Maternal age influence in human colostrum fat composition

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Abstract

**Introduction:** Colostrum is the first milk secretion produced and contains the nutrients necessary for the development of the neonate.

**Objectives:** To compare the chemical level of colostrum fats from adolescent nursing mothers and those of advanced maternal age and to describe their gestational characteristics.

**Methods:** A prospective cross-sectional study, including 98 nursing adolescent mothers (up to 24 years of age) and 33 nursing mothers of advanced maternal age (over 35) attending the Maternity Hospital of Presidente Prudente. Colostrum samples were obtained through manual extraction between 48 and 72 hours postpartum. The determination of fats and calories was performed by means of the Creamatocrit technique. Statistical analysis was performed using the Student's t-test or Mann-Whitney test.

**Results:** The mean age of the nursing mothers of advanced maternal age was 37 years, statistically higher than that of the adolescent mothers with a mean age of 20 years. In total, 66.3% of the adolescent nursing mothers were primiparous and 66.7% of the mothers of advanced maternal age underwent a cesarean delivery. Gestational BMI was statistically higher in the women of advanced maternal age (31.3±5.6 vs 26.06±3.6). Although the colostrum fat and cream composition was significantly higher in nursing mothers of advanced maternal age, the number of calories was similar between the groups.

**Conclusion:** Advanced maternal age influenced gestational BMI and the biochemical composition of colostrum and its components.

**Introduction**

Gestation is the result of a complex network of events, regulated by a variety of mechanisms and mediators of the endocrine, nervous, and immune systems. Three distinct and interdependent processes characterize the moment when gestation ends: cervical remodeling, myometrial contractility, and weakening and rupture of the fetal membranes, which, together, enable the expulsion of the fetus after the 37th week of gestation, characterizing a term gestation\(^1\)\(^-\)\(^3\). The gestational period is a physiological phenomenon which, in most cases, develops without
complications. However, despite the numerous studies and existing technology, the pathophysiology of gestation has not yet been fully elucidated, making it difficult to diagnose and recognize complications that may occur during this period. In this context, High Risk pregnancies are defined as “those in which the life or health of the mother and/or the fetus and/or the newborn are more likely to be affected than those of the average population considered”. These clinical conditions are associated with unfavorable socio-demographic conditions, such as maternal age less than 15 years or greater than 35 years, low educational level, mental disorders, conflictive affective situations, undue or accidental exposure to toxic or teratogenic agents, smoking, alcoholism, and contact with illicit drugs. Nowadays, incidences of pregnancy at the extremes of reproductive life, before the age of 20 years and after the age of 35, are a reality. Adolescent pregnancy is considered a substantial public health problem in some developing countries, with social and biological repercussions.

Gestation during adolescence is highly relevant due to its increasing incidence and maternal repercussions, such as social isolation and discontinuation of studies. In addition, due to biological immaturity involving the growth and development of adolescents, the perinatal outcomes can be adverse, such as an increase in newborns with low birth weight (LBW, < 2500 g), risk of intrauterine growth restriction (IUGR), an Apgar score less than 7 in the fifth minute, and a high rate of preterm birth (< 37th gestational week).

Pregnancy after the age of 35 is considered as late and has become increasingly frequent due to improved birth control, advances in assisted reproductive technology, and late marriages, among others. Pregnancy in mothers of advanced maternal age may lead to a higher risk of developing hypertension, preeclampsia, and diabetes, as well as being associated with fetal complications. The most frequent of these are chromosomal anomalies, fetal distress, neonatal death, low birth weight, and IUGR. Although these gestational and perinatal outcomes are well elucidated in the literature, no studies were found associating maternal age with the colostrum composition of these mothers.
The main strategy of the World Health Organization (WHO) to reduce unfavorable perinatal outcomes and infant mortality is the promotion of breastfeeding around the world\textsuperscript{12}. Breastfeeding can be considered a traditional practice with recognized nutritional, immunological, cognitive, economic, and social benefits for the mother/newborn binomial\textsuperscript{13}. Colostrum is the first milk secretion produced, secreted between the second and third day postpartum, and contains the nutrients necessary for the development of the neonate\textsuperscript{14}. The main components of colostrum are fats, immunoglobulins, proteins, carbohydrates, vitamins, leukocytes, lymphocytes, cytokines, lactoperoxidase, lactoferrin, and lysozyme, in addition to hormone and peptide growth promoters\textsuperscript{14,15}.

