THE RHEOLOGICAL BEHAVIOR OF RAW HUMAN MILK

by

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*This thesis is dedicated to*
*my mother, who gave me the best*
*start in life by breastfeeding me*
*and my father, who supported me*
*even when my dreams did not*
*match his dreams for me.*

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Milk is a biological fluid produced in the mammary glands of all mammals during and after pregnancy. Like other biological fluids, the transport of milk in the ductal system of the breast is essential to good health during lactation. Content of mammalian milk is species-specific with variations throughout lactation and between individuals. Unlike other biological fluids found in humans, the rheological properties of human milk have not been comprehensively studied.

The present study reviews previous work done on the rheology of milk from various mammals, including humans, defines the flow conditions found within the human breast, examines the content of human milk as it relates to the flow properties, and presents experimental work performed on raw human milk. The results of the experimental work demonstrate that raw human milk is a time-dependent shear-thinning non-Newtonian fluid with gel-like behavior at rest. The findings of this study indicate the need for further research which is currently underway.

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# CHAPTER 1

# INTRODUCTION AND MOTIVATION

The transport of bio-fluids is necessary for life. The flow behavior of these bio-fluids is related to various diseases in the human body; so understanding the flow characteristics of bio-fluids is crucial to advancing science, in general, and particularly bio-engineering. Rheology is the study of the deformation and flow behavior of materials. Fluids are generally characterized by their viscous behavior and solids for their elastic behavior. However, most fluids and solids exhibit characteristics of both viscous and elastic behavior. Additionally, the viscous behavior of fluids may vary depending on the temperature, shear stress (non-Newtonian), and length of time the fluid has experienced shear stress (thixotropic or rheopectic). The rheology of some bio-fluids, like blood, is subject to all these factors [1]. These studies help understand the origin and treatment of diseases [2] and develop various bio-medical devices [3].

Human milk is a unique bio-fluid in that its flow is essential for two separate living beings, mother and infant. Milk is a complex fluid suspension produced by diffusion of blood components through alveoli (mammary glands) in the breast [4]. The milk is then transported through a branching ductal system in the breast [5] into the oral cavity and gastric system of the infant. The life-long health and economic benefits of breastfeeding to both infants and mothers have been well documented [6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16]. Health benefits increase in response to longer durations of breastfeeding [13, 14].

Despite the numerous benefits to breastfeeding, challenges exist that may cause a mother to stop breastfeeding. Approximately 60% of mothers stop breastfeeding earlier than they desire [17]. Among the common reasons reported for early cessation of breastfeeding included overfull or engorged breasts, breast infection, perceived insufficient milk production,

and difficulty initiating milk flow. It is important to note that successfully establishing an adequate milk volume requires effective milk removal during the first few days after birth [18] while maintaining an adequate milk supply is based on demand; removing more milk results in producing more milk [19]. All of the previously mentioned reasons women gave for early cessation of breastfeeding involve effective milk flow within the breast. Both engorgement and breast infections are forms of inflammation with an element of milk stasis [20]. Milk stasis is a predisposing factor for breast inflammation that can be caused by oversupply of milk, blocked duct, insufficient drainage of the breast, scheduled or missed feedings, and external pressure on the breast. Regardless of etiology, the treatment for all forms of breast inflammation during lactation involves reversal of milk stasis, maintaining supply, and continued breastfeeding. This treatment requires frequent and regular drainage of the breast. A study of the rheology of raw human milk will facilitate understanding of milk stasis and develop ways to improve milk flow within the breast.

The flow of milk is also important for infants. Premature infants face many challenges when it comes to nutrition intake. The simple process of coordinating suck-swallow-breath pattern can be impaired and may require milk to be thickened or fed via gastric tubes [21]. Thickening of human milk is difficult to standardize because the rheological properties of human milk are not well understood. A major problem with tube feeding is the significant loss of protein (up to 10%) and fat (over 50%) [22]. A better understanding of the rheological properties of human milk will greatly improve the methods of delivering milk to premature and sick infants.

Mammalian milk is species-specific [6]. While considerable work on bovine milk has been conducted, this work does not adequately model human milk flow behavior [23]. Despite the importance of human milk to health, its rheological properties are poorly understood and have not been comprehensively studied. A survey of literature demonstrates this lack and will be presented in Chapter 2.

# CHAPTER 2

# PREVIOUS RESEARCH ON MILK RHEOLOGY

## 2.1 Bovine and Other Dairy Animal Studies

The rheological study of milk from dairy animals has been investigated for over a century, primarily with focus on processed milk and other dairy products [24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37]. The majority of work treated milk as a Newtonian fluid. However, early in the twentieth century – when pasteurization and homogenization were still relatively new – research found significant variations in milk viscosity when flowing through capillary tubes while applying pressure [24]. The authors of that study noted the lack of information provided by previous researchers and concluded that it was impossible to compare the work of one experimenter with another because "one does not know whether or not their results are independent of the dimensions and peculiarities of the apparatus they used".

The viscosity of bovine milk has been related to pressure, temperature, pH, fat content, protein content, concentration, pasteurization, and homogenization [24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34]. Similar research has been conducted on the milk of other mammals frequently used in the dairy industry – such as goat, sheep, and camel – although not to the same extent as bovine [35, 36, 37]. The rheological properties of milk in the dairy industry are used in a variety of manners beyond simple pumping and transport modeling [25, 26, 27, 28, 31, 32].

With the advent of pasteurization and homogenization in the dairy industry, the majority of research on raw bovine milk viscosity occurred in the early twentieth century. Due to the variety of test conditions and methods utilized by past researchers, Bateman and Sharp [24] performed a systematic study with a Bingham viscometer of the effect of varying pressure on milk viscosity and what changes in viscosity of milk were due to changes in the fat verses

changes in the skim-milk phase. They demonstrated that raw milk, both skim and whole, is a shear-thinning non-Newtonian fluid. The variability of the milk behavior was greater at low pressures. When the milk was run repeatedly through the capillary tube at a constant pressure, the skim milk viscosity remained constant while the whole milk viscosity decreased with repeated runs. The decrease in viscosity with repeated runs was even more evident as the age of the milk increased. Homogenized whole milk demonstrated no change with repeated runs similar to skim milk. The authors attributed the decrease in viscosity in raw whole milk from repeated runs through a capillary to clumps of fat globules breaking up. The effect of aging on viscosity of raw skim milk was investigated since previous studies found aging to increase the viscosity of raw whole milk. Refrigeration of skim milk increased the milk viscosity but freezing showed an initial drop in viscosity that increased with longer freeze times. Bateman and Sharp concluded that bovine milk viscosity was dependent on pressure (shearing force), age, method of storage, and, for raw whole milk, mechanical agitation. All viscosity values were determined at 25°C, which is below the melting temperature of bovine milk fat [38].

As the dairy industry grew, the need to model the relationship between viscosity and factors such as content and temperature led to the development of a series of regression equations. The usefulness of each equation depends on the application for which it was developed. A number of these regression equations are shown in Table 2.1.

Snoeren et al. found a relationship between the voluminosity of bovine milk proteins and the dynamic viscosity of commercial skim milk [28]. They showed that viscosity of heat-treated skim milk is a function of the volume fractions of casein, native whey protein, denatured whey protein, and the viscosity of the medium. Based on Bateman and Sharp's research on raw bovine milk [24], Snoeren et al. should have considered the shear rates used in testing due to the presence of native whey protein – which is found in raw bovine milk – yet no mention of shear rate is provided. Additionally, no data concerning the temperature

Table 2.1. Regression Equations for Bovine Viscosity and Density

| **Author** | **Regression Equation** |
|---|---|
| Snoeren et al. [28] | $$\mu = \mu_{ref}[1 + \frac{1.25(\phi_c + \phi_{nw} + \phi_{dw})}{1 - \frac{\phi_c + \phi_{nw} + \phi_{dw}}{\phi_{max}}}]^2$$ $\mu_{ref} : viscosity\ of\ medium\ (cP)$<br>$\phi_c : volume\ fractions\ of\ casein$<br>$\phi_{nw} : volume\ fractions\ of\ native\ whey\ protein$<br>$\phi_{dw} : volume\ fractions\ of\ denatured\ whey\ protein$<br>$\phi_{max} : maximum\ volume\ fractions\ of\ all\ protein$ |
| Jebson and Chen [29] | $$ln\mu = 3.911 + 0.0202(\frac{S - 482.5}{0.85}) - 0.1291(\frac{T - 52.5}{7.5})$$ $S : solids\ content\ (g/kg)$<br>$T : temperature\ (K)$ |
| Phipps [31] | $$log_{10}\mu = [1.2876 + 11.07 \times 10^{-4} T_C][F + F^{\frac{5}{3}}] + \frac{0.7687 \times 10^3}{T_K} - 2.4370$$ $F : fat\ content\ (\%)$<br>$T_C : temperature\ (^oC)$<br>$T_K : temperature\ (K)$ |
| Bakshi and Smith [30] | $$ln\mu = -8.9 + 0.1F + \frac{2721.5}{T}$$ $$\rho = 0.3T - 0.03T^2 - 0.7F + 1034.5$$ $F : fat\ content\ (\%)$<br>$T : temperature\ (K)$ |

of the milk used by Snoeren et al. was provided, even though studies show that temperature affects the viscosity of skim milk [32].

The affect of temperature and fat content on milk viscosity was investigated by Jebson and Chen [29]. They noted that whole milk has a higher solid content and higher viscosity in comparison to skim milk. In their research on the evaporation of bovine whole milk, for concentrates of solids content $> 450\frac{g}{kg}$, they adapted a relation for viscosity as a function of temperature and concentrate total solid. Similarly, Phipps [31] examined the relationship between viscosity, bovine cream fat content up to 50%, and temperature variations of 40 $^oC$ to 80 $^oC$. He determined a regression equation for viscosity for creams with fat content, $F < 0.4$, as a function of temperature and fat content. Neither research considered shear rate in their experiments or equations.

More recent work with raw bovine milk was completed by Bakshi and Smith [30]. They performed several experiments on bovine milk to find a regression equation for the experimental value of viscosity based on the variable parameters of fat content and temperature. They noted that homogenized milk has a higher viscosity than raw milk which they attributed to the fine, dispersed state of the fat when homogenized. The temperature range tested was 0 $^oC$ to 30 $^oC$ and fat content range was 0.1% to 30%. At 30 $^oC$ Bakshi and Smith found the viscosities of skim milk and whole milk to be about the same, approximately 1.25 mPa·s. At lower temperatures the effect of fat percentage on viscosity is greater. Similarly, they found a relationship between density, temperature, and fat content. All experimental work was performed at temperatures when milk fats are solid [38]. No regression equations were determined for raw milk nor was shear rate disclosed.

The aforementioned research involving the development of regression equations for bovine milk viscosity lacks sufficient disclosure of test conditions and methods to allow for adequate comparisons of results. Despite the known influence of shear rate on viscosity, modern researchers fail to consider shear rate in the development of their regression equations. However, the basic rheological behavior of bovine milk in regards to temperature appears to be

consistent; milk viscosity decreases as temperature increases. This decrease is dependent on whether or not the proteins have begun to denature [28]. Additionally, the concentration of proteins and fats affect viscosity. Are these findings similar for human milk?

## 2.2 Human Milk Studies

Rheological studies on raw human milk are very limited. Waller et al. looked at the decreases in kinematic viscosity of prenatal secretions and milk during the first 10 days of lactation, when the composition of milk changes the most [39]. They tested the samples at 37 °C when milk fat is liquid. A significant drop in kinematic viscosity was observed during the first 10 days postpartum that corresponded to the drop in total nitrogen content. A linear relationship between the log of kinematic viscosity (centistokes), $\nu$, and total nitrogen content (g/100ml), $c$, was expressed in Equation 2.1.

$$\log\ \nu = 0.65c - 0.07 \tag{2.1}$$

Further investigation examined the relationship between two protein nitrogens – casein and globulin (a subcomponent of whey) – which undergoes a significant change during the first 14 days of lactation before becoming almost constant. This change in ratio between whey protein and casein during early lactation was confirmed in 1992 by Kunz and Lönnerdal [40].

