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Comparison of Microbiotic Pattern in Gastointestinal Tract from Neonatus Born by Spontaneous Delivery with and without Early Breastfeeding.

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ABSTRAK

Kejadian kematian neonatal dapat ditekan sekitar 22% pada bayi dengan ASI awal pada satu jam pasca kelahiran, mungkin karena berpeluang lebih besar untuk mendapatkan kolostrum. Mulai pada saat bayi lahir, usus steril akan segera dihuni mikroba dalam beberapa jam. Usia kehamilan, cara kelahiran, dan diet merupakan faktor penting yang mempengaruhi pola mikrobiota. Tujuan dari penelitian ini adalah untuk menguji perbedaan pola mikrobiotik pada saluran pencernaan neonatus yang lahir dengan persalinan spontan pada ibu yang menyusui dini dan yang tidak. Sampel apusan rektum diperoleh dari neonatus lahir spontan di Rumah Sakit Dr Soetomo dengan usia kehamilan cukup waktu, 30 sampel dari neonatus dengan 30 sampel menyusui dini dari neonatus tanpa menyusui dini awal 2 jam, 12 jam dan 24 jam setelah melahirkan, dan kemudian diuji untuk keberadaan mikroba aerobik, anaerobik dan mikroaerofilik dengan metode standar di Laboratorium Mikrobiologi, Dr Soetomo, selama bulan Februari-Mei, 2012. Perbedaan yang signifikan ditemukan dalam jumlah koloni mikroba aerobik, dimana hitungan pada kelompok menyusui dini lebih tinggi dibanding kelompok yang tidak menyusui dini. Jenis mikroba adalah E. coli, Enterobacter, Staphylococcus epidermidis *dan Streptococcus* faecalis. *Pada sampel 12 jam, koloni hitungan Lactobacillus lebih tinggi pada kelompok menyusui dini daripada kelompok yang tidak mrnyusui dini. Pada 24 sampel jam jumlah koloni mikroba anaerob di kelompok bukan menyusui dini secara signifikan lebih tinggi dibandingkan dengan kelompok menyusui dini, dan jenis bakteri adalah* Bacteroides, Bifidobacteria *dan* PeptoStreptococcus. *Kesimpulan, prosedur menyusui dini menguntungkan dalam hal pola mikrobiotik, baik dalam variasi jenis dan jumlah koloni mikroba.* ***(MOG 2012;20:23-29)***

Kata kunci: *pola mikrobiota, neonatus, kelahiran spontan, menyusui dini,* Lactobacilli

ABSTRACT

Incidence of neonatal death is suppressed about 22% on babies with early breastfeeding in one hour post birth, possibly due to bigger opportunity to get colostrum. Starting at the time the baby was born, the sterile gut will be colonized soon by microbes in several hours. Gestational age, different modes of delivery, and diet are important factors which influence the pattern of microbiota. The aim of this study was to examine the difference of microbiotic pattern in gastrointestinal tract of neonatus born by spontaneous delivery between the ones with and the ones without early breastfeeding. Rectal swab samples were obtained from neonatus born by spontaneous delivery at Dr. Soetomo Hospital with termed gestational age, 30 samples from neonates with early breastfeeding and 30 samples from neonates without early breastfeeding at 2 hours, 12 hours and 24 hours after delivery, and then were tested for the presence of aerobic, anaerobic and microaerophylic microbes with standard methods at Microbiology Laboratory, Dr. Soetomo Hospital, during February –May, 2012. In 2-hour samples significant difference was found in the count of aerobic microbial colony, in which the count in early breastfeeding group was higher than that in non-early breastfeeding group. The microbial types were E. coli, Enterobacter, Staphylococcus epidermidis *and Streptococcus* faecalis. *In 12-hour samples, colony count of Lactobacillus was higher in early breastfeeding group than in non-early breastfeeding group. In 24 hour samples the count of anaerobic microbial colony in non-early breastfeeding group was significantly higher than that in early breastfeeding group, and the bacterial types were* Bacteroides, Bifidobacteria *and* PeptoStreptococcus. *In conclusion, early breasfeeding procedure is beneficial in terms of microbiotic pattern, either in types variation and numbers of microbial colony.* ***(MOG 2012;20:23-29)***