After birth the neonate requires a very high energy intake and to satisfy this energy need, colostrum has a fundamental component, which represents approximately 50% of the energy content of human milk, fats\textsuperscript{16}. The concentration of fats increases during breastfeeding, so the final milk of the feed (posterior milk) is richer in energy\textsuperscript{17}. Moran et al., 2013\textsuperscript{18}, demonstrated that the concentration of fats in the colostrum of mothers with a maternal age above 35 years was higher when compared to the group of mothers under 35 years. This finding was possibly due to the synthesis of fat and milk excretion or decreased water content, or even an association between them, however the study did not consider the extremes of reproductive age.

When comparing transitional milk between nursing mothers aged over 37 years with transitional milk of mothers under the age of 37 years, a lower fat content was observed in mothers under the age of 37\textsuperscript{19}, thus demonstrating the relevance of investigating the composition of fats in the milk of nursing mothers in the adolescent and advanced maternal age groups. Some studies in the literature relate the nutritional composition (fats) of the colostrum of nursing mothers with pathological alterations during the gestational period, such as gestations complicated by diabetes or obesity\textsuperscript{14,20}. In pregnancies affected by diabetes, the amount of fat in the colostrum of diabetic mothers is lower when compared to non-diabetic mothers\textsuperscript{14}, and among obese pregnant women a higher concentration of fat in the colostrum was identified \textsuperscript{20}. Furthermore, some studies evaluated the
concentration of fats in colostrum with the gestational age of the newborn, categorizing them into preterm (< 37th gestational week) and term (≥ 37th gestational week). The results found no relevant difference in colostrum fat composition; however the studies included mothers with an average age of 28 years. As no studies were found in the literature that relate maternal age during the period of pregnancy, adolescent mothers and those of advanced maternal age, with the colostrum fat composition of these women, investigating the composition of the milk of these mothers becomes extremely important, mainly to reinforce the incentive to breastfeed in these mothers in order to avoid premature weaning and to promote the quality of life of both mothers and their children and to stimulate the mother/newborn bond. This study aims to compare the chemical level of colostrum fats from adolescent nursing mothers and nursing mothers of advanced maternal age and to describe the gestational characteristics.

Materials And Methods
A cross-sectional study was conducted with adolescent mothers and those of advanced maternal age and their newborns who attended the maternity unit of the Regional Hospital of Presidente Prudente, SP, Brazil, from March 2017 to July 2018. The work was approved by the Research Ethics Committee of the Universidade do Oeste Paulista, UNOESTE, Presidente Prudente, SP, Brazil, CAAE 67772617.8.0000.5515. The participants signed the informed consent form and the person responsible for the nursing mother under the age of 18 authorized the participation in the research by signing the Informed Consent Form and the Free and Informed Consent Form according to the Ethics in Research with Human Beings criteria as per Resolution no. 466/2012 of the National Health Council.

Subjects
Nursing mothers and their newborns were recruited using the following inclusion criteria: gestational age at birth between 37 and 41 weeks and negative serological reactions for hepatitis, human immunodeficiency virus (HIV) and syphilis. Twin pregnancies, fetal malformations or loss of data regarding delivery and the neonatal period were the exclusion and/or discontinuity criteria. Subjects were divided in two groups of study: adolescent mothers (between 10 and 24 years old).
and advanced maternal age (over 35 years of age). Mothers aged between 25 and 34 years old were considered the control group.