The work done by Waller et al. [39] is difficult to compare with other researchers. Some samples were previously refrigerated while others were evaluated immediately. The time between collection and testing varied. Also, density differences were not discussed. The testing procedure used by Waller et al. is described by Blair [41]. He noted the average kinematic viscosity of normal milk (mature milk) was 1.3 centistokes with a gradual fall in viscosity with rising shear rates. In his work, he discounted differences in sample densities and neglected to disclose the shear rate used to determine the average kinematic viscosity.

The viscosity of human milk has been examined in a few additional studies with no mention of shear rate used [21, 41, 42, 43, 44, 45]. Almeida et al. tested human milk viscosity to determine if any changes occurred as it aged at body temperature [21]. They used a Q 280 Ford Viscometer cup and determined viscosity by the flow time of the milk from gravitational force through the viscometer. They also used standards for Newtonian fluids and determined that no significant changes occurred when previously frozen human milk was reheated and then kept at 37 °C over 9 hours. One study looked at the effect of temperature on defatted human milk and demonstrated a decrease in viscosity as temperature increased [42]. No shear rate was provided for testing the defatted human milk, even though bovine skim-milk viscosity is known to be non-Newtonian [24]. Another recent study of human breast milk viscosity by Fondaco et al. used previously refrigerated milk [45]. They tested the milk from 0.5 $s^{-1}$ to 500 $s^{-1}$ at 37 °C and noted non-Newtonian fluid behavior but do not show the data. They reported no changes in viscosity at an arbitrarily chosen 20 $s^{-1}$ in relation to a drop in pH from 6.5 to 4.0. The density of the samples was not disclosed. Based on the lack of information provided by researchers in the past, the viscosity of human milk in each study cannot be subjected to a quantitative comparison with other published works nor can it be used to model flow behavior in the breast.

# CHAPTER 3

# FACTORS IMPACTING HUMAN MILK RHEOLOGY

## 3.1 Relationship between Milk Content and Viscosity

The internal structure of a fluid defines its flow behavior. This section will provide a brief overview of the internal structure of mammalian milk as it pertains to its influence on rheology. As seen in Sections 2.1 and 2.2, the rheological behavior of milk has been linked to different content normally found within milk. The major components used to characterize the rheology are proteins and lipids. While milk contains over 100 individual components, the majority of the components can be grouped into four main categories: salts (or minerals), lactose (or sugars), proteins, and lipids (or fats)[1]. Since mammalian milk is species-specific [6], the concentration and chemical structures of these components vary depending on the species. Thus researchers must consider the milk content of the species of interest before determining if research on another species can be utilized.

The concentration of content of human milk varies greatly from other mammals. A comparison of some major components of mature milk content for humans, goats, and cows can be seen in Figure 3.1. Variations in content for human milk vary over 24 hours and over the entire course of lactation [47]. These differences in concentration are important because the concentration of certain salts and lactose affect the molecular size or volume fractions of proteins and fats [25, 48]. Solutions of salts and lactose are Newtonian while the proteins

[1]For the purpose of simplicity, the terms provided in parenthesis will be used interchangeably throughout this document unless otherwise specified. It is important to note that by definition the terms may not be 100% equivalent on a chemical level. For example, lactose is the main sugar found in milk but other sugars can be found in small percentages [46].

Table 3.1. Mature Milk Content for Different Mammals

| **Component** (gm/100 ml) [38] | **Human** | **Goat** | **Cow** |
|---|---|---|---|
| Fat | 3.8 | 4.1 | 3.7 |
| Lactose | 7.0 | 4.7 | 4.8 |
| Protein | 1.2 | 3.3 | 3.3 |
| Calcium | 33 | 130 | 125 |
| Chlorine | 43 | 159 | 103 |
| Magnesium | 4 | 16 | 12 |
| Phosphorus | 15 | 106 | 96 |
| Potassium | 55 | 181 | 138 |
| Sodium | 15 | 41 | 58 |
| Sulfur | 14 | 16 | 30 |

and fats are the components associated with the non-Newtonian behavior of milk. Hence the impact of these intercomponent interactions are important.

In addition to differences in concentration of content, the chemical structure of components of milk differs between species. Human milk proteins can be separated into three major components - casein, whey, and mucins [49]. Mucins contribute a small fraction of total protein content and are associated with the fat globules. The ratio of casein to whey varies throughout lactation and are the components generally used in viscosity equations for skim or defatted milk [28]. Although three forms of casein have been detected in human milk, the $\beta$-casein is the major constituent [49] whereas the major casein constituent in bovine milk is $\alpha_{s1}$ [50]. The structure of human $\beta$-casein micelles is highly porous with a mean Gaussian diameter of 100 nm, which is smaller than bovine micelles [51]. In animal studies, the size of caseins varied with the concentration of cations, like calcium [52]. Since human $\beta$-casein is associated with the solubility of calcium and may facilitate the absorption of other cations [49, 53], the concentration of cations in human milk is likely to affect the voluminosity of $\beta$-casein that will then affect the viscosity of the milk. Similarly, human whey proteins bind different minerals [49]. For example, human lactoferrin is a major whey protein that

becomes more compact with iron binding [54]. The full impact of mineral concentration on whey structures and sizes is unknown.

The effect of salts and lactose on fat globule size is unclear. As previously noted, mucins – a protein component in milk – form a membrane around fat globules called the milk lipid globule membrane (MLGM) [49]. Since other protein voluminosities are affected by concentrations of milk components, the size of the fat globule membrane is likely also affected but to what extent is unknown. Structural differences between bovine and human MLGM exist [55] that may make models developed from bovine studies incompatible for use with human milk.

Fat globules, whose sizes vary greatly, form an oil-in-water emulsion [56]. In raw milk, the fat globules are not uniform in size which accounts for a lower viscosity than homogenized milk [30]. In the human body, these globules are liquid droplets that are deformable. Large deformation testing breaks down the structure of fat globules whereas small deformation techniques can define a material's response to stress while keeping the structure intact [57]. Within the breast, milk that is expressed at the beginning of a feed contains less fat than milk expressed at the end of a feed. The cause of this phenomena is unclear. One theory was that the milk fat globules at the end of a feed were larger than at the beginning, but research has shown that no significant differences exist between globule sizes based on time expressed [58]. However, milk flow coats the walls of tubes used in medical delivery and result in significant losses of fat [22]. An unanswered question is whether or not coating occurs within the breast ductal system and may result in uneven fat distribution reports. The shear stresses experienced within the breast ductal system are extreme (see Section 3.2), which suggests that milk fat globules may experience a breakdown in structure in the smaller ducts and coat the ducts. Then as milk continues to flow over the coated ducts, the coating fats are released and complete their journey through the remaining ducts resulting in an overall higher fat content within the milk expressed at the end of a feed. To determine if this theory is valid, the effect of shearing on fat globules must be investigated in the future.

### 3.2 Fluid-Duct Wall Interaction Dynamics

The flow of milk through the human breast duct is periodic and driven by the mechanical forces exerted by infant suckling. The breast duct can be treated as a porous medium [59]. The breast duct geometry is comprised of a branching structure of microfluidic ducts with an average of 25 bifurcations that originates in the alveoli and terminates in the nipple which contains approximately $5 - 9$ circular duct openings. Using a idealized mathematical modeling, the duct diameter varies from 6 $\mu$m to 2 mm with an overall length of 38 mm [5]. The milk fat globule average diameter size ranges from 4 $\mu$m in advanced lactation to 8.9 $\mu$m in colostrum [60]. The effect of this flow geometry and periodic nature of the flow on the milk is undefined.

The wall shear rate experienced by a Newtonian fluid through a circular duct can be modeled by Equation 3.1 as given by Son [61].

$$\dot{\gamma_a} = 4Q/\pi r^3 \tag{3.1}$$

While calculating wall shear rate for non-Newtonian fluids is not as straightforward, this equation coupled with the mathematical modeling by Mortazavi et al [5] provides a rough estimate of the wall shear rates possible in the breast ductal system.

### 3.3 Pressure Forces during Breastfeeding

Infant sucking involves four stages that alters the shape of the breast and creates a moving boundary layer [62]. Infants apply two types of pressure to the areola and nipple of the breast – negative intra-oral vacuum pressure by sucking and positive oral peripheral pressure by deforming and compressing the nipple. The intra-oral vacuum has been studied along with ultrasound imaging that demonstrates the relationship between milk expression, intra-oral vacuum, and breast movement – particularly nipple deformation and compression [62]. The positive oral peripheral pressure has been neglected and not studied prior to this work.

Since the positive oral pressure is vital to modeling the moving boundary layer and responsible for compression of the ducts located in the nipple-areola complex, preliminary work was completed that captured these forces in conjunction with the negative vacuum. Data was captured (as per IRB 16-41) at the Hartmann Human Lactation Research Group in Western Australia as part of the NSF EAPSI[2] Award #1614350. A total of 12 mother-infant dyads participated. Intra-oral vacuum and ultrasound imaging was captured as outlined by Geddes et al. [62]. The positive oral pressure was captured using Tekscan pressure sensors 9801-5 and 9830-10. Figure 3.1 shows the results of one dyad.

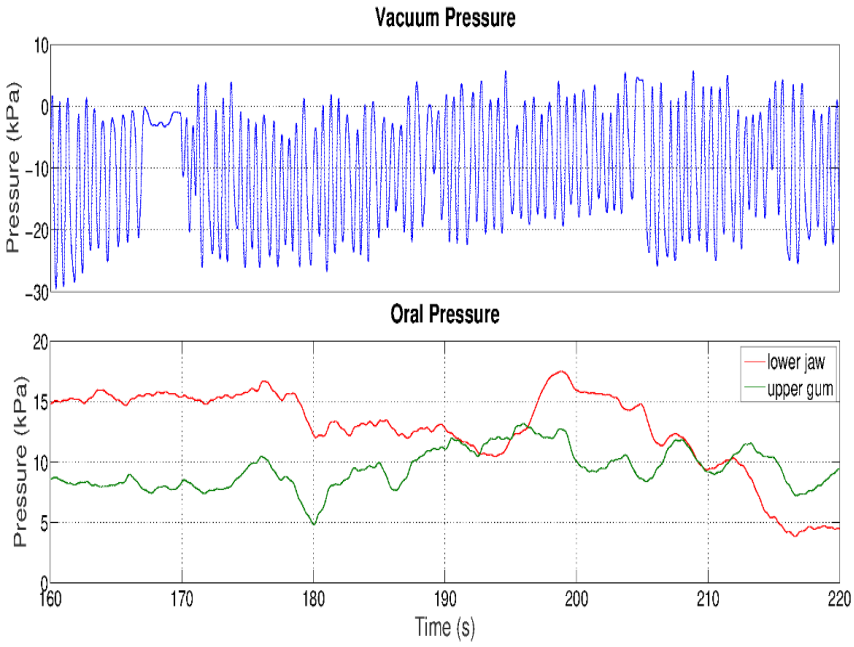


Figure 3.1. Comparison of Infant Applied Pressures

While the data is still being processed and further work is planned, the preliminary results demonstrate that the positive pressure values are not insignificant. As regards the viscosity of human milk within the breast, the positive pressure causes deformation of the ducts within the nipple-areola complex. The change in dimension of the ducts will change the shearing stresses experienced by the milk during ejection.

[2]East Asia and Pacific Summer Institutes for U.S. Graduate Students

# CHAPTER 4

# EXPERIMENTAL SETUP AND PROCEDURE

A portion of this chapter was adapted from the author's conference paper [23].

## 4.1 Participant Recruitment and Sample Collection

Human breast milk was expressed (as per IRB Protocol 15-10) on the same day as the initial experiment. Six participants were initially recruited and up to 30 ml of milk from each breast was saved for testing. Details are shown in Table 4.1. The entire volume and mass of each expression was used to calculate initial density. The initial pH was measured on 10 of the 12 collected samples. A portion of milk was frozen for later chemical analysis (results not discussed in this paper). The remaining milk was separated into 2 amber glass containers, shown in Figure 4.1, to minimize UV exposure. One container was stored at 37 °C and the other refrigerated at 4 °C until tested.

Figure 4.1. Samples Stored in Amber Bottles

Two additional participants were recruited later to provide fresh samples of pre-expression and post-expression milk[1] for additional testing. One participant expressed milk from a single

[1]Pre-expression milk is often referred to as fore milk and post-expression milk as hind milk. Pre-expression milk as used in this study refers to the first 30 ml or less of milk expressed prior to feeding or pumping. Post-expression milk refers to the milk expressed after feeding or pumping. The breast may not be completely emptied when collecting the post-expression milk.

