Maternal age influence in human colostrum acidity composition in a Maternity in an country side city of São Paulo State

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Abstract

Introduction:
Objective: To analyze sociodemographic and gestational characteristics and to compare colostrum acidity content in adolescent and advanced maternal age mothers.

Methods: This cross-sectional study included 98 adolescents (up to 18 year-old) and 33 advanced maternal age (more than 35 year-old) mothers admitted at the maternity of Presidente Prudente Regional Hospital, Brazil. An approximated volume of 10 mL of colostrum was obtained by manual expression of the breasts within 72h postpartum and stored at -20°C up to processing. Acidity of colostrum was evaluated in Dornic degrees. Statistical analysis was performed using Mann-Whitney test or X².

Results: The median age adolescents and advanced maternal age mothers were 20 years (12-24) 37 years (35-45), respectively. The majority of the adolescents (66.3%) were primiparous and C-section delivery had occurred in 66.7% of advanced age mothers. Gestational body mass index (BMI) was statically higher in advanced maternal age women (32.17±5.6 vs 26.09±3.6, p< 0.0001). Acidity measurement was similar between the studied groups.

Conclusions: Sociodemographic and gestational characteristics of adolescents and advanced age mothers showed population heterogeneity, thus, specific follow up and orientations should be applied at each one. Colostrum constitution was constant regarding casein and proteins content, independently of maternal age.

Keywords: maternal breastfeeding, maternal age, colostrum, pregnancy, acidity.
INTRODUCTION

Gestations at the extremes of reproductive ages are characterized as high-risk pregnancies, defined as “those in which the mother and / or the fetus life or health are more likely to be affected than the average of considered population”6. Nowadays, pregnancies at the extremes of reproductive life incidence, before 20’s and after 35’s, are a reality.

Adolescent pregnancy is a significant public health problem in some developing countries with social and biological impacts2. Gestation during this period is extremely relevant due to its increasing incidence and maternal effects, such as social isolation and interruption of scholar education. In addition, as a result of the biological immaturity that surrounds the growth and development of adolescents, the perinatal outcomes may be adverse, which include low birth weight newborns (LBW <2500g), intrauterine growth restriction (IUGR), apgar less than seven in the fifth minute, and high rate of preterm deliveries (<37 weeks of gestation)9.

Pregnancy after 35 years-old is considered as late pregnancy and has become increasingly frequent due to improvements regarding birth control, advances in assisted reproduction technology, late marriages, among others3. However, pregnancy in mothers with advanced maternal age may lead to a higher risk of developing hypertension, preeclampsia, diabetes and is also associated with fetal complications, being the most frequent ones the chromosomal anomalies, fetal distress, neonatal death, LBW and IUGR3.

The adverse gestational and perinatal outcomes are well elucidated by the literature, however, there are few studies associating maternal age with other gestational factors that may influence such unfavorable consequences, such as the colostrum composition from these mothers at the extremes of the reproductive age.

The main strategy from the World Health Organization (WHO) to reduce negative perinatal outcomes and infant mortality is the promotion of breastfeeding throughout the world6. Breastfeeding is considered a longstanding practice with recognized nutritional, immunological, cognitive, economic and social benefits for the binomial mother-newborn2. Colostrum is the first milk secretion, produced between the second and third day postpartum and contains the necessary nutrients for the newborn development1. The main components of colostrum are fats, immunoglobulins, proteins, carbohydrates, vitamins, leukocytes, lymphocytes, cytokines, lactoperoxidase, lactoferrin and lysozyme, as well as growth promoting hormones and peptides5,9. It also has specific characteristics such as acidity, pH, osmolarity, buffering capacity, viscosity, among others, which determine the quality of human milk20.

Thus, colostrum is rich in nutrients31, protective factors32 and its quality can be evaluated under the nutritional, immunological and microbiological aspects. Moreover, physical-chemical stability is fundamental in preserving the characteristics of this secretion14. In this context, acidity plays a key role in maintaining such stability and intact nutrients content, besides immunological and biochemical components13-15.

The colostrum presents in its composition the own acidity that is considered as original and, in its composition, casein, mineral salts (among which phosphates and citrates) and whey proteins can be found14. The acidity of raw human milk at the moment of being milked is 1 to 7° Dornic and the lipid content influences the treatable acidity40. Changes this parameter may cause acidity or basicity overload and consequently can cause acidosis or metabolic alkalosis, so when breastfed the newborn can develop necrotizing enterocolitis and this can be aggravated especially if the baby is LBW and premature10,17.

