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The Difference of Creamatocrit in Colostrum between Preterm and Term Delivery Women

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ABSTRACT

Objectives: To demonstrate the difference of fat concentration in colostrum between mothers with preterm delivery and mothers with term delivery, using creamatocrit (CRCT) as an analytic method.

Materials and Methods: The enrollment period was from January to June, 2016. Two hundred and twenty nursing mothers were divided into 2 groups based on gestational age, 44 preterm and 176 term delivery mothers in the first and second groups, respectively. Colostrum was collected from all participants on the third day postpartum before transferred into capillary tubes and sorted into groups to perform CRCT. CRCT were compared between groups using univariable and multivariable regression analysis to clarify indicators that effected CRCT.

Results: The demographic data were no different in terms of age, blood pressure, body mass index, body weight, height, parity and types of delivery. As from CRCT analysis, mean value of CRCT from colostrum in samples from preterm and term groups were 5.89±2.20% and 6.59±2.50%, ($p > 0.05$). Secondary analysis of age, maternal total weight gain, body mass index at delivery, hypertension, diabetes, parity, route of delivery, hematocrit at delivery and newborn birth weight were no difference.

Conclusion: By using CRCT analysis, the outcome of fat concentration for both delivery groups showed no differenceand no effect from age, body weight, total weight gain during gestation, parity, maternal blood pressure and types of delivery.

Keywords: breastfeeding, creamatocrit, CRCT, colostrum, human milk, nursing mother, preterm, term

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การศึกษาความแตกต่างของครีมาโตคริตใน colostrum ระหว่างมารดาที่คลอดก่อนกำหนดและคลอดครบกำหนด

อัครเดช โอภาสพสุ, จรรยา ธำรงวุฒิกุล, วัชรินทร์ เฉิดฉิ้ม

บทคัดย่อ

วัตถุประสงค์: *เพื่อศึกษาหาความแตกต่างของความเข้มข้นของไขมันในน้ำนมมารดา ระหว่างมารดาที่คลอดก่อนกำหนดและคลอดครบกำหนด โดยการใช้ครีมาโตคริต*

วัสดุและวิธีการ: *เลือกตัวอย่างกลุ่มผู้วิจัย จากมารดาที่คลอดในโรงพยาบาลพระปกเกล้า จังหวัดจันทบุรี ระหว่างเดือนมกราคม ถึง มิถุนายน พ.ศ. 2559 แบ่งเป็น 2 กลุ่ม ได้แก่ มารดาที่คลอดก่อนกำหนด 44 ราย และคลอดครบกำหนด 176 ราย ศึกษาโดยการเก็บ colostrum จากมารดาในวันที่ 3 หลังคลอด โดยตัวอย่างน้ำนมจะถูกเก็บใส่หลอดแคปปิลารี่ และปั่นครีมาโตคริต ผลที่ได้จะถูกเปรียบเทียบระหว่างกลุ่มโดยวิธีการทางสถิติ ปัจจัยต่างๆ จากอาสาสมัครจะถูกนำมาวิเคราะห์ทางสถิติเพื่อหาปัจจัยที่อาจส่งผลต่อครีมาโตคริต*

ผลการศึกษา: *การศึกษาทางสถิติไม่พบความแตกต่างอย่างมีนัยสำคัญของข้อมูลเชิงประชากร ไม่ว่าจะเป็นปัจจัยด้าน อายุ ความดันโลหิต น้ำหนักตัว ส่วนสูง ดัชนีมวลกาย จำนวนการตั้งครรภ์ และวิธีการคลอด ค่าเฉลี่ยของครีมาโตคริตจากน้ำนมมารดา คือ 5.89±2.20 ในกลุ่มมารดาที่คลอดก่อนกำหนด และ 6.59±2.50 ในกลุ่มคลอดครบกำหนด โดยไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติ และจากการวิเคราะห์เพื่อหาปัจจัยที่อาจส่งผลต่อครีมาโตคริตไม่พบว่ามีปัจจัยใดจากการศึกษานี้ส่งผลต่อครีมาโตคริตอย่างมีนัยสำคัญ*

สรุป: *จากการศึกษาความแตกต่างของน้ำนมมารดาด้วยครีมาโตคริต ระหว่างมารดาที่คลอดก่อนกำหนดและคลอดครบกำหนด ไม่พบว่ามีความแตกต่างอย่างมีนัยสำคัญทางสถิติ และในการศึกษายังไม่พบปัจจัยทางประชากรที่ส่งผลกระทบต่อครีมาโตคริตอันประกอบด้วย อายุ น้ำหนักตัวมารดา น้ำหนักตัวที่ขึ้นระหว่างตั้งครรภ์ ความดันโลหิต จำนวนครั้งที่ตั้งครรภ์ และวิธีการคลอด*