Table 4.1. Sample Collection Details

| **Participant & Breast** | **Pump Time (minutes)** | **Mass (gm)** | **Volume (ml)** | **Volumetric Flow Rate (ml/min)** |
|---|---|---|---|---|
| 1 Right | 11 | 97 | 100 | 9.1 |
| 1 Left | 11 | 27 | 28 | 2.5 |
| 2 Right | 17 | 145 | 150 | 8.8 |
| 2 Left | 18 | 135 | 140 | 7.8 |
| 3 Right | 9 | 5 | 11 | 1.2 |
| 3 Left | 9 | 10 | 6 | 0.7 |
| 4 Right | 13 | 175 | 180 | 13.8 |
| 4 Left | 13 | 155 | 160 | 12.3 |
| 5 Right | 7 | 20 | 21 | 3.0 |
| 5 Left | 10 | 50 | 52 | 5.2 |
| 6 Right | 9 | 30 | 31 | 3.4 |
| 6 Left | 9 | 40 | 42 | 4.7 |

breast before and after feeding her child. The last participant used an electric pump and stopped pumping after expressing 30 ml of pre-expression milk into a container and then continued with her expression. She again stopped pumping at the end to express the post-expression milk into a new container. Due to the interruptions in feeding and pumping, the total time was not recorded nor was the total mass recorded for the last two participants. The samples were transported back to the lab at 37 °C and then separated and stored in the same manner as with the initial 6 participants.

After all initial testing was completed, the remaining samples were transferred into freezer storage bags designed for breast milk and frozen at -20 °C. When further testing was conducted, samples were thawed in a warm water bath until desired temperature and then gently agitated by compressing the bag 5 to 10 times until the fat was visually mixed. When lengthy testing procedures were conducted, thawed unused samples were kept in the refrigerator and reheated according to the same procedure as needed.

## 4.2 Density Testing

As noted in Section 4.1, the original 6 participants' entire expressions were weighed and measured to obtain initial density. The electronic scale used for those initial mass measurements has an accuracy of ±1 gm. The initial volume was measured in commercially available infant bottles designed for use with each participant's electric pump. The bottles' accuracies are unknown.

Density was retested on the frozen milk for the initial 6 participants by hand measuring a volume of milk using a 3 ml syringe and weighing the sample on an electronic balance. The milk was tested from 5 °C to 50 °C using a water bath to control temperature, as seen in Figure 4.2. After completing all initial testing, the remaining samples were refrozen to -20 °C. The accuracy of the electronic balance is ±0.01 mg. The thermometer accuracy is ±0.5 °C.

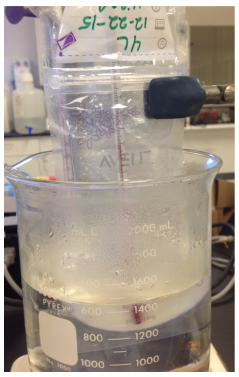

Figure 4.2. Hand Measuring of Density

Density was remeasured from 50 °C to 3 °C on the original samples from Participants 1 - 6 after thawing in a warm water bath and measured from 41 °C to 36 °C on fresh samples from Participant 8[2] using 1.3 ml of milk in an Anton-Paar DMA 4500 M density meter. To prevent the formation of air bubbles during testing, the samples were preheated to 50 °C (45 °C for the fresh samples). The milk was gently mixed as described in Section 4.1. A 2 ml Luer syringe was used to inject the heated milk into the testing chamber. The density meter was programmed to measure when the sample was at temperature equilibrium instead of predetermined to ensure accuracy. After each test, the chamber was cleaned with hot soapy water, flushed with distilled water, and then water and air checks were performed to ensure no trace of the previous sample remained in the chamber. The Anton-Paar DMA 4500 M density meter has an accuracy rating of ±0.00005 g/cm$^3$ and ±0.03 °C.

[2]Participant 7 was unable to provide a large enough sample to test density.

### 4.3 Initial Viscosity Measurements

Samples from each breast from Participants 1 through 6 were tested on an A&D SV-1A vibrational viscometer, shown in Figure 4.3, at 30 Hz in a 2 ml glass sample cup with the first testing occurring within 30 minutes of expression. A 3 ml syringe was used to withdraw the desired sample volume. The samples from the warm container were retested for viscosity two more times over 12 hours. The refrigerated samples were reheated and mixed according to procedure listed in Section 4.1 and tested after 24 hours of refrigeration for viscosity. The temperature of the milks when they were initially tested had dropped significantly within minutes of expression. So for subsequent tests each sample was brought to the original testing temperature. The A&D SV-1A vibrational viscometer has a repeatability of ±1% of reading and temperature accuracy of ±0.1 °C. The viscometer was calibrated according to manufacturer guidelines for 2 point calibration using distilled water and isopropanol.

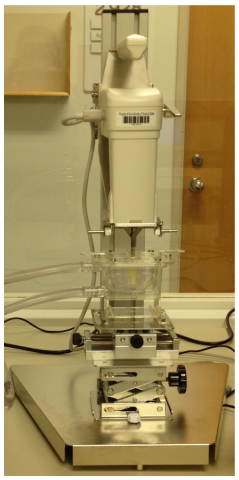

Figure 4.3. Viscometer

### 4.4 Rheology Measurements

The experimental results obtained by Section 4.3 were highly variable and the shear rates of those experiments were unknown. Thus, an Anton-Paar MCR 302 rheometer was obtained for three weeks and additional testing was performed. The Anton-Paar MCR 302 rheometer possesses multiple patented technologies to ensure accurate measurement of torque, deflection angle, and phase shift between electric motor torque and angle response to determine all rheological properties. The Peltier temperature controlled plate and hood provide an accuracy rating of ±0.1 °C between device and sample temperatures. The test parameters were initially determined by testing whole and raw bovine milks and are described below.

Viscosity was determined using a cone-plate measuring system. The cone measured 50 mm in diameter and $0.5^o$ angle. The minimum required sample volume is 0.29 ml but approximately 0.4 ml of each sample was used to ensure complete coverage of testing area. A lower Pelltier temperature controlled plate (P-PTD 200) and Pelltier temperature controlled hood (H-PTD 200) were used to maintain temperatures. Each sample was applied to the plate using a 3 ml syringe, as seen in Figure 4.4, and allowed to rest at least 4 minutes before any testing began.


Figure 4.4. Loading a Sample on the Rheometer

**Shear Rate**. Two separate shear rate ranges were tested. For both tests, samples were brought to 37 °C and held at that temperature for 4 minutes before applying shear. Samples were tested using a linear ramp profile from 1 $s^{-1}$ to 100 $s^{-1}$. Data was read at a constant rate of every two seconds at every shear rate over a period of 200 seconds for a total of 100 data points. The second shear rate range tested was 0.1 $s^{-1}$ to 20 $s^{-1}$ using a linear ramp profile. Data was read over a period of 220 seconds using a linear ramp profile beginning at 10 seconds and ending at 1 second. The total number of data points was 40 with a point density of 2 points/shear rate.

**Temperature**. Three separate temperature sweep ranges were tested – 0 °C to 50 °C, 29 °C to 45 °C, and 36 °C to 43 °C – using a linear ramp profile of 1 °C every minute. All tests occurred with a 4 minute pre-shear of 50 $s^{-1}$ at the initial temperature for the individual

sweep. The shear rate of 50 $s^{-1}$ was chosen after a graph of the results from the shear rate sweeps showed the viscosity verses shear rate curve to be more linear beginning at that shear rate. The viscosity was read after the pre-shear and compared with the first data reading of the sweep. This comparison was made to ensure that temperature was the only variable affecting the viscosity readings during the sweep. Data was read at a constant rate of 1 minute after a temperature increase. Test times varied according to the temperature range tested. The longest test was 55 minutes (4 minutes pre-shear and 51 minutes temperature sweep).

**Loop Test**. The loop test followed the same 4 minute rest period at 37 °C as the shear rate tests. The shear rate was then varied using a linear ramp profile from 1 $s^{-1}$ to 200 $s^{-1}$ and then back down to 1 $s^{-1}$. Data was read at a constant rate of every second at every shear rate over a period of 400 seconds for a total of 400 data points.

**Complex Shear Modulus**. The complex shear modulus was determined using a double gap cylinder measuring system. The bob effective length is 40 mm. The bob inner diameter is 24.66 mm and outer diameter is 26.66 mm. The cup inner diameter is 23.826 mm and outer diameter is 27.592 mm. The needed sample volume is 3.5 ml to 5.0 ml. All tests used 4.0 ml of sample. Samples were brought to 37 °C and held at that temperature for 4 minutes before applying shear strain. The shear strain was applied using a logarithmic ramp from 0.01% to 1000%. The duration of the test was set by the device. A total of 31 data points were recorded with a point density of 6 points per decade.

# CHAPTER 5

# RESULTS & DISCUSSION ON HUMAN MILK RHEOLOGY

A portion of this chapter was adapted from the author's conference paper [23].

The majority of the rheological work performed on mammalian milk has been undertaken by the dairy industry. Due to differences in composition between milk of different species these works are not able to be used for modeling human milk flow within the breast. Studies on human milk rheology conducted by clinicians are limited and lack sufficient data to be useful. The experimental study presented in this chapter demonstrates some basic rheological properties of raw human milk. This study focuses on the effect of temperature and storage on density and viscosity, as well as, shear rate on viscosity.

The nomenclature for the test results is presented in Table 5.1.

## 5.1 Density Results

The initial pH of the samples ranged between 7.0 and 7.4. Density showed some differences with aging and between methods of measurement. Results are shown in Table 5.2. The hand measured density data is subject to a large degree of user error. The initial density was calculated using a balance accurate to 1 gm, and the volume was measured by markings on the collection containers which were commercially available baby bottles. This accuracy is most evident in the initial density of 3R in Table 5.2. While a change in density with aging likely occurs due to evaporation of water or loss of gases, the anticipated change would not be as great as what is seen in 3R. The manual measurements of the aged milk density were performed on a balance with greater accuracy to 0.01 mg and with 3 ml syringes used to administer medications. However, the water bath used to heat the milk was not well

Table 5.1. Nomenclature for Test Results

| | |
|---|---|
| F | Pre-expression or Fore milk |
| H | Post-expression or Hind milk |
| L | Left Breast |
| R | Right Breast |
| $G^\star$ | Complex Shear Modulus |
| $G'$ | Storage Modulus |
| $G''$ | Loss Modulus |
| $\rho$ | Density |
| $\mu$ | Apparent Viscosity |
| $\gamma_L$ | Limit of LVE Region |
| $\tau_f$ | Flow Point |
| $\tau_y$ | Yield Point |

Table 5.2. Effect of Aging on Human Milk Density

| Sample | Initial $\rho$ $(g/cm^3)$ | Manual $\rho$ $(g/cm^3)$ | Meter $\rho$ $(g/cm^3)$ | Temperature (°C) |
|---|---|---|---|---|
| 1R | 0.970 | 1.098 | 1.029 | 26.4 |
| 1L | 0.964 | 1.083 | 1.022 | 24.8 |
| 2R | 0.967 | 1.067 | 1.028 | 25.8 |
| 2L | 0.964 | 1.087 | 1.030 | 26.7 |
| 3R | 0.455 | 1.100 | 1.033 | 24.4 |
| 3L | 1.667 | 1.099 | 1.033 | 24.7 |
| 4R | 0.972 | 1.064 | 1.031 | 26.0 |
| 4L | 0.969 | 1.104 | 1.031 | 27.0 |
| 5R | 0.952 | 1.100 | 1.034 | 24.8 |
| 5L | 0.962 | 1.105 | 1.030 | 25.4 |
| 6R | 0.968 | 1.111 | 1.026 | 26.2 |
| 6L | 0.952 | 1.110 | 1.032 | 26.0 |

controlled. It is possible that the milk sample was not always uniform in temperature and a drop in temperature occurred when the milk was drawn into the syringe. Using this method, density was recorded over a temperature range (data not shown). The density would temporarily increase in certain temperature ranges, most notably between 30 °C and 32 °C, when human milk fat melts [38].