The weight classification of the newborns was small for gestational age (SGA), appropriate for gestational age (AGA), and large for gestational age (LGA) (weight/gestational age ratio) according to the service protocol.

**Colostrum collection**

The colostrum was collected by manual expressing, always in the morning (from 8 to 10 am) and in the interval between two feedings, considering the period between 48 and 72 hours after delivery. Half of the samples from each study group and the control group were collected at 48 h postpartum and half at 72 h postpartum in order to avoid variations in the fat concentrations over time. A maximum colostrum volume of 10 mL was collected into sterile plastic tubes, immediately transported to the laboratory and then frozen and stored at -80°C until analysis.

**Determination of fat concentration**

After collection, the colostrum was submitted to fat determination using the Creamatocrit method as described by Lucas et al. (1978)^24^. The colostrum samples were placed in capillary tubes in triplicate and centrifuged at 12,000g for 15 minutes. After centrifugation, the cream was separated from the whey. The length of the cream column and total product column [cream column + serum column, expressed in millimeters (mm)] were measured. To determine the fat content and the calorie concentration, after performing the cream column and total column measurements, the following formulas were used: 1. Fat content = % cream (mm) - 0.59 / 1.46, where the % cream = cream column (mm) x 100/total column (mm); Kcal/L = (66.8% cream) + 290.

**Statistical analysis**

Data on the sociodemographic and gestational variables were submitted to the $X^2$ or Fisher's exact test, or to the Kruskal-Wallis non-parametric test for comparison between the two study groups, after analysis of data normality by the Kolmogorov-Smirnov test. The concentration of fat and calorie in
colostrum was analyzed by the Kruskal-Wallis test, followed by the Mann-Whitney test test. Statistical analysis was performed using Graph Pad Prism software, version 6.0 (San Diego, CA), and the level of significance adopted for all tests was 5%.

Results

The sociodemographic and obstetric data are described in Tables 1 and 2. Fat and calorie dosages are presented in box plots in Figures 1, 2, and 3.

Table 1 presents the demographic characteristics of the mothers in the extremes of reproductive age: adolescents and advanced maternal age. As expected, the median maternal age was statistically lower in the adolescents ($p<0.0001$) compared to nursing mothers of advanced maternal age. The predominant marital status was a stable relationship in the adolescent group with 66 cases and in mothers of advanced maternal age 30 women were single ($p = 0.008$). The smoking habit was highlighted in the advanced maternal age group (9 cases) compared to the group of adolescent mothers (7 cases) ($p=0.0047$).

Table 1. Socio-demographic characteristics of nursing adolescent mothers and those of advanced maternal age (AMA) included in the study.
| Characteristics                      | Adolescents (n=98) | AMA (n=33) | p      |
|--------------------------------------|--------------------|------------|--------|
| **Maternal characteristics**         |                    |            |        |
| Age (years)
\(^{a}\)                | 20 (12-24)         | 37 (35-45) | <0.0001* |
| Ethnicity\(^{b}\)                   |                    |            |        |
| White                               | 43 (43.9)          | 12 (36.4)  | 0.47   |
| Non-white                           | 55 (56.1)          | 11 (63.6)  |        |
| Civil status\(^{b}\)                |                    |            |        |
| Stable relationship                 | 66 (67.4)          | 3 (9.0)    | 0.008* |
| Single                              | 32 (32.6)          | 30 (91.0)  |        |
| Profession\(^{b}\)                  |                    |            |        |
| Paid                                | 20 (20.4)          | 17 (51.5)  | 0.0006*|
| Unpaid                              | 78 (79.6)          | 16 (48.5)  |        |
| Smoking habit\(^{b}\)               |                    |            |        |
| Yes                                 | 7 (7.2)            | 9 (27.3)   | 0.0047*|
| Mother in Relationship with Smoker\(^{b}\) |                |            |        |
| Yes                                 | 38 (37.8)          | 19 (57.6)  | 0.06   |
| Stylist\(^{b}\)                     |                    |            |        |
| Yes                                 | 2 (2.0)            | 1 (3.0)    | 0.57   |
| Physical activity practice\(^{b}\)  |                    |            |        |
| Yes                                 | 10 (10.2)          | 2 (6.0)    | 0.72   |
| Use of illicit substances\(^{b}\)   |                    |            |        |
| Yes                                 | 5 (5.1)            | 4 (3.0)    | 1.00   |
| Any type of disease\(^{b}\)         |                    |            |        |
| Yes                                 | 17 (17.3)          | 9 (27.3)   | 0.21   |