คำสำคัญ: *ครีมาโตคริต, น้ำนมแม่, มารดาคลอดก่อนกำหนด*

Introduction

Since 2001, World Health Organization (WHO) has promoted "Exclusive Breastfeeding" for at least 6 months due to the fact that breast milk has huge benefit for all infants[1]. Additionally, breastfeeding delivers appropriate nutrition source and immune support for newborn. Especially, for preterm or hospitalized newborns, they need higher energy and nutrient than those infants with term delivery required in order to imitate intrauterine development, prevent starvation and extrauterine growth restriction[2]. Lipid is the main nutrient in breast milk.Its amount varies by gestational age at delivery[3-7]. It can be measured by differnt methods such as mid-infrared spectroscopy which is accurate but required special technique[5].

Breastfeeding is the issue raised internationally. Our neighbor such as Australia and China had reported in similar trend that breastfeeding was precluded due to prematurity. This matter leaded the cessation of breastfeeding and early feed of supplementary food under anxiety of mother and their family[8, 9]. In 1978, the study of creamatocrit (CRCT) was raised. Breast milk samples of term delivery mothers were collected by a simply and painless method. This test required simple equipment such as hematocrit centrifuge, hematocrit reader and capillary tubes[4], which was easy for all health centers to conduct the test. The results showed that fat ratio is directly associated with the energy in breast milk. Similarly, other experiments related to colostrum had concluded in the same way[2-7, 10, 11]. So CRCT analysis is a precise and accurate method for clinical use and research.

Moltó-Puigmartí and colleagues were the very first authors- performing CRCT from frozen human milk, compared between group of very preterm, preterm and term mothers which shows significant difference with small sample number[3]. But temperature may have effect on CRCT[4]. t Vieira et al have shown decreasing effect of freezing process on CRCT[7].

According to the information above, one of the breastfeeding barriers is anxiety of prematurity affect to breast milk nutrition[8, 9]. To eliminate this effect, we had conducted the CRCT from fresh human milk, to prove whether the gestational age would effect the property of colostrum and to identifythe confounder effect from indicators to CRCT.

Materials and Methods

This cross sectional study was performed at the postpartum unit of the Obstetrics and Gynecology Department, Prapokklao Hospital, Chantaburi, Thailand. The Ethics Committee Review Board approved this study. Inclusion criteria were postpartum mothers who want to give breastfeeding, no contraindication of breastfeeding (active tuberculosis infection, HIV infection, or stillbirth) and can communicate in Thai language. Preterm mothers recruited in this study had livebirth newborn with no lower limit for gestational age. We started from recruiting the first case of preterm mother and four of term mothers subsequently, eventually we enrolled 44 preterm and 176 term mothers, all participants were given informed consents. Gestational age was confirmed by comparing last menstrual period calculation with the first gestational age calculated by ultrasonography andwas noted in the antenatal medical record.

Clinical data and were recorded and colostrum samples were collected on the third day of postpartum for creamatocrit (CRCT) testing[3, 4]. Well mixed breast milk was manually collected in glass bottle at least 30 ml from each mother according to the policy of Prapokklao Hospital. Each breast milk sample was divided into 2 capillary tubes and performed CRCT within 30 minutes. Capillary tubes were then occluded with clay for 1 centimeter depth. The hematocrit centrifuge (Boeco Hematocrit CENTRIFUGE Hc-240) was set at 3,500 rpm, and run for 15 minutes. Samples were separated into 2 layers which are creamy white and clear serum. The creamy white part of the sample was immediately measured using hematocrit reader to interpret the percentage of creamy layer by the total level of milk component. The data were recorded in mean

between samples of each participant to reduce error of optical measurement. Fat concentration was calculated from CRCT according to Lucas' equation[4].

The sample size was calculated by using STATA version 12.1, as refer to Moltó-Puigmartí C, et al, 2011[3]. The ratio between preterm milk sample and term milk sample of our study was one to four, and the power of the study was 0.8 with p-value of 0.05. Demographic data of these 2 mother groups were compared. There were 44 mothers in preterm group and 175 mothers in term group. Data were analyzed by STATA version 12.1. Fisher's exact test and t-test were applied for categorizing data and studied continuous data respectively. T-test was was used to compare CRCT and fat proportion. The data would be analyzed using univariable and multivariable logistic regression analysis in order to clarify indicators related to CRCT analysis.

