as natural selection leaves its mark on a species, so does selection for breeding purposes. In the case of dairy, intensive selection for milk traits, e.g. milk production or milk composition, means that dairy cattle have been through genetic bottlenecks to improve production. The study by Utsunomiya et al. (1) set out to look for areas of the genome that carry the tell-tale signs of selection, referred to as a “selective sweep,” by comparing dairy breeds with beef breeds, and including *Bos taurus* and *Bos indicus* breeds.

How to find a selective sweep?

The project to sequence the entire genome of cattle began in 2003–2004, and the first draft of the sequence was published in 2009. This project provided the tools to study cattle genetics in great detail and also dramatically reduced the cost. As a consequence, there are now thousands of individual cattle that have been genetically scanned, providing a wealth of data to look at the impact of selection on dairy cows (3-4).

Utsunomiya et al. (1) collated data from genome scans in a total of 855 animals. They whittled this number down to 136 animals from 4 breeds to balance the breed representation and remove closely related animals. These included Swiss Brown dairy cattle, a breed favored in Europe that originated in Switzerland up to 8,000 years ago; Angus cattle, a beef breed developed in Europe; Gyr cattle, a breed that originated in Northern India, and is used for dairy and beef production; and Nelore cattle, which are an indicus beef breed that are popular in South America. There are about 3 billion letters in the DNA of dairy cows, but these letters can be divided into genetic islands. This is how Utsunomiya and his colleagues (1) approached the problem, they divided the DNA for each animal into 500,000 pieces—still a large number! Then by comparing the DNA of all cattle in one analysis, they found those pieces that were similar within each breed and those that were common among breeds.

Their tests revealed 153 regions in Angus, 212 in Brown Swiss, 3 in Gyr, and 13 in Nelore that had the hallmarks of selection. They looked more closely at the genes in these regions, and what they found in Brown Swiss dairy cattle were genes associated with fertility and one particular enzyme. The fertility genes are linked to pregnancy and lactation hormones, so that makes sense, but what about the enzyme? The gene is called calpain, and it turns out that it may be one of the factors that can determine whether milk production is turned off by the mammary gland.

Could these regions be what distinguish a good dairy cow from a beef breed? Quite likely, but there were surprisingly few regions identified, and perhaps other breeds could add to this tally. The good news is that there is still enough diversity within the cattle genome to continue to improve production by selection (5-6).

When it comes to finding a perfect dairy cow, about 30-40% is determined by the genes passed down from its ancestors. Although genetics can be optimized by selective breeding, the rest of production improvement is up to the farmer.

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Europe's First Cheese Makers

- Dairy farming began in Central Europe approximately 7500 years ago
- Chemical analyses of fatty acid residues preserved in ancient ceramics revealed their use in milk processing, most likely to separate the solid fatty curds from liquid whey during cheese production.
- Cheese is lower in lactose than milk and can usually be digested without side effects by people who are lactose intolerant
- The ability to process milk into foods like cheese would have enabled the establishment of dairy culture in a lactose intolerant population

Today, nearly one-third of the world's adults are lactose tolerant. If you are a part of this group, you most likely can trace your ancestry to a population with a long history of dairy agriculture, such as that in Scandinavia where nearly 100% of the people carry a gene that maintains the manufacturing of lactase (the enzyme that breaks down lactose) into adulthood. This lactase persistence gene (LP) was probably quite rare, however, when humans first began to practice dairy agriculture ca. 10,000 years ago. In order to take advantage of this novel and nutritious food source, Neolithic farmers would have been hard-pressed to come up with a way to reduce the lactose content in milk. One clever way to do this is by allowing bacteria to digest the milk sugar for you, which also just happens to be a critical first step in the production of cheese. But just how ancient is the art of cheese making?



Alas, cheese does not preserve in the archaeological record, but ceramic materials used in cheese production do. A new paper by Salque et al. (2013) reports on the earliest “cheese strainers” identified to date. At 7500 years old, these ceramic fragments from Poland are nearly as old as the first evidence for dairy agriculture in this region, suggesting that humans wasted no time in figuring out the recipe for cheese.

Ancient cheesecloth?

The first Neolithic populations known to use domesticated animals and cultivated plants in central Europe are associated with the central European Linear Pottery, or *Linearbandkeramik* culture. Artifacts associated with this culture appeared in Central Europe between 7500 and 8000 years ago, including the site of Kuyavia, Poland. In addition to pottery vessels believed to have been used for cooking and food preparation, this site yielded numerous fragments of perforated bowls, similar in appearance to a colander or sieve.

When archaeologists first unearthed these ceramic fragments in the 1970s, there were several hypotheses about their function, ranging from flame covers to honey strainers. However, the abundance of domesticated cattle remains at the site, combined with the similarity in structure between these vessels and historic ceramic cheese-strainers, was highly suggestive of their role in cheese making (Salque et al., 2013). Specifically, researchers believed that these bowls were used to separate the solid cheese curds from the liquid whey, an integral step in the cheese making process.