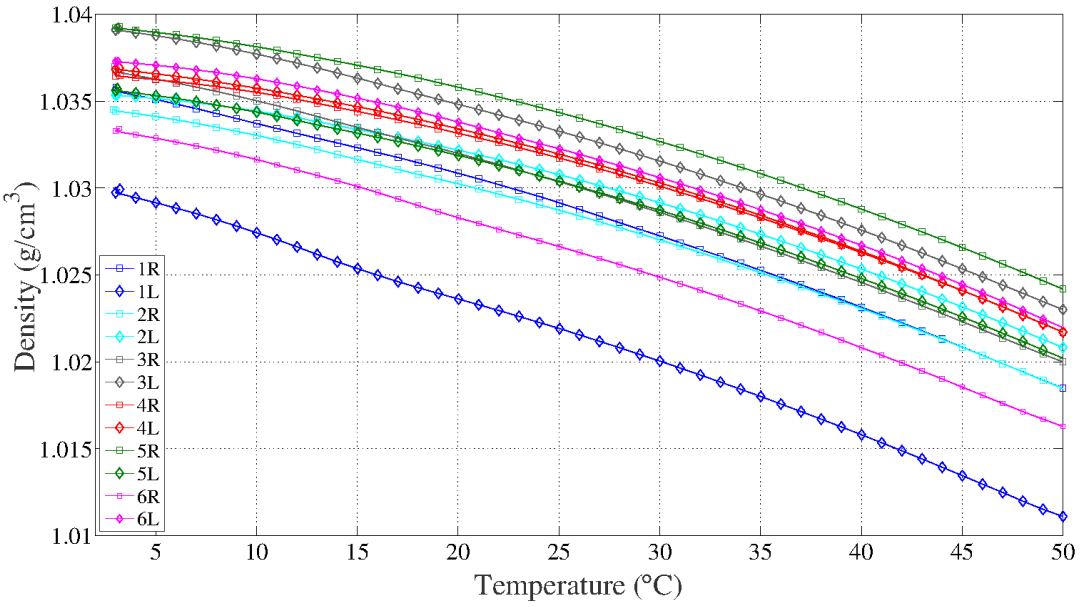


Figure 5.1. Effect of Temperature on Previously Frozen Human Milk Density

The density meter has an accuracy rating to 0.00005 g/cm$^3$ and temperature accuracy to 0.03 °C. The density meter showed the effect of temperature on the milk and the range of variation of density between breasts, between pre- and post-expression milk, and between women. Figure 5.1 shows the density values for the first 12 samples, which had been frozen prior to testing. Figure 5.2 shows the density of fresh milk at the beginning and end of an expression and between breasts. The temperature range displayed in Figure 5.2 is the normal range of body temperature that can be expected to occur in the breast either naturally or with the use of topically applied heat. Due to the time necessary to test the density over this temperature range, the 8L post-expression milk had aged 15 hours since expression at the start of testing. All fresh samples were held at 37 °C until time of testing.

All the milk showed a decrease in density as the temperature increased. The decrease was approximately 0.02 g/cm$^3$ over 47 °C for each sample in Figure 5.1. With the meter set to measure at temperature equilibrium, changes in density during phase changes in the milk would not be apparent. Differences in density of milk between breasts of some participants

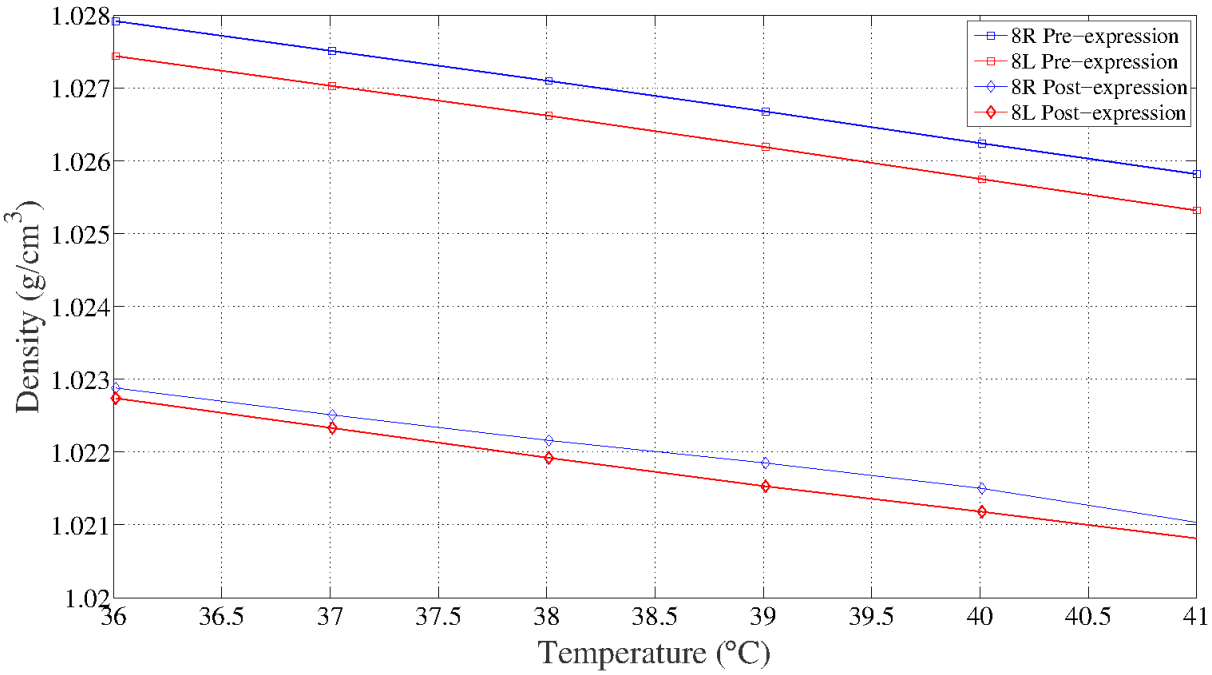


Figure 5.2. Differences between Pre- and Post-Expression Fresh Human Milk Density

were noted, especially 1, 3, 5, and 6. Participant 4 had no significant differences between breasts. The difference between pre-expression and post-expression fresh milk density is significant in Figure 5.2. Research regarding milk composition between breasts and over the course of an expression shows that for some mothers the composition can varying greatly whereas other mothers have few differences [47]. The variations in composition would account for the differences in density and should be taken into account when modeling human milk flow.

## 5.2 Viscometer Results

The viscometer results measured for the initial 6 participants show significant differences in viscosity in response to aging as seen in Figure 5.3. Some sample viscosities increased while others decreased. Similarly, Table 5.3 shows that after 24 hours of refrigeration the viscosity of the milk changed, either increasing or decreasing from its value at the time

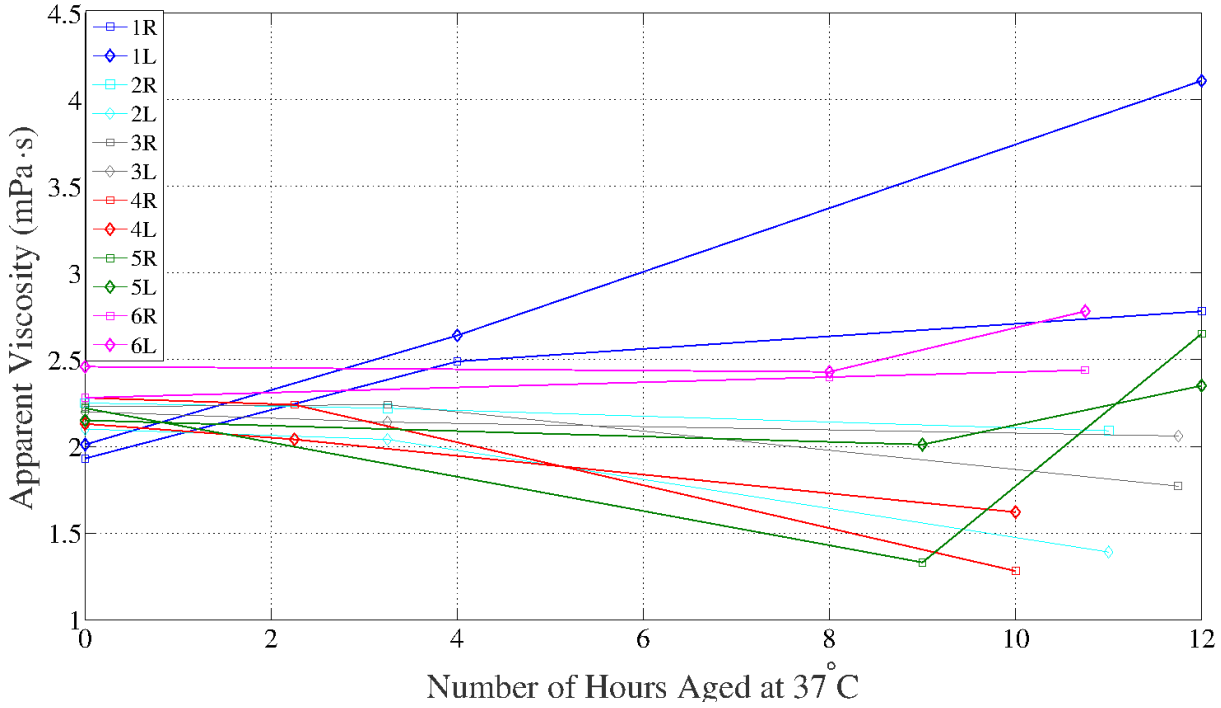


Figure 5.3. Effect of Aging on Human Milk Viscosity

of expression. Some of the samples were immediately retested when large changes were noted, either rerunning the same loading or drawing a fresh loading to retest. Often the sample viscosity decreased when retested (data not shown). These results differed greatly from previous research by Almeida et al. [21]. As noted in Section 2.2, the determination of kinematic viscosity utilized in that study was for Newtonian fluids. Their milk was previously frozen as compared with this study using fresh milk. Comparing their results with Bateman and Sharp [24], the length of time that milk is frozen affects the viscosity and may account for the lack of variation in viscosity readings by Almeida et al.

## 5.3 Shear Rate Results

The experimental results obtained by the rheometer confirm that raw human milk has similar behavior as other raw mammalian milks from dairy animals. The effect of shear rate on human milk demonstrated shear-thinning non-Newtonian behavior as reported by Fondaco

Table 5.3. Effect of Refrigeration on Human Milk Viscosity

| Sample | Initial $\mu$ (mPa·s) | Post 24 Hours $\mu$ (mPa·s) | Temperature (°C) |
|---|---|---|---|
| 1R | 1.93 | 3.26 | 26.4 |
| 1L | 2.01 | 4.27 | 24.8 |
| 2R | 2.25 | 2.10 | 25.8 |
| 2L | 2.10 | 1.36 | 26.7 |
| 3R | 2.23 | 2.98 | 24.4 |
| 3L | 2.20 | 2.11 | 24.7 |
| 4R | 2.28 | 2.28 | 26.0 |
| 4L | 2.13 | 1.52 | 27.0 |
| 5R | 2.22 | 1.97 | 24.8 |
| 5L | 2.15 | 2.39 | 25.4 |
| 6R | 2.28 | 2.79 | 26.2 |
| 6L | 2.46 | 2.92 | 26.0 |

et al. in regards to human milk [45] and Bateman and Sharp in regards to bovine milk [24]. This behavior was especially visible at low shearing rates less than 20 $s^{-1}$ and can be seen in Figures 5.4 and 5.5. As shear rate decreases, the viscosity increases toward infinity. Thus raw human milk has a yield point and a firm texture when at rest. As the shear rate increases, the slope of the curve decreases, although it never appears to develop true Newtonian behavior over the shear rates tested. This decrease in slope at high shear rates likely accounts for the many researchers who assumed Newtonian behavior for milk. However, currently no research exists concerning human milk that could help define at what shear rate Newtonian behavior begins.