Keywords: *microbiotic pattern, neonatus, spontaneous delivery, early breastfeeding,* Lactobacilli

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INTRODUCTION

Incidence of neonatal death is suppressed about 22% on babies with early breastfeeding in one hour post birth. Clemens et al. in 1999 in his research in Egypt concluded that early breastfeeding was associated with the declining rate of diarrhea in 6 first months of baby's age, possibly due to bigger opportunity to get colostrum, and the initiation of development in colonization of normal microbes in neonatus gastrointestinal tract earlier, so the immunity of neonatal gastrointestinal mucosa will rise earlier.[1]

The mechanism which underlies how the early breastfeeding can avoid the neonatal death has not been clearly explained yet. It has been noticed that neonates are susceptible to infection because their immune system has not yet completely mature. The development of body's immune response depend on host, environment, and agent factors, indeed needs microbial antigen sensitization to form innate and adaptive immune response to recognize the next pathogen challenge.[2]

Gastrointestinal tract is a massive organ whichdeals directly with microbes in a large number continuously, either from food or environment. With its surface area reaching 300 m^2, gastrointestinal tract mucosa has largest exposure to the world outside, therefore lymphoid system of gastrointestinal tract masters two-third of the whole human immune system.In a number of more than 10^{14}, commensal bacteriahas a big role in gastrointestinal tract immune system.[2]

Intestinal microbiotic pattern is mostly determined in neonatal age, so this period has an important role in the development of microbiotic pattern which will get stable when the kid reaches several months of age. Starting at the time he was born, the sterile gut will be colonized soon by microbes in several hours. Gestational age, different modes of delivery, and diet are important factors which influence the pattern of microbiota. A baby who was born through the vagina will get microbiotic pattern from vaginal flora and maternal feces. While the baby delivered abdominally, the biggest influence comes from the environment such as operation room and the rescuer's gloves.[3]

The goal of the research is to prove that there is indeed difference of microbiotic pattern among the babies which delivered normally with and without early breastfeeding initiation. After the baby is contaminated by the mother's vaginal flora and feces by normal delivery, a baby with early breastfeeding will be exposed to the normal flora of his mother's chest, including the flora from the mother's breast and nipple. By the additional exposition, it is expected that the baby's gastrointestinal tract with early breastfeeding initiation will be colonized by microbiote earlier and with more types of bacteria.

MATERIALS AND METHODS

The study was performed at Soetomo Hospital. Sample collection was performed in Delivery Room andNICU of Emergency Unit, which were expected to have the same environment. After fulfilling the inclusion and exclusion criterions and requesting for the parents' written permission to participate in the study, collection was performed in a blind method, i.e., samples were taken without noticing whether they had run early breastfeeding initiation or not. The samples were obtained by rectal swab on two hours, 12 hours, and 24 hours after birth. The first samples were taken two hours after birth with consideration that if early breastfeeding was done by one hour after birth, bacterial colonization in the rectum would be assesed an hour later. The second collection was done at 12 hours after birth is appropriate with theory that bacterial colonization will reach rectum around 3 to 7 hours, and the third collection, on 24 hrs after birth, is meant for assessment of the growth differ-ence on the optimal colonization time, when bacterial colony could reach the number more than 1014. The periodic sampling methods was done following some previous studies.[3,4]

The sample size was considered according to Central Limit Theorybecause until today no similar study was done previously, i.e., studies about gastrointestinal tract's microbiota of the neonatus with early breastfeeding initiation, so the necessary minimal number of sample was 30 on early breastfeeding initiation group and 30 in control group (non-early breastfeeding group). Each sample collection was performed three times, at two hours, 12 hours and 24 hours after birth, so that the total samples collected was 180 samples.