Results

According to the statistical record of childbirth, there were 1,812 total deliveries from January to June 2016 at Prapokklao Hospital. Among 1,812 mothers, there were 28 mothers did not meet the selection criteria. Moreover, 100 nursing participants from preterm labor mothers refused to join the study, failed to collect their milk on third day postpartum and missing data Participants were separated into two groups, 44 mothers in preterm group, and 176 mothers in term group. (Fig. 1) A total of 220 mothers were enrolled to this study.

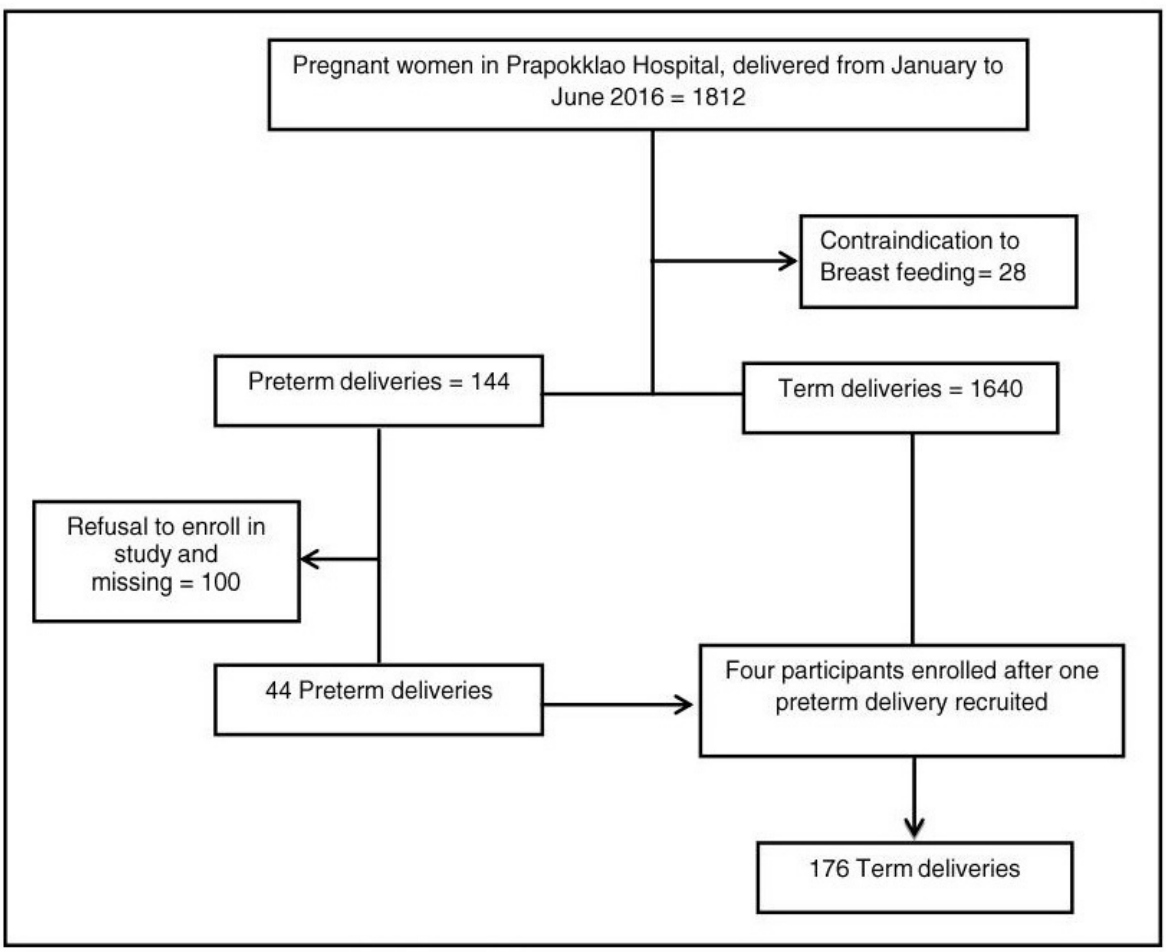

Fig. 1. Participants enrollment.