An oscillatory behavior pattern emerges beginning around 20 $s^{-1}$ and continuing throughout the sweep as seen in Figure 5.5. Bateman and Sharp noted changes in the degree of clumping of the fat globules when milk was repeatedly run through the capillary tubes [24]. This could be the cause of the oscillations. Changes in the fat globules as the shear increases would be a reasonable expectation. However, Bateman and Sharp tested bovine milk at 25 °C while the fats were solids in the milk. They also noted shear thinning behavior in raw

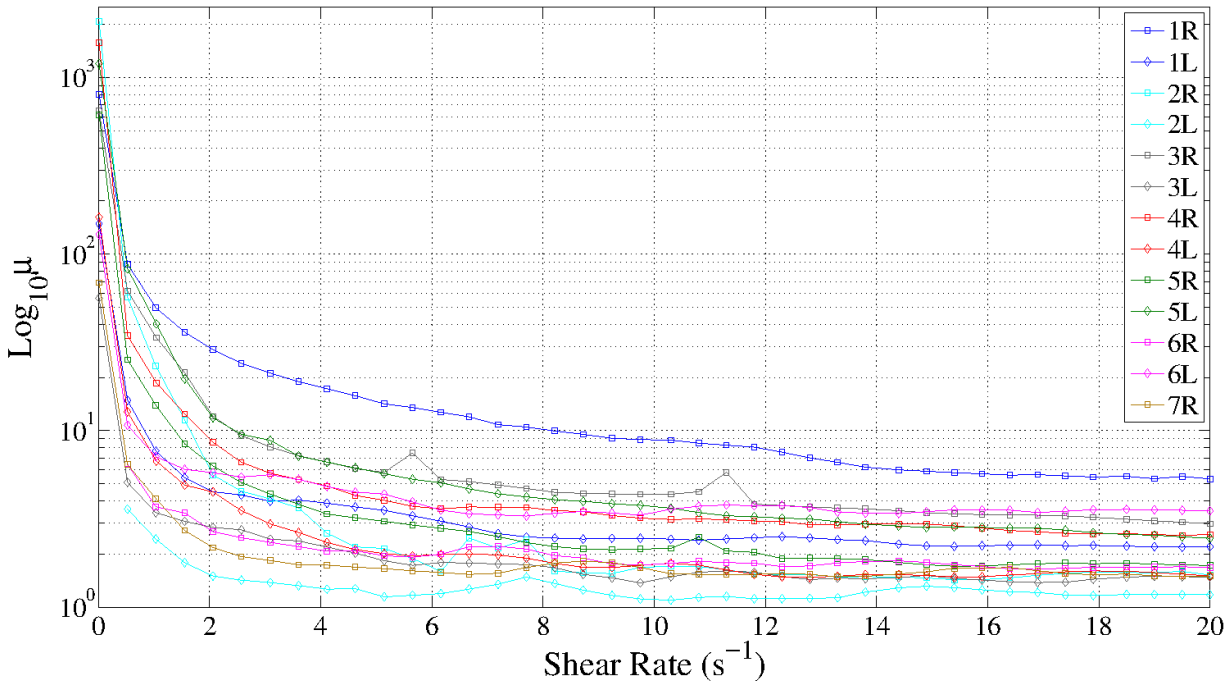


Figure 5.4. Viscosity at Very Low Shear Rates

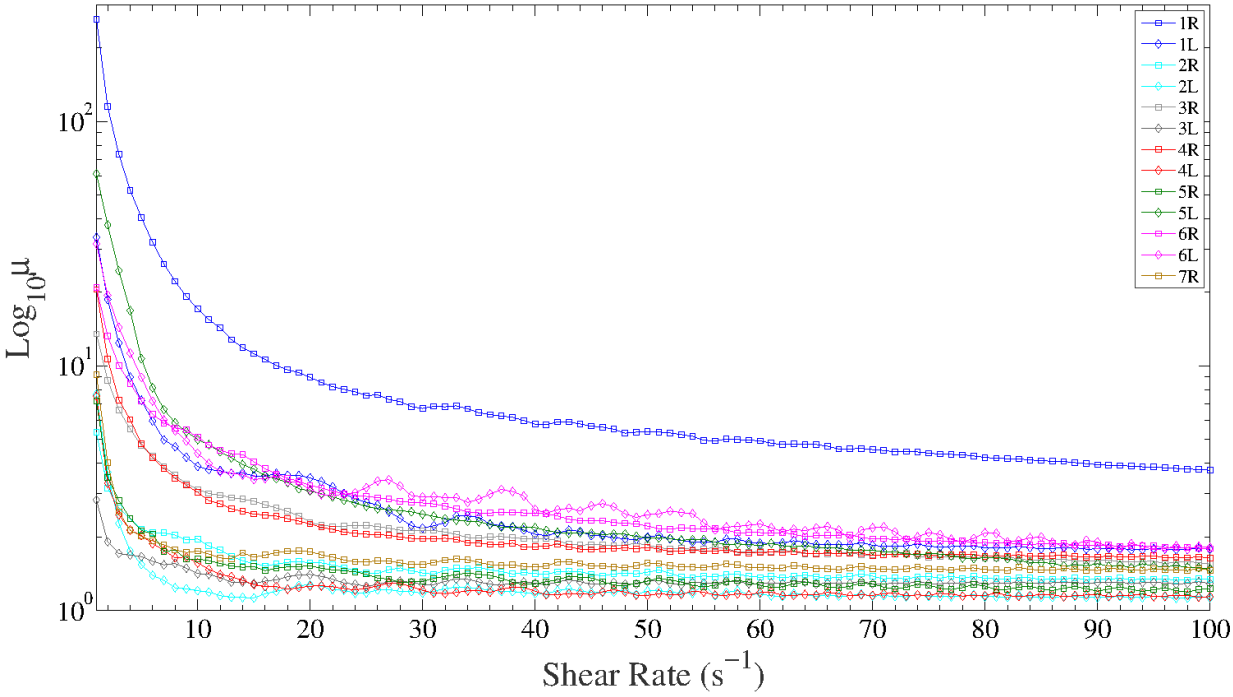


Figure 5.5. Viscosity at Low Shear Rates

skim milk. Thus, the effect of shear rate on proteins in the milk cannot be neglected without further study. So the exact cause of the oscillations is not known but likely due to changes in the particle orientation, shape, and size. Further research to identify the causes is planned.

### 5.4 Temperature

Temperature influences on viscosity were noted by the temperature sweep at a constant shear rate of 50 $s^{-1}$. Prior to testing, a 4 minute pre-shear at 50 $s^{-1}$ was performed with the plate and hood maintaining temperature. At the end of the pre-shear, the viscosity was tested from 0 °C to 50 °C with measurements recorded after 1 minute at each new temperature to allow sufficient time for sample to reach a homogeneous temperature. The pre-shear viscosity value was compared to the first viscosity recorded during the actual temperature sweep (data not shown). All but 2 samples had preshear viscosities similar to the first sweep read with less than 1 mPa·s variation. These results indicate that 4 minutes was sufficient time for the milk particles to orient themselves and to isolate the effect of temperature on the viscosity. Temperatures greater than 50 °C were avoided to prevent denaturization of proteins that would alter viscosity [32]. The results are shown in Figure 5.6.

In general, the increase in temperature decreased the viscosity. However, some samples demonstrated an increase in viscosity over certain temperature ranges before returning to anticipated behavior. All the samples that were tested to 50 °C demonstrated an increase in viscosity between 40 °C and 50 °C, shown in Figure 5.7[1], while a few showed a brief increase between 6 °C and 16 °C as seen in Figure 5.6. The original 12 samples collected were retested with a new loading over a smaller temperature range of 29 °C to 45 °C. Similar rises in viscosity, seen in Figure 5.8, occurred as temperature exceeded normal body temperature. While the pattern of behavior for the samples remained the same, the viscosity values from

[1]For clarity, some graphs were omitted.

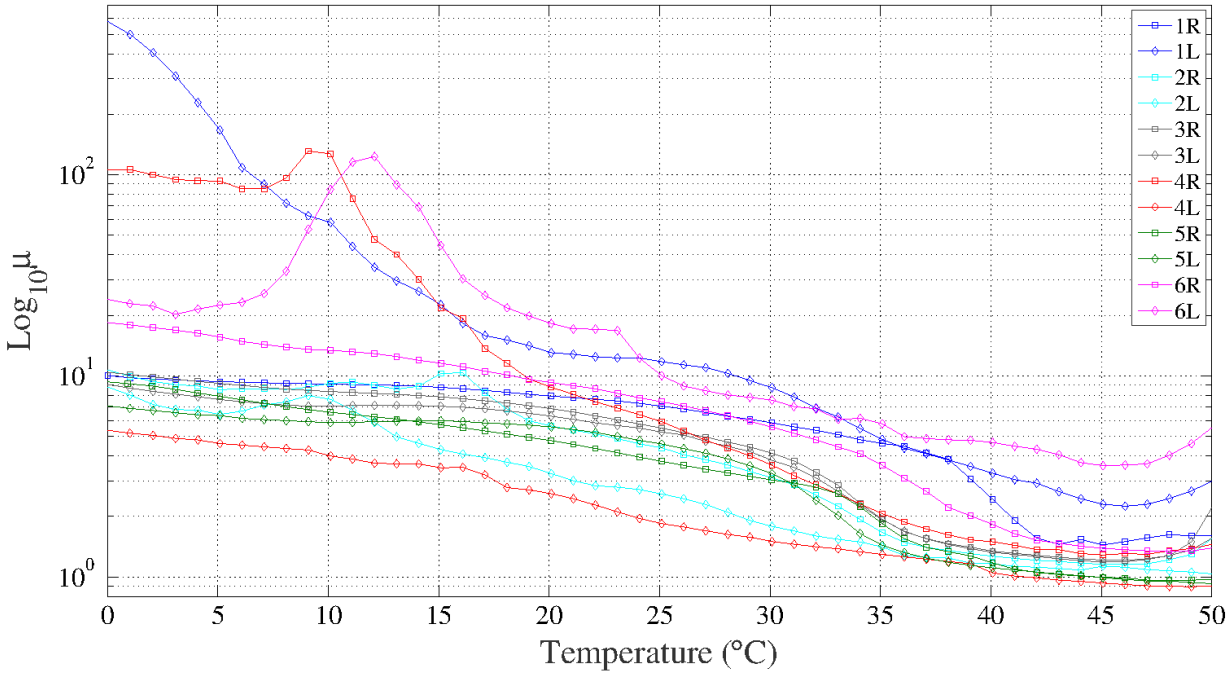


Figure 5.6. The Effect of Temperature on Previously Frozen Human Milk

the second temperature sweep did not match the viscosity values of the original test over the same temperature range.

## 5.5 Aging and Storage

To test for viscosity changes due to aging and storage, the samples provided by the last two participants were tested on the rheometer. Participant 7 provided a hand expressed sample before and after feeding her infant at the breast while Participant 8 provide the first and last 30 ml of her expression via double electric pump. As noted in Section 3.1, post-expression milk generally contains higher fat content than pre-expression milk [58]. Visually, the pre- and post-expression milks appeared different with the post-expression milk looking "creamier" and adhering to the plate better during loading.