Microbiological assessments were done in Laboratory of Clinical Microbiology at 5th floor of Gedung Diagnostik Center, Soetomo Hospital, Surabaya. Determination of dominant bacteria was done by Gram staining followed by culture in media for aerobic, anaerobic and microaerophylic bacteria and colony count (CFU/ml).

RESULTS AND DISCUSSION

Considering that gestational age was one of factors which influence the establishment ofintestinal microbiotic pattern.[5,6] the study was limited to aterm birth with gestational age over 37 weeks and neonatal weight 2500-4000 grams only.

The babies were divided in 2 groups, i.e. the ones with early breastfeeding initiation and the ones without early breast feeding initiation.There was no significant difference on both study groups (=0.087). At the early breastfeeding group, most babies obtained diet by breast milk only, 22 (73.3%), greater than the non-early breastfeeding group which obtained breast milk only was 14 (46.7%). While there was just one baby (3.3%) who obtained formula milk only at early breastfeeding group, less than at non-early breastfeeding group, 4 (13.3%) babies. Walker (7) stated that the earlier and

longer the skin-to-skin contact between the mother and the baby,the longer the breastfeeding period will be.

Together with baby's rooming in, that babies have more opportunity to get breast milk from their mothers, so it is not necessary to give breast milk replacement.The American Academy of Pediatrics recommended to put mother in close distance and in the same room with their babies to facilitate the lactation process.[8] The treatment model was called as rooming in. In the study, rooming in was done by 26 (86.7%) mothers in early breast-feeding group and 23 (76.7%) in non-early breast-feeding group. There was no significant difference on both groups (p=0.317). Separating the mother and the babies at the early period ofpost natal age was associated with the shortened breastfeeding age. According to research report, the rooming in on 60%or more of hospitalstay significantly rose the period of breastfeeding until the baby reached age of four months.[7] Rooming in increased the breast milk production at the fourth-day post natal, associated with the high breastfeeding intensity without formula milk supplementation.[7]

Microbiotic Pattern Found on Early and Non-Early Breastfeeding Groups

The microbiotic pattern on gastrointestinal tract obtained from neonatal rectal swab in the study, totally was commensal bacteria which indeed live in gastro-intestinal organ as normal flora. Descriptively they were grouped into three: anaerobic, micro-aerophilic, and aerobic bacteria. Those included in anaerobic cathegory were *Bacteroides, Bifidobacteria, and Peptostrepto-coccus.* Microaerophilic bacteria was *Lactobacillus*. While the aerobic ones were non-pathogenic *Escher-ichia coli, Enterobacter, Staphylococcus epidermidis,* and *Streptococcus faecalis*.

The absence of pathogenic microbes in the research was caused by exposure to the same birth canal, by vaginal normal delivery, and the same environment, at Soetomo Hospital. The samples obtained from the rectal swab of the neonates did not show the suspected infection of meconium, such as smell odor, either change in color and consistency. Other possibilities which caused no finding of pathogenic microbes was that no species identification has been done, for instant some species of *Bacteroides* could be pathogenic. While *Lactobacillus sp., Bifidobacteria sp., PeptoStreptococcus sp., and Enterobacter sp*., were normal flora of gastrointestinal tract.

Comparison of positive culture results on early and non early breastfeeding initiation in every sampling period showed that Positive culture on the first sample collection on 2 hours after birth was dominated by *Bifidobacteria*, which was found in 14 (46,7%) samples from both early breastfeeding and non-early breast-feeding groups. Culture positive on *Lactobacillus* also found with the same rate from both groups, 8 (26,7%). The positive *Bifidobacteria* and *Lactobacillus* on the very early age showed that the bacteria came from the mother when babies slipped through the delivery canal under normal delivery. From Gram staining preparation could be seen that the dominant bacteria also *Bifidobacteria* and *Lactobacillus*, the beneficial normal flora of the intestine.