\(^{a}\) Mann-Whitney test, median (minimum – maximum)

\(^{b}\) \(X^{2}\) test or Fisher’s Exact test, n (%)

* Statistically significant \((p< 0.05)\)

Table 2 describes the gestational and obstetric characteristics of the adolescent nursing mothers and those of advanced maternal age included in the study. The body mass index at the beginning and
end of gestation was statistically higher in the advanced maternal age group \((p<0.0001)\). The parity variable presented a higher index of multiparous women in the advanced maternal age group and primigravidae in the group of adolescent mothers \((p<0.0001)\). Regarding the type of delivery, there was a contrast between the groups, as in the advanced maternal age group, cesarean delivery predominated and in the adolescents, vaginal delivery \((p = 0.0009)\). The parity variable showed a higher number of primigravidae in adolescent mothers (65 cases).

Table 2. Gestational and obstetric characteristics of nursing adolescent mothers and those of advanced maternal age (AMA) included in the study.

| Characteristics                          | Adolescents \((n=98)\) | AMA \((n=33)\) | \(p\)   |
|----------------------------------------|------------------------|----------------|---------|
| **Gestational Characteristics**        |                        |                |         |
| Contact with toxic substances during pregnancy\(^b\) | 1 (1.0)                | 1 (3.0)        | 0.44    |
| Body mass index (BMI)\(^a\)            |                        |                |         |
| Start of gestation                     | 21.6 (14.3-42.9)       | 28.6 (19.5-40.6) | \(< 0.0001^*\) |
| End of gestation                       | 26.6 (19.2-63.9)       | 31.3 (24.6-51.4) | \(< 0.0001^*\) |
| Weight gain (kg) \(^b\)                | 12 (-1.0 - 2.35)       | 9.5 (-0.55 - 32.0) | 0.056   |
| **Gestational Disease \(^*\)**         |                        |                |         |
| Yes                                    | 45 (45.9)              | 15 (45.5)      | 0.96    |
| **Parity**                             |                        |                |         |
| Primigravida                           | 65 (66.3)              | 2 (6.1)        | \(< 0.0001^*\) |
| Secundigravida                         | 20 (20.4)              | 7 (21.2)       |         |
| Multiparous                            | 13 (13.3)              | 24 (72.7)      |         |
| **Number of prenatal consultations\(^b\)** |                        |                |         |
| \(\leq 7\) consultations              | 29 (49.2)              | 30 (73.2)      | 0.65    |
| > 7 consultations                      | 24 (40.7)              | 9 (21.9)       |         |
| **Obstetric Characteristics**          |                        |                |         |
| Gestational age at birth (weeks, days)\(^a\) | 39w (36w06d – 41s)    | 38w (36w – 41w03d) | 0.18    |
| Type of birth\(^b\)                    |                        |                |         |
| Vaginal                                | 66 (67.4)              | 11 (33.3)      | 0.0009  |
| Cesarean                               | 32 (32.6)              | 22 (66.7)      |         |
Discussion

The present study compared the composition of colostrum fats in nursing mothers at the extremes of reproductive age with term gestation as well as describing the socio-demographic and obstetric characteristics.