Demographic data and results of this study were demonstrated in Table 1 and Table 2 respectively. Range of gestational age of both groups were 25.14 - 36.85 in preterm group and 37.0–42.24 in term group, respectively. There was difference between preterm and term groups for age, weight, height, body mass index, parity, and route of delivery ($p < 0.05$).

This study demonstrated statistical significant difference in some variables ie women with diabetes while pregnant were 11.36% in preterm and 2.45% in term ($p = 0.02$); the data of hematocrit recorded in labor room of preterm and term group were 33.79±3.77% and 35.76±3.57% respectively ($p = 0.0015$).

Table 1. Demographic information.

Characteristic	Preterm (n=44)	Term (n=176)	p value
Age (year)*	26.27±7.73	24.94±6.59	0.24
Gestational age (weeks)*	34.43±2.73	39.13±1.27	<0.01
Maternal body weight (kg)*			
Pre-gestational	56.18±13.69	55.75±11.62	0.83
In labor department	67.14±14.73	68.06±11.41	0.65
Total weight gain (kg)	10.95±6.36	12.62±6.70	0.13
Height (meter)*	1.57±0.06	1.58±0.06	0.15
BMI (kg/m^2)*			
Pre-gestational	27.97±8.5	29.03±8.767	0.46
In labor department	33.51±10.42	35.55±9.88	0.22
Hematocrit on labor day (%)*	33.79±3.77	35.76±3.57	<0.01
Parity†			
Nulliparous (%)	34.09	30.67	0.72
Multiparous (%)	65.91	69.33	
Medical issue†			
Diabetes (%)	11.36	2.45	0.02
Hypertension (%)	9.09	7.98	0.76
Route of delivery†			0.49
Vaginal delivery‡ (%)	52.27	58.9	
Cesarean section (%)	47.73	41.1	
Baby birth weight (gram)*	2230.11±601.04	3068.05±368.47	<0.01

* Mean ± SD, and analyzed with unpaired t-test,
† Analyzed with Fisher's exact test
‡ Included; normal delivery, forceps extraction and vacuum extraction

Primary outcome of the study aimed to demonstrate the difference of CRCT value between preterm and term groups. The authors had analyzed the relation of CRCT and gestational age, as shown in Fig. 2 and Fig. 3. CRCT and gestational age had linear correlation but no statistical significant increment.

A final result was shown in Table 2. CRCT in preterm groups was 5.89±2.20%, in term group was 6.59±2.50% with a p-value of 0.09. In summary, the

analysis had shown no statistical significant. As refer to Lucas' equation, mean of fat were 36.31±15.09 g/l in preterm and 41.02±17.18 g/l in term (p-value 0.09). Average energy requirement in infant is 120 kcal/kg/day and total volume intake is 150 ml/kg/day[12]. Current recommendation for infant nutrition is 30-60% of energy should come from fat[13]. All of the participants' colostrum provided 40.85±16.97 and 46.48±19.39 percent of energy from fat in preterm and term group respectively (Table 2).