Table 1. Sample Characteristics

Cathegory	Early Breastfeeding		Total	p
	Early BF	Non-Early BF		
Maternal Age				
< 20	2 (6.7%)	1 (3.3%)	3. (5,0%)	0.908
20-35	25 (83.3%)	23 (76.7%)	48 (80%)	
> 35	3 (10%)	6 (20%)	9 (15%)	
Gravidity				
Primigravida	12 (40%)	13 (43.3%)	25 (41.7%)	0.793
Multigravida	18 (60%)	17 (56.7%)	35 (58.3%)	
Gestational Age				
37-40	22 (73.3%)	26 (86.7%)	48 (80.0%)	0.197
40-42	8 (26.7%)	4 (13.3%)	12 (20%)	
Neonatal weight				
2500-3000	17 (56.7%)	9 (30%)	26 (43.3%)	0.120
3100-3500	11 (36.7%)	18 (60%)	29 (48.3%)	
>3500	2 (6.7%)	3 (10%)	5 (8.3%)	
Diet				
Breastfeeding	22 (73.3%)	14 (46.7%)	36 (60%)	0.87
Breastf. + form.	1 (3.3%)	4 (13.3%)	5 (8.3%)	
Formula milk	7 (23.3%)	12 (40%)	19 (31.7%)	
Rooming in				
Yes	26 (86.7%)	23 (76.7%)	49 (81.7%)	0.317
No	4 (13.3%)	7 (23.3%)	11 (36.7%)	

After getting contaminated by microbiota during delivery process, the next microbiotic transfer comes from environment, and mother's mouth and skin, to neonatus gastrointestinal tract through baby suckling, kisses and hugs.[9] By early breastfeeding initiation, commensal microbes are transferred from the mother's breast skin to baby's mouth, then swallowed, go further to the neonatus gastrointestinal tract where they build colonies.

Tabel 2. Positive Culture Results

Bactery type	2 hour			12 hour			24 hour		
	Early BF	Non-E.BF	p	Early BF	Non-E.BF	p	Early BF	Non-E.BF	p
ANAEROB									
Bacteroides	1 (3.3%)	2 (6.7%)	0.935	11 (36.7%)	12 (40%)	0.764	7 (23.3%)	10 (33.3%)	0.591
Bifidobacteria	14 (46.7%)	14 (46.7%)		10 (33.3%)	9 (30%)		12 (52.2%)	10 (33.3%)	
Peptostrepto.	9 (30%)	9 (30%)		9 (30%)	8 (26.7%)		11 (36.7%)	9 (30%)	
No growth	6 (20%)	5 (16.7%)		0 (0%)	1 (3.3%)		0 (0%)	1 (3.3%)	
MICROAERO.									
Lactobacillus	8 (26.7%)	8 (26.7%)	1.000	26 (86.7%)	21 (70%)	0.117	24 (80%)	24 (80%)	1.000
No growth	22 (73.3%)	22 (73.3%)		4 (13.3%)	9 (30%)		6 (20%)	6 (20%)	
AEROB									
E. coli non path.	9 (30%)	12 (40%)	0.226	9 (30%)	12 (40%)	0.771	11 (36.7%)	12 (40%)	0.721
Enterobacter	9 (30%)	13 (43.3%)		17 (56.7%)	16 (53.3%)		17 (56.7%)	14 (46.7%)	
S. epidermidis	3 (10%)	2 (6.7%)		2 (6.7%)	1 (3.3%)		1 (3.3%)	1 (3.3%)	
S. faecalis	0 (0%)	0 (0%)		2 (6.7%)	1 (3.3%)		1 (3.3%)	3 (10%)	
No growth	9 (30%)	3 (10%)		0 (0%)	0 (0%)		0 (0%)	0 (0%)	

The positive culture of non pathogen *E.coli* and *Enterobacter sp.* were obtained on two hours sample of non-early breastfeeding group, about 12 (40%) and 13 (43.3%), greater than the early breastfeeding group with 9 (30%) samples. Both microbes were also part ofmother's normal flora.[10] The culture of 2 hours sample, 6 (20%) and 5 (16.7%) samples of early breast-feeding and non-breastfeeding group respectively showed no growth of anaerob microbes. It can be explained that for such fastidious microbes like an-aerobic microbes from vaginal flora, on the early time after birth the number was so few that they does not always grow from the rectal swab samples.[11]