Participant 7′s milk was tested at a constant shear rate of 50 $s^{-1}$ over a small temperature range. As with the initial testing using the viscometer, the effects of aging at body temper-

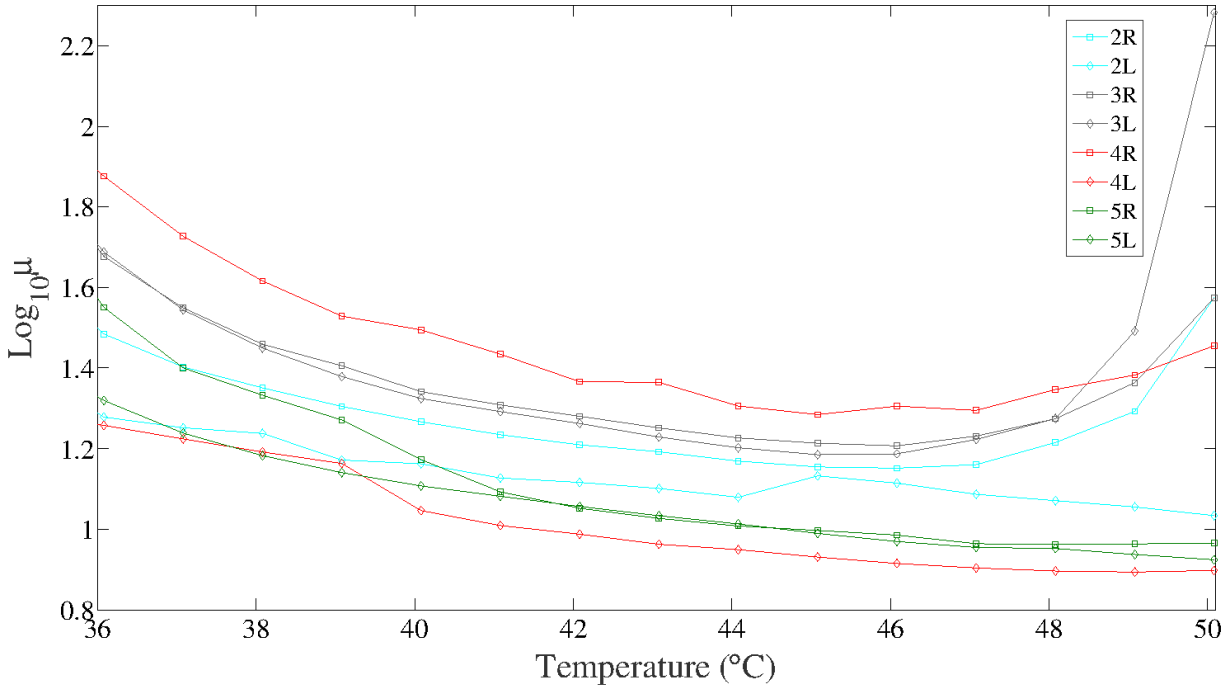


Figure 5.7. Viscosity Changes as Temperature Exceeds Normal Body Temperature

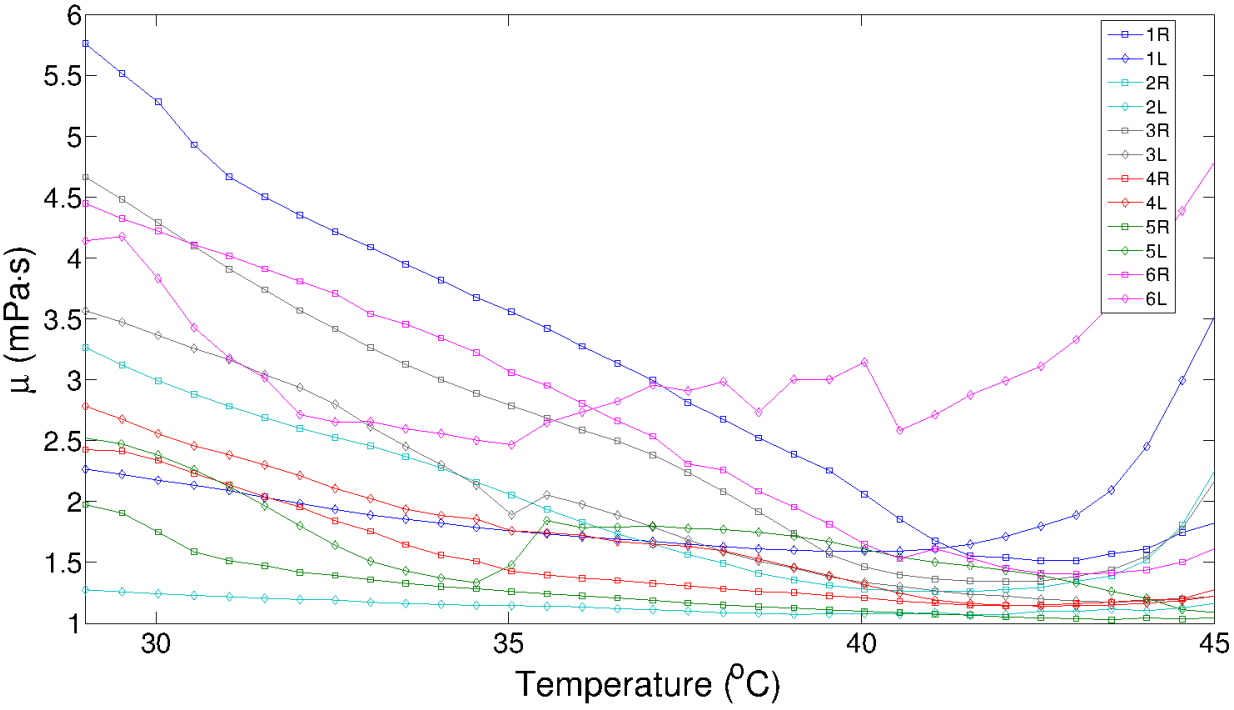


Figure 5.8. Viscosity Changes from 29 °C to 45 °C

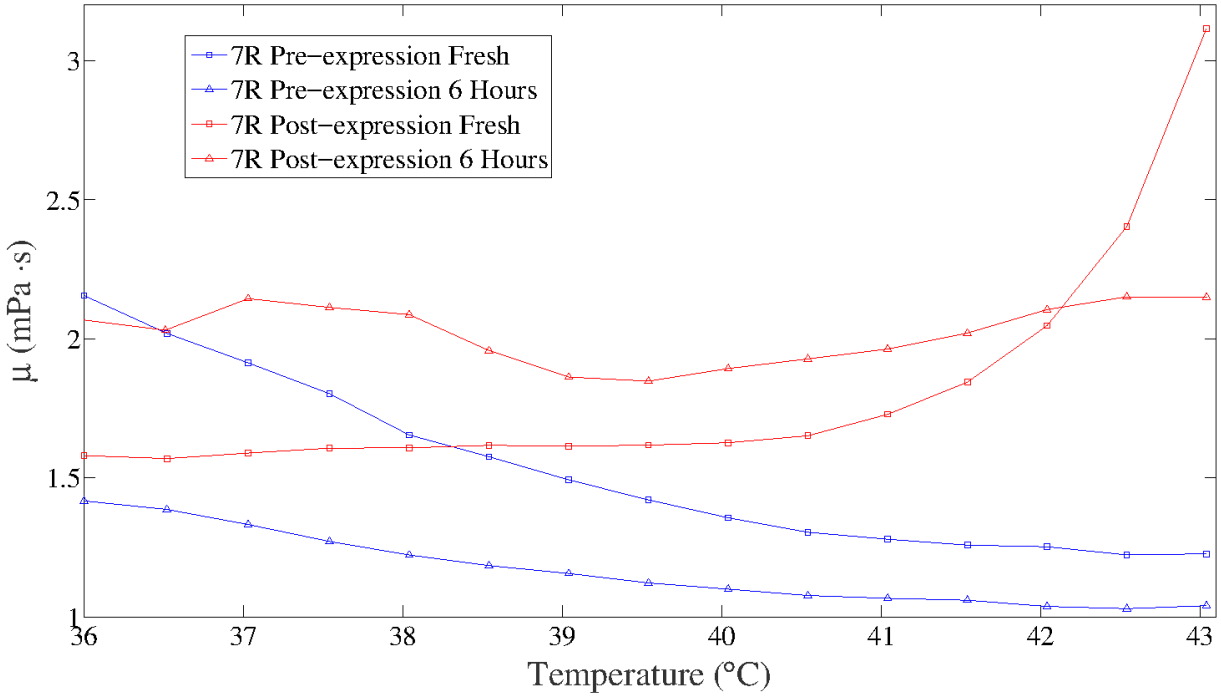


Figure 5.9. The Effect of Aging on Viscosity for Pre- and Post-Expression Fresh Human Milk

ature and refrigeration were investigated. Figure 5.9 shows the results of aging fresh milk at 37 °C over 6 hours. Post-expression milk viscosity increased with aging while pre-expression milk viscosity decreased. Also of interest in Figure 5.9 is that only the post-expression milk viscosity increased when the temperature increased. As seen in Figure 5.7, some samples experienced an increase in viscosity at temperatures above the upper limit of 43 °C used to test Participant 7′s samples. Thus the pre-expression milk may have experienced an increase in viscosity at a higher temperature if tested. Assuming the post-expression milk contains a higher fat content than the pre-expression milk, the effect of heat on the fat globules may have a great impact on overall flow behavior with high heat applications actually having a negative influence. This effect requires further research and could impact the use of heat application to resolve milk stasis within the breast [20].

Participant 8′s milk samples were held at 37 °C and not initially tested until 5 hours after collection. Due to this delay, data regarding changes due to aging at 37 °C were not

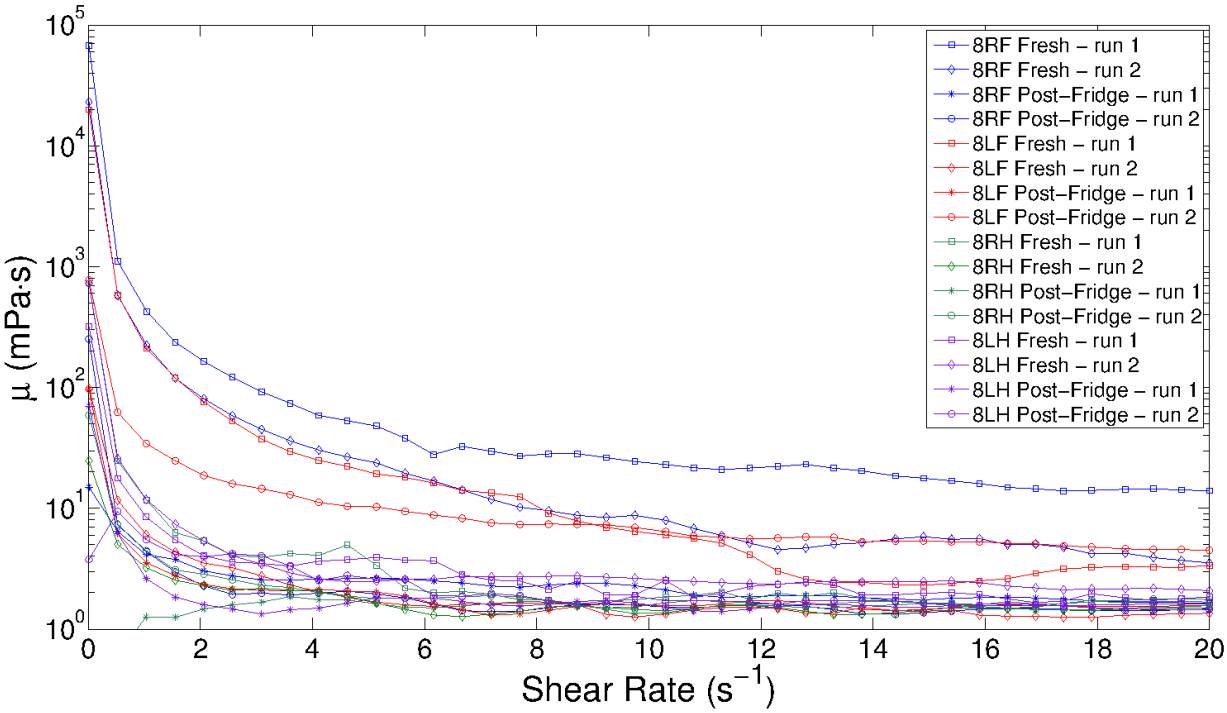


Figure 5.10. Effect of Refrigeration on Human Milk Viscosity at Low Shear Rates

obtained. All samples were tested at 5 hours after expression and after 3 days refrigeration using the shear rate sweeps detailed in Section 4.4. For the fresh and 3 days post-refrigeration tests, each sample was tested twice with a new loading. At very low shear rates, shown in Figure 5.10, the fresh pre-expression milks tested considerably higher during the first loading compared to the second fresh loading. The post-expression fresh milk samples tested closer to the post-refrigeration values.

Looking at the lowest shear rate of 0.01 $s^{-1}$ shown in Figure 5.11[2], all the first runs of fresh samples measured considerably higher than the post-refrigerated samples. Only for sample 8LH was the fresh second run higher than the fresh first run sample. Significant differences between first run and second run samples are seen in 8RF for both fresh and post-refrigerated results.

[2]The value for sample 8R Post-expression Post-Fridge − run 1 is from 1 $s^{-1}$ due to the negative viscosity value read at 0.01 $s^{-1}$.