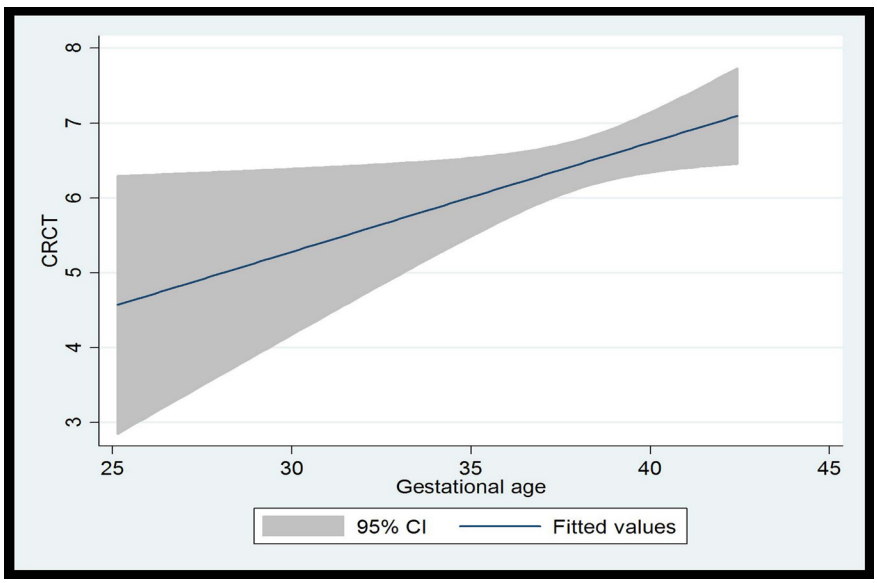

Fig. 2. Linear prediction of gestational age (GA) and CRCT

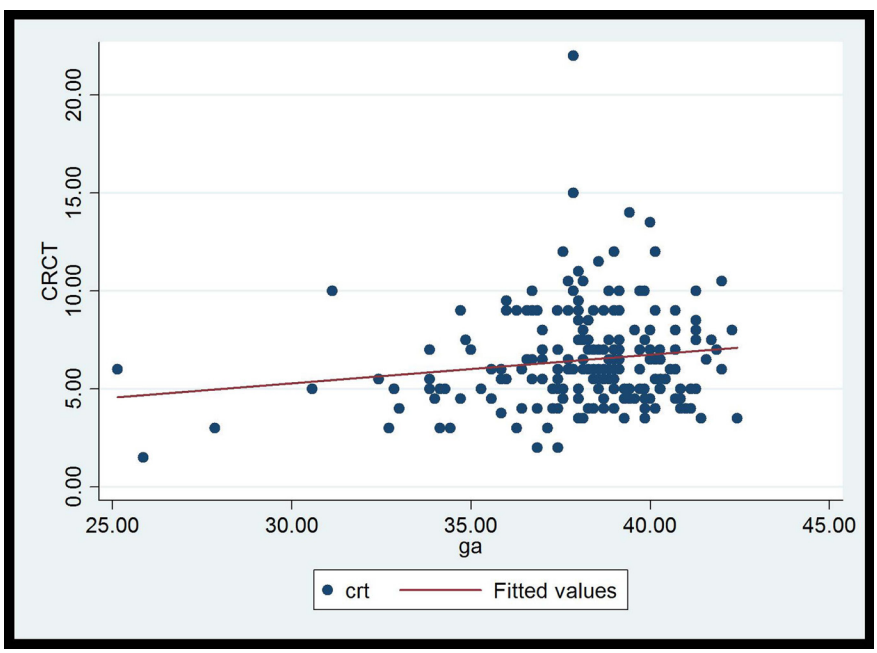

Fig. 3. Scattered plot of gestational age (GA) and CRCT

Table 2. Results of study.

Outcome	Preterm (n=44)	Term (n=176)	p value
Creamatocrit (%)*	5.89±2.20	6.59±2.50	0.09
Fat content (g/l)†	36.31±15.09	41.02±17.18	0.09
Estimate volume intake (ml)‡	334.52±90.15	460.32±55.88	< 0.01
Total fat intake per day (g)	12.73±7.28	19.12±8.36	< 0.01
Percent of energy from fat intake‡	40.85±16.97	46.48±19.39	0.07

* Mean of 2 tubes represented in Mean ± SD, and analyzed with unpaired t-test.
† Based on equation of Lucas A.[4]
‡ Estimated 120 kcal/kg of average total energy requirement[12],
‡ Estimated 150 ml/kg/day of average total volume requirement[12]

Univariate analysis was used for demographic parameters and factors such as diabetes during pregnancy and hematocrit at delivery. Indicators included maternal age, total weight gain, body mass index at delivery, hypertension, diabetes, parity, route of delivery, hematocrit at delivery and newborn birth weight has no statistical significant effect on CRCT. CRCT level would vary in proportional to the change of every 1 unit of study indicators (Table 3), e.g., CRCT will increase for 0.015% of increasing 1% of hematocrit but without statistical significant difference. No indicators affected to CRCT.

The mean value of CRCT of multiparous group was higher than nulliparous group by 0.753 (95%CI 0.024 - 1.532), which practically addressed as tend to be significant difference (p-value 0.058). All of these indicators from multivariable model provided the power of analysis 0.64-0.73. Larger sample size are needed.

Table 3. Multivariable logistic regression analysis.

Factors affected to CRCT*	Coefficient	95% CI	p value
Age	-0.043	(-0.099, 0.011)	0.123
Maternal total weight gain	0.004	(-0.047, 0.054)	0.876
Body mass index at delivery	-0.032	(-0.074, 0.009)	0.125
Hypertension	-0.506	(-1.821, 0.808)	0.449
Diabetes	-0.423	(-2.152, 1.304)	0.629
Parity	0.753	(0.024, 1.532)	0.058
Route of delivery	0.252	(-0.401, 0.907)	0.447
Hematocrit at delivery	0.015	(-0.107, 0.076)	0.735
Newborn birth weight	< 0.001	(-0.000, 0.001)	0.013
Constance	5.047	(-4.204, 14.298)	0.283

* CRCT = creamatocrit

Discussion

Demographic data were no difference. This study showed that both of preterm and term CRCT values of colostrum had no difference (5.89±2.20

and 6.59±2.50 respectively, p= 0.09).