At the culture of 12 hours sample collection, we obtained that the most positive culture were *Lactobacillus*, about 26 (86.7%) on early breastfeeding group and 21 (70%) on non-early breastfeeding group, followed by *Enterobacter*, positive on 17 (56.7%) of early breastfeeding group and 16 (53.3%) of non-early breastfeeding group. E. coli was found growing on 9(30%) and 12(40%) of early breastfeeding and non-early breastfeeding samples respectively. *Staphylo-coccus epidermidis* grew from 2(6,7%) and 1(3,3%) samples of early breastfeeding and non-early breast-feeding respectively. No samples of both groups showed no microbial growth on 12 hours sampling collection. Cultures of 24 hours sample collection, the highest positive culture showed *Lactobacillus*, about 24 (80%) for each of early and non-early breastfeeding groups. There was positive *Bifidobacteria* on 12 (52.2%) on early breastfeeding group and 10 (33.3%) on non-early breastfeeding group.

Bourlioux (2002) reported that in the first 12-24 hours of neonatal life outside the uterus, the first bacterial colonization showed up were *Eschericia coli* and *Enterococcus sp*. The next one would be dominated by *Bifidobacterium sp*., followed by some *Bacteroides sp*, for the breastfed babies. On the contrary, among the neonates who obtained formula milk, the culture was predominated by *Bacteroides sp*. followed by *Bifido-bacterium sp*.[2] The 12 and 24 hours samples from early breastfeeding groups were potentially dominated by *Lactobacillus* and *Bifidobacteria*, accompanied by the change of gastrointestinal tract's microbiotic pattern. This phenomenon gives benefits for stimulation process of innate and adaptive immunity.[12]

Comparison of Microbial Colony Counts on Early and Non-Early Breastfeeding Initiation Groups on various Sample CollectionTime

On two hours after birth sample collection, there was significant difference (p=0.029) on aerobic microbial colony count, averagely 266.95 + 518.37 on early

breastfeeding group, compared to non-early breastfeeding group with average of 195 + 127.01. This was in accordance with Mackie's study (1999) which reported that aerobic microbes such as *E. coli* and *Streptococcus* were the first microbes which showed up in gastrointestinal tract of a vaginally-delivered neonate. The number of colony on the early breastfeeding samples was possibly useful for the development of mucosal immune response by sensitization of common antigen.

Tabel 3. Colony Counts

Bacteria type	Early BF	Non-Early BF	P
Anaerob			
2 hours	400 ± 590.8	250 ± 175.11	0.837
12 hours	13173.91 ± 23464.96	18250 ± 28290.16	0.296
24 hours	3582782.60 ± 5516604.67	6262500 ± 6651202.89	0.014
Micraerophilic			
2 hours	60.86 ± 111.75	125 ± 235.23	0.804
12 hours	295869.56 ± 1242365.20	18062.5 ± 24001.3	0.189
24 hours	4469565.21 ± 5894021.22	16143750 ± 26800233.1	0.093
Aerob			
2 hours	266.95 ± 518.37	195 ± 127.01	0.029
12 hours	31000 ± 47556.66	20312.5 ± 26777.4	0.289
24 hours	4952173.91 ± 6797851.9	4906250 ± 5295969.37	0.186

By early breastfeeding initiation, the commensal microbes which looked like the pathogenic microbes such as *E. coli* and *Streptococcus* could stimulate macrophages earlier to recognize the pathogenic microbes which invade later.[12] Morelli (2008) stated that the baby could also be contaminated by potentially pathogenic aerobic bacteria coming from the external environment like *Enterobacteriaceae, Streptococci, and Staphylococci*.The microbes perhaps were not the best choice for the health of neonates, but the metabolism of these microbes was assumed to play role in preparing the condusive environment for development of microbes which were potentially useful for gastointestinal tract. Breast milk was also additional source of microbiota for neonates, because it contained up to 10^9 microbes/L on a healthy mother.[9]