Despite recent studies on the subject, little is known about the influence of maternal age on the composition of colostrum fats at the extremes of reproductive age. Lubetzky et al., 2015\textsuperscript{25}, identified in their study that a maternal age greater than 35 years influences the composition of human colostrum, corroborating with the findings of the present study which demonstrated a difference in fat composition and percentage of cream in nursing mothers of advanced age ($p < 0.0001$), while for Costa and Sabarense, 2010\textsuperscript{26} maternal age is a positive modulator in the micronutrient composition of human milk, thus highlighting the data found in the present study.

Recently, transformations in family structures have resulted in the growth of uniparental families, especially single women, and as a consequence there has been an increase in maternal responsibility. Our study revealed that in mothers with advanced maternal age, the predominant marital status was single (91\% of mothers) compared to adolescent mothers ($p = 0.008$), similar to the findings described by Marin, A. & Piccinini, C. A., 2009\textsuperscript{27}. This may have occurred due to the high divorce rates or, alternatively, the option of these mothers to have children while single.

When analyzing the variable profession of nursing mothers, it can be observed that in the adolescent group unpaid activity prevailed ($p = 0.0006$); unfavorable social conditions are directly related to the quality of maternal nutrition due to the high costs of balanced nutrition\textsuperscript{28}. During the gestational period, food needs increase to support the growth and development of the fetus and maintain the maternal metabolism. Thus, dietary and nutritional recommendations should be adapted to each pregnant woman considering individual differences\textsuperscript{29}, and therefore the adolescent nursing mothers in our study may have presented a decrease in colostrum cream and fat concentrations due to
unfavorable conditions and consequent incorrect eating during the gestational period, as well as due to the characteristics of adolescence.

Recent studies have demonstrated that maternal smoking influences the composition of human colostrum due to the presence of nicotine, which may alter the formation of some cytokines present in human milk, and thus, the children of smokers are more likely to develop infections, added to which nicotine alters milk production by reducing the amount of milk produced, hindering the breastfeeding process and reducing breastfeeding time in nursing mothers.\textsuperscript{30,31} Agostini et al., \textit{2013} \textsuperscript{32} in their study, analyzed colostrum of nursing mothers and identified a reduction in the content of breast milk fat. However, this study did not consider the extremes of reproductive age. In the present study, 27.3\% of mothers of advanced maternal age had a smoking habit and this group of women, even considering age and smoking, presented an increase in the composition of colostrum fats, diverging from the data presented by the above study.

Among the main factors that contribute to the composition of colostrum and maternal milk are socio-demographic factors, ethnicity, diet, age, gestational variables such as body mass index, parity, duration of gestation, and stage of lactation. The study performed by Sinanoglou et al., \textit{2017} \textsuperscript{33} evaluated the possible impact of socio-demographic variables on the composition of colostrum fatty acids and fats in Greek nursing mothers and showed that the fatty acid and colostrum fat profile depended mainly on nationality and age, not on the type of delivery or maternal gestational BMI, and also found that the colostrum of mothers with advanced maternal age presented lower fat content. These findings differ from those found in the present study as we demonstrated that these maternal variables were not significant to alter the colostrum composition of the study population and, also in divergence, the mothers over 35 years old presented a higher fat concentration in the colostrum.

Mangel et al., \textit{2017} \textsuperscript{34} affirm in their study that the composition of human colostrum fats is not influenced by maternal BMI and also, for Sinanoglou et al., \textit{2017} \textsuperscript{33} the composition of colostrum may not be influenced by BMI. In the present study, we diverged from the above findings as we found a difference between the BMI of adolescent mothers and advanced maternal age as well as in the
colostrum of these mothers ($p < 0.0001$).

Azeredo, 2013$^{35}$, in their study, state that parity does not influence the composition of human milk fats. Our findings demonstrate that there were a greater number of multiparous women in the advanced age group compared to adolescent mothers, and considering parity and age, there was a difference in the colostrum composition of these nursing mothers.