The colostrum chemical composition may be different depending on the stage of gestation, volume of excreted milk, maternal diet and among different periods of lactation18. Still, gestational adversities such as diabetes and obesity may influence the nutritional composition of colostrum18-20.

In pregnancies affected by diabetes, the total amount of protein is reduced20, which may lead to the acidity alteration in human colostrum; however, there are studies that have shown the amount of colostrum fat from diabetic mothers is lower when compared to non-diabetic mothers4, factors that can modulate the acidity profile of human colostrum18.

Nevertheless, there are few studies relating the biochemical composition and colostrum acidity profile to maternal age during pregnancy, so the knowledge regarding acidity content becomes extremely important. The understanding regarding colostrum characteristics reinforces the incentive for breastfeeding, in order to decrease early weaning rates, favor quality of life for the mother-child binomial, and still stimulate the mother/newborn bond. Thus, this study aimed to compare the chemical composition by the titratable acidity of colostrum from adolescent and advanced maternal age mothers and to analyze the sociodemographic and gestational characteristics.

METHODS

This is a cross-sectional prospective study21, including nursing mothers attended at the maternity unit of Regional Hospital, Presidente Prudente, from February to December 2017. The study was submitted and approved by the Research Ethics Committee of Universidade do Oeste Paulista, UNOESTE through Plataforma
Brasil, (protocol 55688216.8.0000.5515) and all the included mothers has signed the Informed Consent Term.

Inclusion criteria were gestational age at delivery between 37 and 41⁶/₇ weeks, negative serological reactions for hepatitis, HIV and syphilis, and absence of gestational adversities. Pregnant women diagnosed with fetal malformations and twin pregnancy were excluded.

The sample size estimation was based in the number of births occurred in the service, around 120 births/month. Moreover, it was considered the age mean difference in adolescents and advanced maternal age groups and the smaller difference between the results. Therefore, 131 nursing mothers were included, distributed in adolescent nursing (n = 98) and nursing mothers with advanced maternal age (n = 33).

Sociodemographic and obstetric data were collected from an interview using a specific questionnaire for this study, and additional data were collected from medical records.

Approximated 10 mL of colostrum was obtained by manual expression of the breasts within 72h postpartum, always in the morning during the interval between two breast-feedings. Samples were immediately stored at -20°C up to processing.

The acidity was evaluated by the Dornic method (D). Colostrum samples were separated into three 1 mL aliquots for triplicate evaluation. A drop of phenolphthalein indicator solution (1%), for pH (hydrogen ion concentration) identification, was added to the aliquots, next, the titration was performed by adding dropwise of sodium hydroxide (NaOH) N/9. During the titration, the test tube containing milk was permanently and softly agitated, in order to avoid the air incorporation to the product. The procedure was interrupted when the coloration changed to a light pink when compared to the “in natura” milk. Subsequently, the volume of sodium hydroxide used to perform the titration was quantified, given that each 0,01 mL NaOH N/9 corresponds to 1,0 ºD. The calculation of the final value of Dornic acidity was based on the triplicate arithmetic mean. Values between 1.0 to 7.0º D range were considered normal for human milk10.

The sociodemographic and obstetric data were stored in spreadsheets of Excel 2010 software, and compiled for studied variables analysis using Mann-Whitney, X² or Fisher exact test. All tests were performed in Graph Pad Prism software, version 6.0 and the significance level adopted was 5%.

**RESULTS**

Adolescent and advanced maternal age mothers’ sociodemographic data are presented in Table 1 and, as expected, there was a significant difference regarding maternal age (p <0.0001). It is noteworthy that the majority of adolescent pregnant women reported having a stable union and unpaid activities. On the other hand, a higher percentage of pregnant women of advanced maternal age reported a smoking habit.

Regarding the gestational and obstetric characteristics (Table 2), a body mass index (BMI) increase was observed in both groups considering the beginning and at the end of gestation, but the weight gain was not significantly different, although it trended to significance (p=0.056). In adolescent group, the majority was primiparous and also underwent vaginal delivery.