Martin (2003) stated that breast milk could be a relevant source of *Lactobacillus* for a neonatus; by DNA fingerprinting he found the same strain of *Lactobacillus* at the stool samples of the mother and the baby. There was a unique ecological relationship between the oral cavity of the newborn and the maternal skin around the nipple and the nipple itself.[13]

The microbiotic pattern of neonatus gastrointestinal tract which delivered with early and non-early breastfeeding initiation on colony count at 12 hours sample, colony count of microaerophilic microbes like *Lactobacillus* was found more in early breastfeeding group with average of 295.869,56 ± 1.242.365,20, compared to non-early breastfeeding group with average of 18.062,5 ± 24.001,3, although there was no significant difference between both groups. The non significant difference could be possibly caused by colony count of > 10^5. On 12 hours sample, aerobic microbes in the early breastfeeding group also showed more colony count with average of 31.000 ± 47.556,66, compared to non-early breastfeeding group with average of 20.312,5 ± 26.777,4; although statistical test also showed no significant difference between both groups, the faster colony growth of *Lactobacillus* in early breastfeeding group indicated that there was particular mechanism on early breastfeeding procedure which could potentiate the potential-useful microbes such as *Lactobacillus* and *Bifidobacteria* to develop more at 12 hours after birth. The mechanism might be played role by secretory IgA (sIgA) which was found on colostrum which assisted to enhance the early recognition of microbial common antigen, so the gastrointestinal environment was more condusive for the growth of *Lactobacillus* and *Bifidobacteria*. Secretory IgA (sIgA) was the most important immunoglobulin in breast milk, secreted by maternal plasma cells and released on breast milk. The antibody bound the microbial structure on the mucosal surface of the mother, either the commensal or pathogen-ic organisms, and also antigen from diet.[14] The microaerophilic milieu changed further along with the more suitable metabolism of epithelial cell of mucosal surface on gastrointestinal tract, accompanied by nutritional support of breast milk and collostrum. The baby's gastrointestinal tract who got breastfeeding showed low pH, about 5.1-5.4, build up the high acidity environment, so in the first six weeks, gastrointestinal microbiota was dominated by *Bifidobacteria*; while on the other side, there was low amount of pathogenic bacteria, such as *E. coli, Bacteroides, Clostridia,* and *Streptococci*. The baby who had formula milk showed high pH, about 5.9-7.3. The baby who got combination of breast milk and formula milk obtained pH of 5.7-7.0 on the first four weeks, then it declined to 5.45 at the sixth week.[7]

Descriptively, the trend of colony count was higher on early breastfeeding group than on non-early breastfeeding one, but statistical test showed there was no significant difference between both groups; it could be caused by the low number of n samples, or its range of amount which was too wide.

The anaerobic bacterial colony on the 24 hours sample collection of early breastfeeding group was averagely 3.582.782,60 ± 5.516.604,67 the amount was significantly (p = 0.014) less, compared to anaerob colony on non-early breastfeeding group which averaged

6.262.500 ± 6.651.202,89. The explanation for this situation was that the anaerobbacteria which examined on the research was groups of *Bacteroides*, *Bifidobacteria*, and *Peptostreptococcus*, and no identification until the level of species. The *Bacteroides* group had commensal and pathogenic species, but the reasearch could not identify the *Bacteroides*species because there has not been tool available to evaluate it. While in 24 hours colonization had ran optimally, so the colonization of pathogenic species from *Bacteroides* could occur. If early breastfeeding was not performed, there would not be a source of sIgA from colostrum and the baby wouls be less sensitized by common antigen from the mother's breast, so the development of pathogenic species was unsupressed.

The weakness of the research was the colony counting system which was not specific toone particular germ, but was performed for the whole anaerobic, microaerophilic, and aerobic microbes at once. It caused that certain germ, for instant *Bacteroides* which could have both commensal and pathogenic species, was not categorized further.