Luckily, methods now exist to directly test the function of pottery through organic residue analysis. Because pottery is porous, fats from foods cooked or stored in the pots are absorbed and trapped inside the pores. Chemical analyses of these ancient fatty residues tell researchers the types of foods that came in contact with the vessels, allowing a test of the cheese-strainer hypothesis.

Hidden fats

Salque and her team (Salque et al., 2013) removed the mysterious perforated fragments, along with some other proposed cooking vessels, from storage and subjected them to three methods of chemical analysis. First, they determined the types of fatty acids present in the ceramics and found most fats were saturated with either 16 or 18 carbons (palmitic and stearic acid, respectively). This information alone is not sufficient to determine the pot's function, as both body fat and dairy fat contain these types of saturated fatty acids. Therefore, they performed two additional tests that would allow them to identify the specific isotopic structure of the fats (i.e., the number of neutrons in the carbons that make up the individual fatty acid chains). Fats derived from body tissue have an isotopic signature distinct from fats derived from milk, allowing researchers to distinguish between ceramic vessels used for cooking or preparing animal fats and those used exclusively for dairy processing (Evershed et al., 2008).

All of the perforated fragments but one contained fatty acids that exhibited the isotopic signature associated with dairy fat. In contrast, the ceramics believed to be cooking vessels had a completely different signature that was within the range predicted for fats derived from adipose tissue. Their use in cooking was also confirmed by data that suggested the fats within the pots had been heated to a very high temperature.

Nearly 40 years after these ceramics were excavated, Salque's research group demonstrated that the perforated vessels had a specialized function related to processing dairy products. And because butter and yogurt do not require a sieve-like tool in their production, it seems most likely that these perforated bowls were used to make cheese, collect the curds, and filter out the whey.

Now that's what I call aged cheese

These prehistoric cheese strainers date from between 5400 and 4800 BC, making the practice of cheese making, at least in Northern Europe, nearly 7500 years old. Cheese making may be an even older practice, however. Milk residues have been found on pottery vessels dated to 8500 years ago from sites in Southeastern Europe, Turkey, and the Middle East, using methods identical to those employed by Evershed et al. (2008).

Unlike the sieve-like containers found in Kuyavia, Poland, the ceramics found in Southeastern Europe, Turkey, and the Middle East lacked any telltale signs of cheese manufacturing. Regardless, the researchers know the containers must have been used in milk processing because fat from raw milk does not preserve in ceramics. As was true at Kuyavia, there appeared to be no lag in time between the appearance of dairy culture and the practice of milk processing, suggesting that these Neolithic peoples quickly figured out methods for reducing the lactose content of milk in order to take advantage of this important food source.

Don't underestimate the importance of cheese

The LP gene is believed to have co-evolved with dairy agriculture, such that as dietary emphasis on dairy increased, so too did the selective pressure favoring the presence of LP. With its lowered lactose concentration (anywhere from 0 to 2.5%, compared with nearly 5% in fresh milk), cheese and other processed milk products such as yogurt would have permitted the consumption of calorie-rich dairy foods without the adverse side effects of lactose intolerance (e.g., diarrhea, gas). That is, no biological adaptation would have been required for the adoption of dairy culture as milk processing was quickly implemented. Indeed, many populations, such as Greeks and Southern Italians, that focus more on cheese and yogurt than on fresh milk have very low frequencies of the LP gene.

Cheese also has another big advantage over raw milk in that it can be stored for a much longer period of time. In the Neolithic, storage was key—should crops or animal herds have let you down, it would have been comforting to know you had pounds and pounds of cheese in your storage shed to get you through the rough patches.

A cheesy ending

The next time you bite into a creamy Havarti or a stinky Limburger cheese, take a moment to think about the impact that cheese production may have had on dairying culture in Europe and the Middle East. Had the populations of these areas not stumbled onto methods for processing milk into cheese, those lactose intolerant farmers may have moved to other food products and abandoned their emphasis on dairy. Doesn't this make you love cheese just a little bit more?

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Great Expectations for Milk Metabolomics

- **Metabolomics, the youngster of the “omics” family, offers new opportunities for the dairy industry.**
- **The milk metabolome is a snapshot of the metabolism of the cow and provides new predictors for a cow’s health.**
- **Measuring metabolites in milk might help to produce better cheeses and milk rich in oligosaccharides.**
- **Integrating different “omics” techniques will have a synergetic effect, independent of the original application field.**

A quick internet search with the words “genomics” retrieves about 14 million hits, whereas the same search with the word “metabolomics” retrieves a mere 1.1 million hits. This alone is a fair indication that within the “omics” family there are different generations, and metabolomics is one of its youngest members.