The acidity of colostrum was similar between studied groups (p = 0.37), suggesting that colostrum constitution in casein and proteins appears to be constant regardless of maternal age.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Adolescents mothers (n=98)</th>
<th>Advanced maternal age mothers (n=33)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)a</td>
<td>20 (12-24)</td>
<td>37 (35-45)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Ethnicityb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>43 (43.9)</td>
<td>12 (36.4)</td>
<td>0.47</td>
</tr>
<tr>
<td>Non-white</td>
<td>55 (56.1)</td>
<td>11 (63.6)</td>
<td></td>
</tr>
<tr>
<td>Marital statusb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stable union</td>
<td>66 (67.4)</td>
<td>3 (9.0)</td>
<td>0.008*</td>
</tr>
<tr>
<td>Single</td>
<td>32 (32.6)</td>
<td>30 (91.0)</td>
<td></td>
</tr>
<tr>
<td>Professionb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paid-activity</td>
<td>20 (20.4)</td>
<td>17 (51.5)</td>
<td></td>
</tr>
<tr>
<td>Unpaid activity</td>
<td>78 (79.6)</td>
<td>16 (48.5)</td>
<td>0.0006*</td>
</tr>
<tr>
<td>Smoking habitb</td>
<td>7 (7.2)</td>
<td>9 (27.3)</td>
<td>0.0047*</td>
</tr>
<tr>
<td>Mothers living with smokersb</td>
<td>38 (37.8)</td>
<td>19 (57.6)</td>
<td>0.06</td>
</tr>
<tr>
<td>Alcohol useb</td>
<td>2 (2.0)</td>
<td>1 (3.0)</td>
<td>0.57</td>
</tr>
<tr>
<td>Physical activity practice b</td>
<td>10 (10.2)</td>
<td>2 (6.0)</td>
<td>0.72</td>
</tr>
<tr>
<td>Use of illicit substances b</td>
<td>5 (5.1)</td>
<td>4 (3.0)</td>
<td>1.00</td>
</tr>
<tr>
<td>Other diseasename</td>
<td>17 (17.3)</td>
<td>9 (27.3)</td>
<td>0.21</td>
</tr>
</tbody>
</table>

*a Mann-Whitney test, median (min – max); b X² or Fisher test, n (%); * Statistically significant (p< 0.05)

Table 1: Sociodemographic characteristics of adolescent and advanced maternal age mothers.
Table 2: Gestational, obstetric and colostrum acidity characteristics of adolescent and advanced maternal age mothers.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Adolescents mothers (n=98)</th>
<th>Advanced maternal age mothers (n=33)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contact with toxic substances during pregnancyb</td>
<td>1 (1.0)</td>
<td>1 (3.0)</td>
<td>0.44</td>
</tr>
<tr>
<td>Body mass index (BMI)a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beginning of pregnancy</td>
<td>21.6 (14.3-42.9)</td>
<td>28.6 (19.5-40.6)</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td>Ending of pregnancy</td>
<td>26.6 (19.2-63.9)</td>
<td>31.3 (24.6-51.4)</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td>Weight gain (kg) b</td>
<td>12 (-1.0 - 2-35)</td>
<td>9.5 (-0.55 – 32.0)</td>
<td>0.056</td>
</tr>
<tr>
<td>Pregnancy disease*</td>
<td>45 (45.9)</td>
<td>15 (45.5)</td>
<td>0.96</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>65 (66.3)</td>
<td>2 (6.1)</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td>2</td>
<td>20 (20.4)</td>
<td>7 (21.2)</td>
<td></td>
</tr>
<tr>
<td>&gt;2</td>
<td>13 (13.3)</td>
<td>24 (72.7)</td>
<td></td>
</tr>
<tr>
<td>Number of prenatal appointmentsb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤7</td>
<td>29 (49.2)</td>
<td>30 (73.2)</td>
<td>0.65</td>
</tr>
<tr>
<td>&gt; 7</td>
<td>24 (40.7)</td>
<td>9 (21.9)</td>
<td></td>
</tr>
<tr>
<td>Obstetrics Characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age of delivery (weeks, days)a</td>
<td>39w (36w06d – 41w)</td>
<td>38w (36w – 41w03d)</td>
<td>0.18</td>
</tr>
<tr>
<td>Deliveryb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td>66 (67.4)</td>
<td>11 (33.3)</td>
<td>0.009*</td>
</tr>
<tr>
<td>Cesarean section</td>
<td>32 (32.6)</td>
<td>22 (66.7)</td>
<td></td>
</tr>
<tr>
<td>Colostrum aciditya</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colostrum acidity titration (values in Dornic degrees)</td>
<td>4 (0-7)</td>
<td>4 (0-9)</td>
<td>0.370</td>
</tr>
</tbody>
</table>

a Mann-Whitney test, median (min – max); b X2 or Fisher test, n (%); * Statistically significant (p< 0.05)