Comparison of Dominant Microbes by using Gram Staining on Early and Non-Early Breastfeeding Groups at Various Sampling Periods

On Gram staining on two hours samples, the highest result was samples which were dominated by Gram positive Bifidobacilon samples from early breastfeeding group, i.e. 15 (50%), while on the non-early breastfeeding group was 14 (46.7%). It showed that *Bifidobacteria* could be included to the anaerobic microbe which first showed upin neonate digestive tract because they were exposed during vaginal delivery process and the early breastfeeding procedure which involved normal flora of mother's breast. The breastfed babies who obtained additional microbiota from breast milk, which contained up to 109 microbes/L on the healthy mother. The microbes that often found were *Staphylococci, Streptococci, Corynebacteria, Lactobacilli, Micrococci, Proprionibacteria* and *Bifidobacteria*. Morelli (2008) thought that these bacteria came from mother's nipple and the skin around, and from the milk ducts inside the breast.

On Gram staining of 12 hours samples, the most resultwas domination of Gram negative *bacilli*, i.e12 (40%) on early breastfeeding group and 11 (36.7%) on non-early breastfeeding group. *Lactobacillus* was one of microbes in the group. The presence of *Lactobacillus* on Gram staining, associated with colony count on 12 hours samples, indicated that more colonies were obtained on the early breast feeding group than on the non-early breast feeding one.

The higher results of domination of potential useful microbes on early breastfeeding group was also shown on samples collected on 24 hours, i.e. domination of Gram positive Bifidobacil (*Bifidobacteria*) and Gram positive bacilli (*Lactobacillus*), both were 10 (33.3%), compared to 7 (23.3%) and 11 (36.7%) on non-early breastfeeding group. The results of microscopic examination supported the culture results, solved the obstacles caused by fastidious microbes such as anaerobic and microaerophilic microbes.

The study could show that by normal delivery, the baby was exposed to potential-useful microbes from maternal birth canal. If itwascontinued further with early breast feeding, potentiation would occur. This can be seen from the results of positive cultures on two hours and 12 hours (Table 1). After early breastfeeding, the baby would have breast milk, formula milk, or combination of both. The diet would also infuence the baby's microbiotic pattern. The weakness of the study was that the type of diet could not be controlled tightly, because the choise of the baby's dietary was decided by the baby's mother. The volume of a 24-hours-old baby's gaster was just 7 ml, relatively small than those of 48-hours-old baby (14 ml) and those of 4-days-old baby (58 ml); so it was presumable that the dietary difference in first 24 hours did not givesignificant influence.[7]

The absence of significant differences on both study groups, could also be caused by the same mode of delivery, i.e. normal delivery, so that both groups had obtained the same good microbiotic pattern. Zahra (2009) showed difference microbiotic pattern between babiesborn normally with a babies delivered by elective cesarean section; the samples of normal delivery showed more potential-useful microbes. There has not been any study to compare themicrobiotic pattern on the babies who was born by elective cesarean with and without early breastfeeding.

The role of microbiota in mucosal immune system could not be desperaged, especially in a neonatal gastrontestinal tract which has not been developed perfectly. The gastrointestinal tract which was exposed to lots of antigens from the outside world needed antibody which suitable with the acidity of gastrointestinal tract, the Immunoglobulin A. The antibody was produced in breast milk, especially the colostrum which contained high sIgA. By early breastfeeding, a baby had more opportunity to get colostrum.

Early breastfeeding gave early information to colonization of gastrointestinal tract microbes included in

common antigen to build the innate and adaptive immunity response in gastrointestinal tract mucosa earlier. The examination of two hours sample showed some beneficial effects of early breastfeeding such as variety of normal flora, domination of *Lactobacillus* and *Bifidobacteria*, and earlier opportun-ity to get collos-trum. Colostrum contains high sIgA and plays role in mucosal immunity system, and composed of simple carbohydrates which was easy to be metabolisized to create the condusive microaerophilic milieu for the growth of *Lactobacillus* and *Bifido-bacteria*.

CONCLUSION

Early breastfeeding procedure showed more benefit than non-early breastfeeding,.

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PAGE 1

PAGE 2

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