Moltó-Puigmartí et al (2011) had done the study on CRCT values from processed human milk according to gestational age. Their results showed that the CRCT values in colostrum of very preterm (< 30 wk of gestation), preterm (30-37 wk of gestation) and term (> 37 wk of gestation) were 4.05 ± 1.62, 2.58 ± 1.88 and 2.60 ± 1.48, respectively (p < 0.05)[3]. However, there was no difference on colostrum's energy and nutrition among groups in this study, after control for temperature. Vieiraet al compared fresh human milk with processed milk under freezing and found that CRCT was marked decrease from 8.9% in fresh human milk to 3% in processed milk[7]. Temperature has major effect on CRCT[4, 7].

Lucas et al s (1978), introduced the use of CRCT for evaluating fat concentration of breast milk[4]. Despite of the precision of CRCT, the other experimenters had doubted that CRCT might reveal overestimated fat. O' Neill EF et al (2013) suggested that mid-infrared spectroscopy is more accurate than CRCT[5]. However, we do not have facility for spectroscopy. CRCT is a simple method requirusing simple equipment[3-7] with high accuracy. Currently there has been a new method to measure CRCT with the same accuracy, less noise and lighter[10, 11]. Above all, traditional CRCT is still a very simple method and is suitable for all health centers.

According to pediatric nutritional practice, average energy requirement in infant is 120 kcal/kg/day, total volume intake is 150 ml/kg/day. Bovine-base milk formula for term and preterm infant were tried to be the same as human milk. Bovine-base milk formula gives 20 kcal/oz. for term formula and 24 kcal/oz. for preterm formula[12-15]. This study showed that most of breast milk provides more energy than formula milk. About 30-60% of energy for preterm infant should come from fat[13]. Colostrum provided 40.85±16.97% and 46.48±19.39% of energy from fat in preterm and term group, respectively. Another neonatal nutrition issue is necrotizing enterocolitis mostly occurred to infants who were fed with bovine-base milk[14, 16]. Importantly, according to our findings, breastfeeding should be the first energy nutrient option for all infants.

Liu P et al, reported that mothers who worry about insufficient milk supply has HR of 1.25 - 1.66 to conclude breastfeeding and early supplement feed at only 6 months after childbirth[9]. Our results should reduce maternal concernabout breast milk nutrition of preterm newborn.

The hematocrit centrifuge using in this study wasn't general available in other departments. Further study should compare the outcome of other types of centrifuge.

In developed country, milk bank and milk donor plays an important role as supplement nutrition[2, 3]. Moltó-Puigmartí advised to give donor milk of the same gestational age to infants[3]. However, both nursing mother groups can provide breast milk with non-significant fat concentration, represented as CRCT. We suggest that every infant should receive breastfeeding as primary nutrition supplement from their own mother and giving donor milk regardless of gestational age severe malnutrition babies. Adequate energy intake is not the only factors for newborn health condition. Investigation on others nutrition or modifiable factors affected CRCT for neonatal nutrition development need to be examined.

Further study may show that breast milk has different state in postpartum period included colostrum, transitional milk and mature milk[3, 12], following up and further CRCT testing to these variant should encourage. More accurate timing of milk sample collection is interesting. The authors avoid of nursing activity increment according to this hospital based study.We integrated this study protocols in regular hospital nursing activity.

Moreover, obstetricians should take a key role of encouraging mother to achieve exclusive breastfeeding[1, 17]. As mentioned, breast milk is the best nutrition source and immune support for every single infant[2-7]. Moreover, lactation has a benefit to maternal health such as reduce retention weight after delivery, decrease risk of cardiovascular event, delay

the reduction of bone mineral density[18-21]. Apart from health benefit, breastfeeding is a very cost-effective nutrition of newborn[22, 23].

Conclusion

This study used CRCT as a quantitative nutrient measurement in breast milk. Women can provide adequate nutritional support to their own child, because there was no difference on CRCT between groups of mothers at different gestational age. Both groups of infant were fed with proper requirement by body weight ratio as recommendation[12].

Acknowledgement

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Potential conflicts of interest

The authors declare no conflict of interest.

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