DISCUSSION

The sociodemographic, obstetric and titratable acidity profile of colostrum were evaluated in nursing mothers at the extremes of reproductive age with full term pregnancy in a maternity hospital in the interior of the State of São Paulo. The analysis identified that median maternal age reached statistical significance, as expected, being lower in the group of adolescent mothers compared to the advanced maternal age group.

It was observed the prevalent marital status was the stable union in the adolescent group, while higher number of single mothers was observed in the advanced maternal age group, similar findings were described by Marin & Piccinini22. However, the unpaid occupation was highlighted in the group of adolescent mothers whereas paid activity was more frequent in advanced maternal age. In relation to the marital status, Queiroz et al.23 has suggested gestation may be a preponderant factor for conjugality among adolescent couples, which may explain the stable union of more than 50% of pregnant adolescents observed in this and other studies24,25. In this context, it is understood why the majority of adolescent pregnant women do not perform paid activity, since the financial and emotional dependence on the partner and family, besides baby care, still overcomes for them in this period23.

The smoking habit was more frequent in the advanced maternal age group when compared to adolescents. Adversely, previous studies emphasize the last ones are more vulnerable to smoking, alcoholism and illicit drug use, and the influence of friends and public places are the predominant factors for such habits26. Thus, we observe a particularity of the studied population, which differs from other places in the country.

According to the Brazilian Health Ministry, the recommended total weight gain during pregnancy is 11.5 to 16.0 kg for pregnant women with initial adequate body mass index (BMI) (ie, BMI between 20.0 and 24.9)27.
The advanced maternal age group presented a higher BMI, either at the beginning and at the end of gestation, than adolescents, moreover, they presented a median BMI above the recommended range for the beginning of gestation, thus classified as overweight in that period. In a previous study, pre-natal overweight and/or obesity were identified as a risk factor for maternal and perinatal adversities\textsuperscript{28}, including prematurity, LBW, pre-eclampsia, among others. These data reveal the importance of evaluation and knowledge of these parameters, in order to provide adequate guidelines to pregnant populations regarding weight control deficiency and its possible complications.

Advanced maternal age mothers presented a higher number of multiparous women while primigravida were in higher number in adolescents. In this regard, Costa & Sabarence\textsuperscript{29} have reported that parity does not influence the composition of human milk.

The predominant type of delivery was vaginal in adolescent pregnant women, data similar from other studies in the literature\textsuperscript{34}, which is opposite to the advanced maternal age group, in whose cesarean was predominated with rates above than the recommended by the World Health Organization, estimated around 15%. This organization recommends medical intervention to be performed only when necessary, in order to guarantee the health of the mother/newborn binomial. However, Brazil still has high rates of cesarean deliveries, which implies a greater surgical risk for the mother in cases without necessity, greater recovery time and, consequently, high costs to the Unified Health System\textsuperscript{30}.

Regarding colostrum acidity, no differences were observed between the samples from mothers at the extremes of the reproductive ages, which show adequate amounts of casein and proteins, since the acidity is a direct marker of these molecules concentration\textsuperscript{4}. Colostrum casein is one of the adjuvants that help to protect the newborn against gastrointestinal infections, preventing the adherence of bacteria to intestinal mucosal cells\textsuperscript{31}, given that the total protein/casein ratio is 80/20\textsuperscript{32}. Thus, we can infer the maternal age did not influence the acidity of colostrum in the studied mothers, and their neonates, therefore, would not suffer any loss in the immunity conferred by the expected acidity. Accordingly, previous data show colostrum of different nursing mothers does not present changes in the acidity profile when the measurement is right after milking or after a short period of storage\textsuperscript{33,34}, corroborating with data found by our study. On the other hand, Lubetzky et al.\textsuperscript{35} identified that maternal age over 35 years influences the chemical composition of human colostrum, but the authors evaluated other parameters than acidity, such as fat and carbohydrate content.