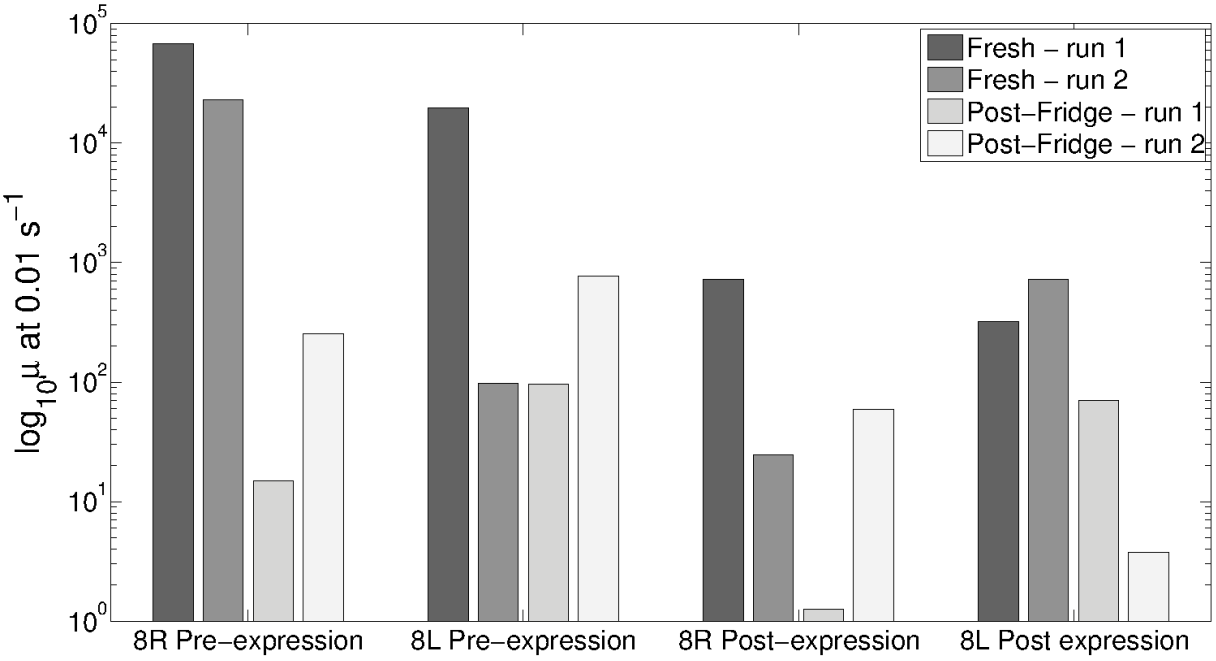


Figure 5.11. Variations in Viscosity at 0.01 $s^{-1}$

The post-expression milk samples had lower viscosities than pre-expression samples at low shear rates. Figure 5.11 shows the extreme variations. This trend is also seen when the samples were tested over a higher shear sweep in Figure 5.12. However, with the exception of sample 8RF Fresh – run 1, no extreme differences are seen at 100 $s^{-1}$. While the number of samples is insufficient to make absolute statements, the results demonstrate interesting flow behaviors. These behaviors are similar to what Bateman and Sharp reported [24].

As discussed in Section 2.1, previous researchers focused on fat content as the main determining factor for viscosity and only looked at protein content with defatted milk. However, for fresh raw human milk, the pre-expression milks – which statistically contain less fat content – had higher viscosities than post-expression milks at low shear rates. Since protein concentration throughout the expression is generally stable, the pre-expression milk has a higher protein to fat ratio. In Section 2.2, the protein composition was found to heavily influence viscosity during early lactation. These results indicate that the protein content may have a stronger influence on viscosity at low shear rates than previously thought. While

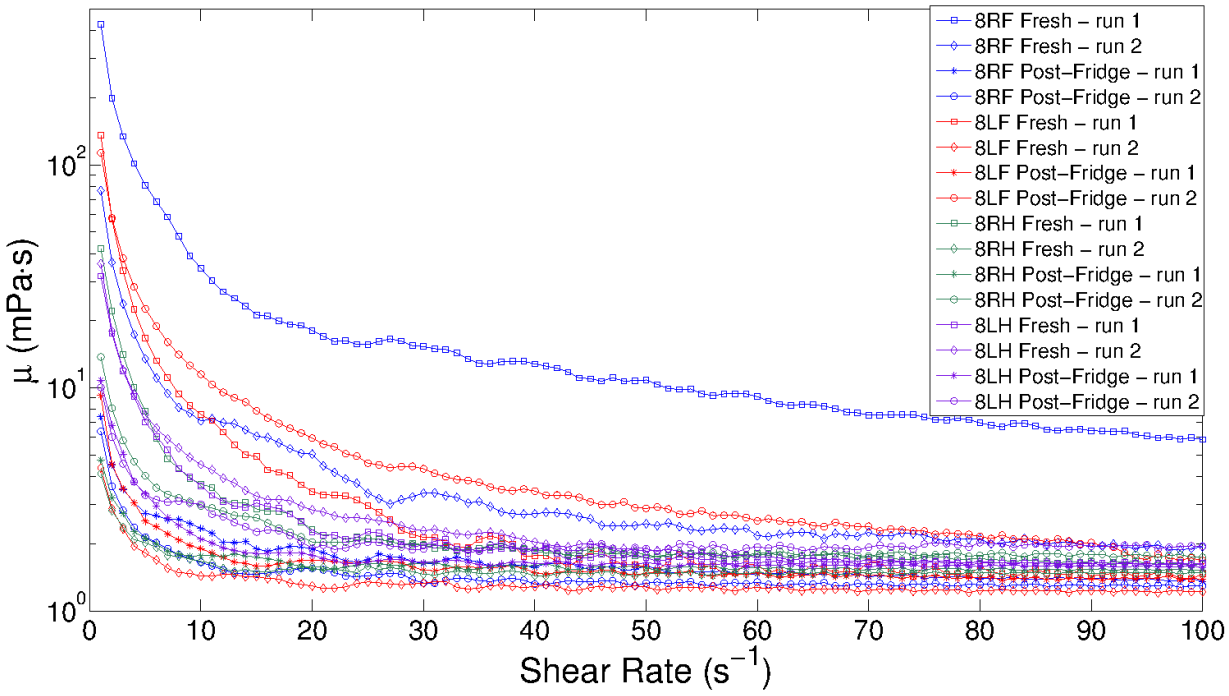


Figure 5.12. Effect of Refrigeration on Human Milk Viscosity as Shear Rate Increases

testing for the milk yield point was not conducted on these samples, the extremely high viscosity values at 0.01 $s^{-1}$ for the pre-expression milk may denote higher internal cohesion forces when the protein to fat ratio is higher. Further study is planned.

## 5.6 Differences between Breasts

Differences between the right and left breast were noted and can been seen in Figures 5.3 through 5.12. As with density differences, the viscosity differences likely originate from differences in composition [47] with additional influence from differences in particle size and distribution [63]. While the composition of human milk differs from bovine milk, the two major influences on viscosity in bovine milk − protein and fat − are likely the same causes in human milk viscosity variations [28, 29, 30, 31].

## 5.7 Time Dependence

The lack of repeatability indicates that human milk viscosity has time dependence behavior. Despite building in rest time for each sample and minimizing pre-testing handling, no two loadings of the same sample produced the same results. A prime example of this lack of repeatability is seen in sample 8R pre-expression and can easily be visualized in Figures 5.10 and 5.12. During these experiments, the temperature and handling of samples before separating a loading from the main sample was varied. Some loadings were drawn from cold liquid samples when the milk fat was solid. Most loadings came from samples that were warmed above the fat melting temperature. The 4 minute resting period before testing was used to ensure the sample temperature was uniform throughout and matched the temperature of the plate and hood. Despite the extreme care shown in handling the samples, repeatability was not achievable.

To confirm the presence of time dependence, a loop test from 1 $s^{-1}$ to 200 $s^{-1}$ back to 1 $s^{-1}$ was performed on the post-refrigerated milk from Participant 8. The result of each sample was similar and all results are shown in Figure 5.13. The loop tests confirm the time dependence behavior. Since this milk had aged at 4 °C for over 5 days, the extent of the time dependence may have altered from when it was fresh. Further investigation is planned.

## 5.8 Complex Modulus

The shear sweep testing demonstrated that raw human milk is a viscoelastic material. As noted in Section 5.3, the raw milk has a yield point which defines the limit of the linear viscoelastic (LVE) region. When the shear stress is less than the yield point, the milk experiences elastic deformation behavior and is able to return to its former shape when the stress is removed.

The complex shear modulus, $G^\star$, is used to represent the entire viscoelastic behavior of a material. The complex shear modulus can be further broken down into two components: the

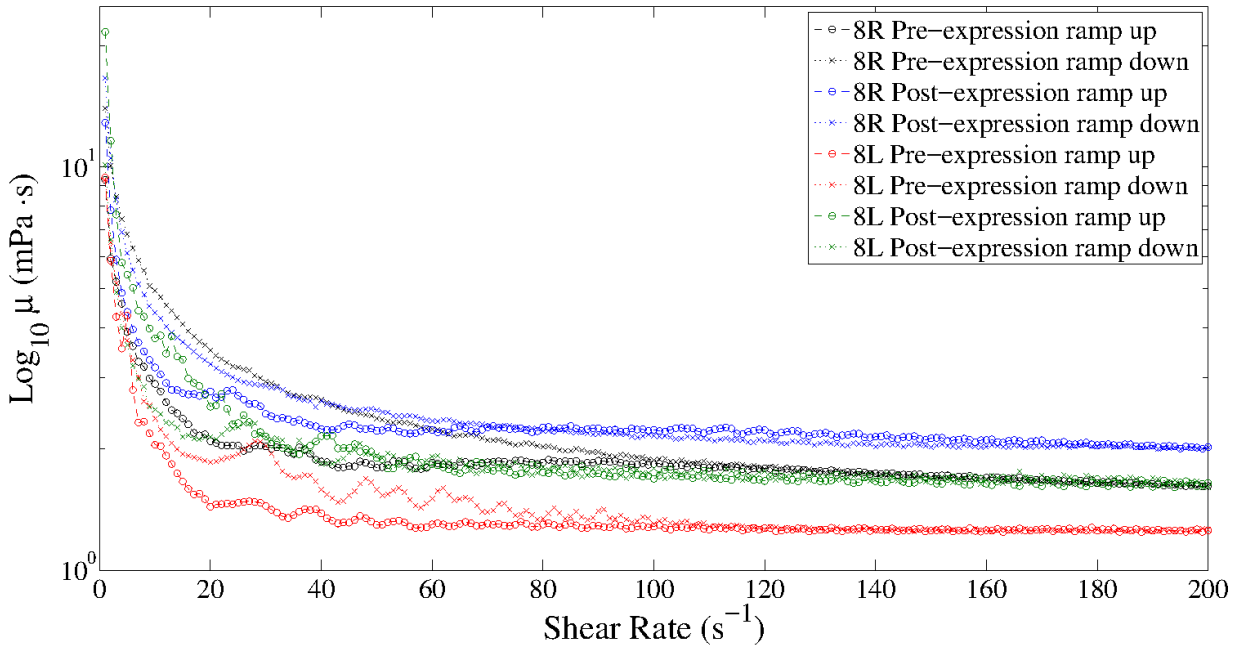


Figure 5.13. Loop Test Demonstrating Time Dependence

storage modulus, G′, that characterizes the elastic behavior and the loss modulus, G″, that characterizes the viscous behavior. For a material to flow, the applied forces must exceed the internal forces or molecular bonds that exist in the material at rest. In Section 3.1 the intercomponent interactions of human milk were discussed. The results of an oscillatory test with controlled shear strain applied is used to understand the viscoelastic behavior of raw human milk.

Samples from 3 participants were tested at 37 °C and constant angular frequency of 5 $s^{-1}$. The oscillating shear strain was applied with a logarithmic ramp from 0.01% to 1000% using a double gap measuring system with 4 ml of sample. All samples had G′ values greater than G″ in the LVE region and can be seen in Figure 5.14. This relationship classifies raw human milk as viscoelastic solids or gel-like. The LVE region is difficult to define because G′ is increasing for half of the samples. For consistency between samples the limit of the LVE region was determined at the highest G′ value before the slope of the curve became negative.