When comparing the delivery method, Dizdar et al., 2013$^{36}$ demonstrated that the composition of human colostrum fats is similar, but the study did not consider the extremes of reproductive age. Our study demonstrated that mothers with advanced maternal age had a predominance of cesarean delivery and still had a higher concentration of fats in colostrum when compared to the group of adolescent mothers with 67.4% vaginal deliveries and a lower concentration of colostrum.

Dritsakou et al., 2016$^{37}$ demonstrated in their study that advanced maternal age is related to the composition of human colostrum and showed an increase in fats and other components. These findings corroborate with the data found in the present study which also showed a higher concentration of fats in the colostrum of mothers with advanced maternal age. The increase in the fat concentration in the colostrum of mothers over 35 years old may be related to the reduction in milk secretion and, thus, it is still considered that the biochemical composition is not determined by this factor alone.

Fat composition may be influenced by obstetric variables such as gestational age and gestational diseases. Studies have shown that colostrum fat composition is decreased in diabetics and hypertensives$^{14}$. When comparing gestational age in preterm and term deliveries, there is no difference in colostrum fat composition$^{21}$. These studies are similar to the data elucidated by the present study where obstetric variables such as gestational age, number of prenatal consultations, gestational diseases, and contact with substances were not statistically significant ($p > 0.05$).

Therefore our findings provide knowledge on colostrum fat composition at the extremes of reproductive age, considering the scarcity of data in the literature dealing with the subject. We also show that, unlike the initial hypothesis of the project, the colostrum of nursing mothers with advanced
maternal age had a higher concentration of fats and cream, but did not differ in number of calories when compared with adolescent mothers, this fact may have occurred due to the biological immaturity of adolescent nursing mothers.

Conclusion

Advanced maternal age influenced gestational BMI and the biochemical composition of colostrum and its components. However, further studies should be conducted on this theme to provide better understanding of colostrum composition at the extremes of reproductive age.

Clinical Significance

Our study brings new information about socio-demographic, gestational outcomes and colostrum fat content by adolescent and advanced maternal age pregnancies. Our findings demonstrated that maternal age influenced the mode of delivery, BMI and colostrum composition and its components.

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**Declarations**

**Ethics approval and consent to participate:** The work was approved by the Research Ethics Committee of the Universidade do Oeste Paulista, UNOESTE, Presidente Prudente, SP, Brazil, CAAE 67772617.8.0000.5515. The participants signed the informed consent form and the person responsible for the nursing mother under the age of 18 authorized the participation in the research by
signing the Informed Consent Form and the Free and Informed Consent Form according to the Ethics in Research with Human Beings criteria as per Resolution no. 466/2012 of the National Health Council. Confidentiality was ensured by assigning an identification number to substitute personal identifiers.

**Consent for publication.** Not applicable.

**Availability of data and materials:** All datasets used and/or analysed during the current study are available from the text and other informations you are need just ask to corresponding author.

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**Figures**
Box plot with respect to quantification of the percentage of colostrum cream of adolescent and advanced maternal age (AMA) mothers. In the box-plot, the quadrants represent 25 to 75% of the values, the median is represented by the horizontal trace and the bars correspond to the 10th and 90th percentiles (*Mann-Whitney test, p=0.004).
Box plot with respect to quantification of the % of colostrum fats of adolescent and advanced maternal age (AMA) mothers. In the box-plot, the quadrants represent 25 to 75% of the values, the median is represented by the horizontal trace and the bars correspond to the 10th and 90th percentiles (*Mann-Whitney test, p=0.05).
Figure 3

Box plot with respect to quantification of the Kcal of colostrum of adolescent and advanced maternal age (AMA) mothers. In the box plot, the quadrants represent 25 to 75% of the values, the median is represented by the horizontal trace and the corresponding error bars the 10th and 90th percentiles. (*Mann-Whitney test, p=0.63).