It is important to point out that acidified milk may not meet nutritional requirements and the acidity increase with consequent protons release from the ionization of lactic acid provoke the destabilization of soluble proteins and casein micelles. Such alterations may incite milk coagulation, increase osmolarity and alter taste and odor, as well as impede calcium availability\textsuperscript{17}. In addition, lipolytic and proteolytic reactions increase acidity with reduced energetic and immune value of colostrum.

The smoking habit may influence the human colostrum composition by reducing its nutritional value due to substances such as nicotine, and may also modify the colostrum acidity profile, since nicotine is an alkaloid substance\textsuperscript{36}. Adolescents showed a 7.2% smoking rate, while 27.3% of mothers with advanced maternal age reported this habit. However, the colostrum acidity was not associated with smoking, and our findings do not corroborate previous data that show changes in colostrum composition in smokers\textsuperscript{36,37}.

Results from Mangel et al.\textsuperscript{38} and Sinanoglou et al.\textsuperscript{39} suggested the composition of human colostrum is not influenced by maternal BMI, so our data corroborate with these authors, as we have demonstrated that even in advanced maternal age presenting high BMI, this variable did not modify the casein and colostrum proteins content.

Our findings have improved the knowledge about the colostrum chemical composition at the extremes of reproductive ages, considering the data scarcity in the literature. The colostrum acidity was not influenced by maternal age, so the constitution of casein and proteins remained the same in both groups. However, the sociodemographic and gestational characteristics were shown to be divergent when compared adolescents with advanced maternal age mothers.

This research carried out some limitations, especially considering the cross-sectional design, and a small number of samples from advanced maternal age nurses, mainly due to characteristic of population attended in the studied maternity. Still, in this group, we experienced complications related to the difficulty of breastfeeding due to fissures, engorgement and low milk production, which made the collection impossible in some nursing mothers and, therefore, a lower number of samples could be included.

Our results contribute to public health actions development aiming the encouragement and support for breastfeeding, in addition to elucidate that there is no difference in the casein and proteins composition, evaluated by the acidity, in human milk at the extremes of the reproductive ages, enabling the health professionals a broad knowledge related to the composition of human milk.

Gestations during adolescence and at advanced age imply a higher maternal and perinatal risk. Recent data from the “Nascer no Brasil” study\textsuperscript{40} confirm that the health system is fragile in avoiding gestation in adolescence. This scenario aggravates the option of women with greater control of their reproductive life that opts to postpone pregnancy beyond the age of 35, most of the time due to work activities.

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REFERENCES


Resumo

Introdução:

Objetivo: Analisar as características sociodemográficas e gestacionais e comparar a acidez do colostro de nutrizes adolescentes e idade materna avançada.

Método: Estudo prospectivo transversal, que incluiu 98 nutrizes adolescentes (até 18 anos) e 33 nutrizes com idade materna avançada (maior que 35) atendidas na Maternidade do Hospital Regional de Presidente Prudente. Amostras de aproximadamente 10 mL de colostro foram obtidas por meio da ordenha manual entre 48 a 72h após o parto, e armazenadas a -20°C até o processamento. A acidez foi verificada por meio da titulação e calculada em graus Dornic. A análise estatística foi realizada utilizando-se o teste de Mann-Whitney ou X².

Resultados: A mediana da idade materna das nutrizes adolescentes foi de 20 (12-24) anos, e das nutrizes com idade materna avançada foi de 37 (35-45) anos. A maioria das nutrizes adolescentes (66,3%) eram primíparas e a via de parto cesárea ocorreu em 66,7% das nutrizes em idade materna avançada. O índice de massa corpórea (IMC) gestacional foi estatisticamente maior em nutrizes com idade materna avançada (31,3±5,6 vs 26,06±3,6, p< 0,0001). A acidez do colostro das nutrizes nos extremos da idade reprodutiva não apresentou diferença significativa entre os grupos de nutrizes.

Conclusão: As características sociodemográficas e gestacionais de adolescentes e mães com idade materna avançada evidenciam heterogeneidade dessas populações e consequentemente, atendimento e condutas específicas devem ser aplicados a cada uma. A constituição do colostro em caseína e proteínas apresentou-se constante, independente da idade materna.

Palavras-chave: aleitamento materno, idade materna, colostro, gravidez, acidez.