Metabolomics defined

Metabolites are small molecules with functions as diverse as signaling, inhibition, and defense. Metabolites can be intermediates or products of synthesis or degradation processes and pathways. A typical example of degradation involving metabolites is drug metabolism; any compounds originating from the initial active compound of a drug is a drug metabolite. On the other hand, metabolites are also amino acids, organic acids, sugars, etc., all of which originate from metabolic processes allowing the degradation of a drug. In other words, metabolites are ubiquitous compounds involved in every process in the body or organism.

The word “metabolism” originates from the Greek word Μεταβολισμος, meaning “change.” It is the dynamics of metabolites, either intermediate or final products of metabolism, that scientists measure. All metabolites—the metabolome—can be measured both in tissues and in biofluids involved in providing and removing metabolites. Examples of biofluids used in metabolomics include saliva, blood, and urine, and also milk.



The catalyst of metabolomics

Identification of metabolites in readily available biofluids is a long-standing practice. In ancient China, sweet-tasting urine was recognized as an indicator of a disease, later recognized as diabetes. Nowadays, cutting edge techniques allow the simultaneous measurement of approximately 200 metabolites, each assigned to a different class. With these resources, metabolomics is attempting to unravel different metabolic pathways as soon as possible, aiming at answering different questions.

Probably due to economic availability, the metabolomics concept and techniques have initially been applied in the field of cancer, where it has provided great help in identifying biomarkers of specific carcinogenic pathways. These have been pinned down and integrated into diagnosis and therapeutic approaches. If these biomarkers for carcinogenicity or therapeutics could be analyzed by a faster, more accessible and affordable technique, this could mean a significant improvement in health care.

Milk, the white gold of metabolomics

So why not adopt the same line of thought to dairy? Milk is a readily available fluid and can be considered to mirror the health status of the cow and milk synthesis process. Therefore, metabolites could be used as biomarkers to better understand both the cow’s health and milk production.

In one line of research, milk metabolites are used to predict economically important milk traits. Poor coagulation properties of milk result in low cheese yields and low-quality cheeses. Looking at particular metabolites, the work of Suskindle et al. (2011) has correlated a higher concentration of citrate, a molecule that binds calcium, with poorer coagulation properties of milk, possibly by disrupting the casein micelle, a protein-structure paramount in cheese making. The same work has correlated the well-studied choline and carnitine with milk coagulation properties.

In another line of research, milk metabolites are used to assess animal health. Metabolic profiles of milk correlate organic acids hippurate and lactate with subclinical mastitis—infection of the udder. Whereas lactate is probably produced by bacteria presence in milk, the presence of hippurate is likely associated with the protective capability of certain organic acids against bacterial growth. Importantly, there is a need to identify biomarkers predictive of metabolic status and health. Klein et al. (2012) identified in healthy cows that the ratio of glycerophosphocholine to phosphocholine can be used to predict ketosis development. This is a major advance since current methods can only identify ketosis during the acute stage.

Metabolomics gets a little help from the other members of the “omic” family

If a metabolic approach is so promising, what are the possibilities if its information is joined with that gathered from other “omic” techniques? Recently, the work of Lu et al. (2013) showed that integrating metabolomics and proteomics provided a better understanding of the mammary physiology of cows in negative energy balance (NEB). In dairy cows, after calving, feed intake cannot support energy needed for body maintenance and sudden requirements for milk production. Cows enter and remain in NEB for several weeks, a status that is often associated with several metabolic diseases. When feed intake can again fulfill energy requirements, cows are at a positive energy balance (PEB). Proteomics and metabolomics results indicated glucose-1-phosphate and stomatin, a protein present in the milk fat globule membrane, as possible biomarkers for NEB. Importantly, it was the synergetic collaboration between these techniques that suggested the integrity and organizational properties of the epithelial cells of cows in NEB and PEB are different.

Further, metabolomics can be integrated with genomics and used in the identification of specific traits of animals. The work of Wittenburg et al. (2013) combined the milk metabolome with genetic information, and although several milk metabolites may not be suited to breeding strategies, some have significant genetic variability. By using metabolite concentration as a selection factor, breeding strategies may be used to improve animal robustness to specific disorders.

Future perspectives

While genomics and transcriptomics identify genes and pathways that are important in specific processes, proteomics incorporates the knowledge of function and structure of proteins. Understanding milk synthesis and its relation to animal health (metabolic status) is an intricate and dynamic concept. Metabolomics can be the missing link.

If it is possible to identify specific milk components that are important to human (or cow) health and to better understand how and why they are present in milk, it may be possible to apply specific management or breeding strategies to dairy farming. However, it is imperative that different “omics” techniques be incorporated to allow a greater profit not only for the dairy industry, but also for the improvement of both cow and human nutrition and health. For example, bovine milk oligosaccharides (BMO) resemble human milk oligosaccharides and share similar beneficial properties. Animal management and breeding strategies may favor the presence of important metabolites paramount in the production of BMO. These findings could aid in prioritizing, selecting, and isolating BMO to add to infant formula aiming at mimicking the benefits of human breast milk in infants' gut flora. Expect applications of milk metabolomics to continue to grow.

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