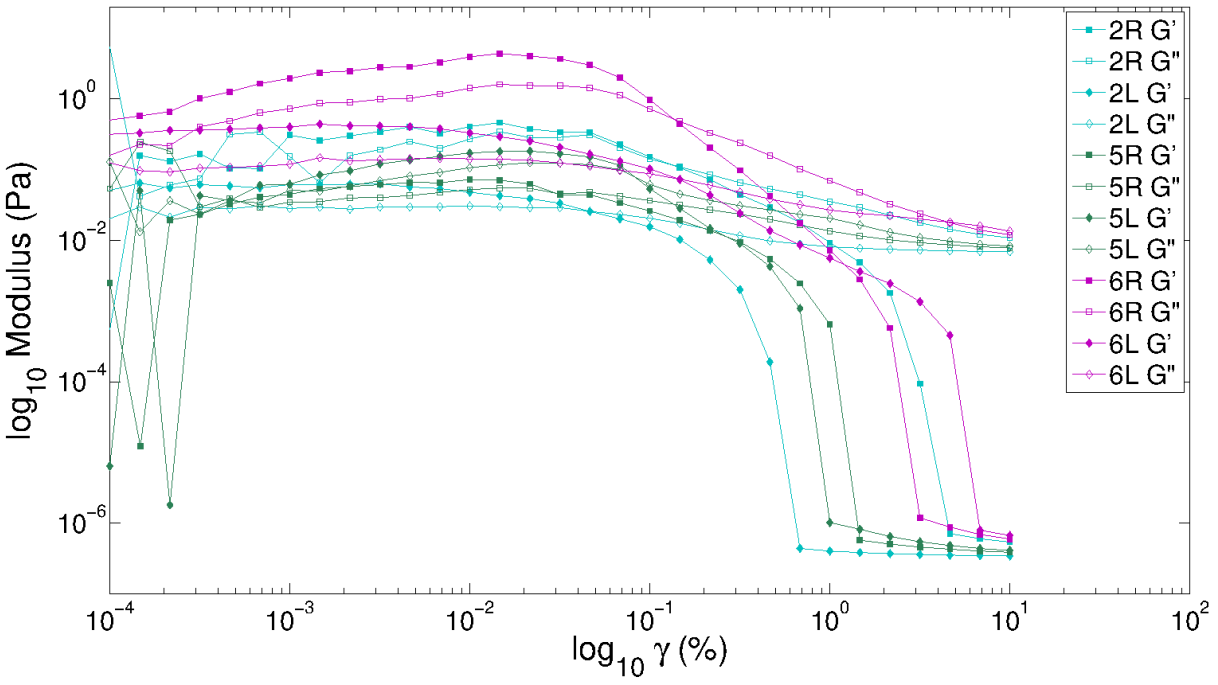


Figure 5.14. Complex Shear Modulus as a Function of Shear Strain for Raw Human Milk

The yield point for each sample varied. Differences between breasts ranged from a factor of 5 to a factor of 100. These disparities indicate large differences in the structural strengths or gel strengths of the milk between breasts which means one breast requires greater force to deform the milk within the ductal system. This gel-like structure likely helps keep particles from settling when the milk is at rest. The decrease of the G′ curves after the LVE region indicates a slow decline in structural strength which demonstrates a smoother, more homogeneous flow behavior.

A gradual rise in G″ after the LVE region occurred in 4 of the 6 samples tested. This indicates that deformation energy was transformed into friction heat due to internal viscous friction while elastic behavior dominated. Figure 5.14 shows the crossover points for G′ and G″ where viscous behavior begins to dominate for each sample. This crossover point is called the flow point. Both the yield point and the flow point can be determined from Figure 5.15.

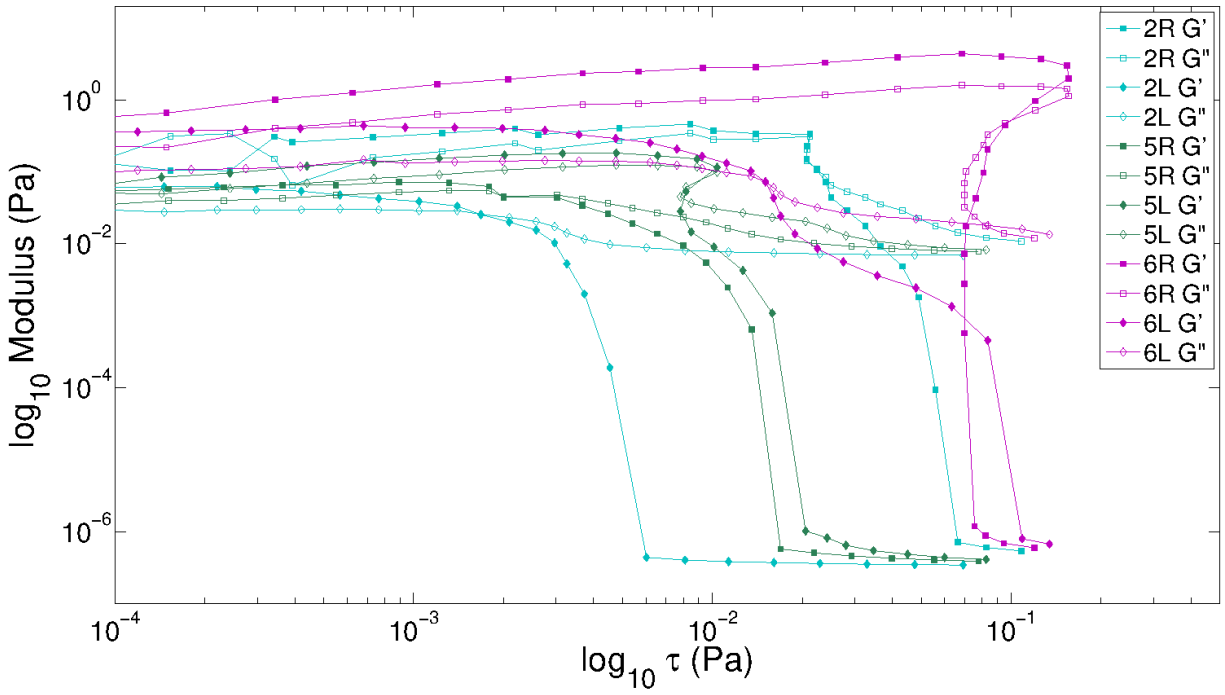


Figure 5.15. Complex Shear Modulus as a Function of Shear Stress for Raw Human Milk

The flow transition index (FTI) is the ratio of the flow point to the yield point. This value describes the transition behavior of the milk from the LVE region until flow begins. The FTI, $\gamma_L$, $\tau_y$, and $\tau_f$ (mPa) are provided in Table 5.4. The closer the FTI is to 1, the greater the tendency for brittle fracturing or non-homogeneous breakdown to occur past the yield point. Only sample 6R has a FTI close to 1. From Figure 5.14, 6R showed the steepest curve upon leaving the LVE region and had the highest G′ value. So 6R experienced a large amount of internal viscous friction as it transitioned from elastic dominated to viscous dominated flow behavior.

Table 5.4. Important Points that Characterize the Flow Properties of Raw Human Milk

| Sample | $\gamma_L$ (%) | $\tau_y$ (mPa) | $\tau_f$ (mPa) | Flow Transition Index |
|---|---|---|---|---|
| 2R | 1.47 | 8.428 | 22.366 | 2.65 |
| 2L | 3.17 | 0.22 | 1.679 | 7.63 |
| 5R | 1.00 | 0.891 | 2.0 | 2.24 |
| 5L | 2.16 | 4.766 | 9.26 | 1.94 |
| 6R | 1.47 | 68.433 | 95.303 | 1.39 |
| 6L | 0.147 | 0.67962 | 15.065 | 22.17 |

# CHAPTER 6

# CONCLUSION

The flow of human milk within the ductal system of the breast is essential to the health and well-being of both mother and child. Previous research has not adequately characterized the flow behavior of human milk. Modeling derived from other mammalian milks prove inadequate for modeling the flow behavior of human milk due to differences in composition and processing.

The experimental results demonstrated property and flow characteristics similar to other mammalian milks. The density of human milk increased slightly with aging and storage. This increase is likely due to the exchange of gases at the gas/liquid interface. As temperature increased, the density of milk decreased as expected.

Viscosity of human milk showed a strong dependence on shear rate, particularly at lower shear rates of $\leq$ 20 $s^{-1}$, and demonstrated non-Newtonian shear-thinning behavior. An oscillatory behavior was noted as the shear rate increased and is likely due to reorientation of particles within the milk. The viscosity generally decreased as temperature increased with exceptions noted in temperature ranges from 6 $^{o}$C to 16 $^{o}$C and $\geq$ 45 $^{o}$C. The increase in viscosity at higher temperatures indicates a need for caution when applying heat to the breast to relieve milk stasis. If too high of a temperature is applied, then milk viscosity could increase instead of decrease and compromise the flow further. The causes of these behaviors are under investigation.

The viscosity of milk expressed from both left and right breasts showed differences, as well as, milk expressed pre- and post-expression. These differences likely stem from differences in concentration of contents and varied between participants. Aging and storage also affected

viscosity, however the time dependence of human milk viscosity inhibited the experiments from isolating the effects of aging and storage.

Human milk viscosity displays time dependence. The importance of time dependence becomes apparent when modeling milk flow within the breast ductal system discussed in Section 3.2. Using the modeling developed by Mortazavi et al. [5] and Equation 3.1 for wall shear rate from Son [61], an approximate wall shear stress can be determined. The volumetric flow rate from sample 3L of 6 ml/9 minutes would give a wall shear rate of 7.6 $s^{-1}$ at the duct opening in the nipple of a breast containing 9 ducts. Comparatively, the wall shear rate at the alveoli connecting duct in a breast containing 5 ducts would be 91650 $s^{-1}$ for sample 4R with a volumetric flow rate of 180 ml/13 minutes. Since the milk initially experiences very high shear rates when entering the ductal system from the alveoli, the final milk viscosity would appear to be lower than if the milk had entered directly into the terminating duct in the nipple.

The time dependence behavior also affects modeling milk delivery using various feeding apparatus. Since the milk viscosity appears to change the longer it experiences a constant shearing, modeling of milk delivery through tubes and artificial nipples may not be accurate if using a Newtonian fluid model. Even flow within the digestive track should consider the time dependence of human milk when discussing delivery methods and gastrointestinal mobility. Further investigation is planned.

The complex modulus testing further defined the flow characteristics of raw human milk. The structural strength of the milk samples varied with most samples exhibiting a slow decline in structural strength as deformation increased. All the tested samples demonstrated gel-like structures and flow points to indicate how much deformation and shear stress was required before the milk behaved as a fluid. These findings demonstrate the importance of applying sufficient shear stress to transition milk from elastic dominated to viscous dominated flow behavior when trying to alleviate milk stasis within the breast and to optimize flow

within feeding apparatus. A more thorough understanding of raw human milk rheology will assist in optimizing the flow of milk within the human breast ductal system and maximize the delivery of fats and proteins via tube feedings to premature and sick infants.

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# VITA

Diana L. Alatalo completed her Bachelor of Science in Electrical Engineering cum laude from The University of Texas at Dallas (UTD) in May 2015. During her undergraduate education, she conducted research in the Advanced Research in Thermo-Fluid Systems (ARTS) lab under Dr. Fatemeh Hassanipour, her current advisor. Her research on human milk rheology commenced the summer before matriculating into her graduate program thanks to a Jonsson School Undergraduate Research Award. Her undergraduate work investigated the effect of heat application on the viscosity of human milk and was showcased as a poster presentation at the 2015 BMES Annual Meeting in Tampa, FL in October 2015.

Ms. Alatalo cross-tracked from Electrical Engineering to Mechanical Engineering and formally commenced her graduate studies in the Fall of 2015 at UTD. Her concentration area is Thermal and Fluid Sciences. In the summer of 2016, she was awarded a NSF East Asia and Pacific Summer Institute Fellowship for U.S. Graduate Students which enabled her to conduct research on infant vacuum and oral peripheral pressures at the Hartmann Human Lactation Research Group in Western Australia under the guidance of Dr. Donna Geddes. Her experimental work on density and viscosity of raw human milk earned her first place in the Young Engineer Paper Award at the 2016 ASME International Mechanical Engineering Congress and Exposition in Phoenix, AZ in November 2016. In the Fall of 2016, Ms. Alatalo received the UTD Excellence in Education Doctoral Graduate Fellowship and began her Ph.D. studies at UTD. She plans to continue investigating the rheological properties of human milk.