International Journal of Nutrition and Metabolism Research
Vol.2, No.1, pp.1-13, 2024
Print ISSN: 2399-7052
Online ISSN: 2399-7060
Website: https://www.eajournals.org/
Published by European Centre for Research Training and Development UK

Microbial Analysis and Optimal Storage Conditions of Manually Expressed Breast Milk from Healthy Lactating Mothers

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doi: https://doi.org/10.37745/ijnmr.16/vol2n1113 Published November 10, 2024

Citation: Onwuchekwa C.O., Eghafona N.O., Kalu E.I., Okojie R., Enwere B.I., Ike N.M., and Omorotionmwan B. (2024) Microbial Analysis and Optimal Storage Conditions of Manually Expressed Breast Milk from Healthy Lactating Mothers, *International Journal of Nutrition and Metabolism Research*, Vol.2, No.1, pp.1-14

ABSTRACT: *The Human breast milk is a dynamic physiological fluid, which contains different living cells. The nutritional constituents of this fluid make it desirable and ideal for new born babies and infants. Possible microbial contamination of expressed human breast milk was observed from this research, and was more likely owing to its rich composition nutritionally. The aim of this study was to examined the presence of bacterial contaminants present in expressed breast milk sample. Sixty (60) breast milk samples were collected from different nursing mothers attending five (5) different Health facilities at Umuahia Metropolis in Abia State for post-natal care. Ten millilitres (10ml) of breast milk was manually extracted from the nursing mothers into universal bottles and transported immediately to the laboratory for analysis. Questionnaires and consent forms where administered to nursing mothers prior to sample collection. Specimens were aseptically cultured on dried plates of blood agar, chocolate agar and later incubated overnight. Isolates were identified by standard microbiological techniques. Bacterial growth was recorded more in samples collected from urban (55%) and educated (48%) mothers than those from rural (25%) and non-educated (35%) nursing mothers studied respectively ($p<0.05$). Isolates recovered included Escherichia coli 18 (25%), Staphylococcus aureus 17 (24%), Enterococcus faecalis 7 (10%), Streptococcus species 11 (16%), Micrococcus species 6 (9%) and Lactobacillus species 11(16%). Direct breast feeding was rarely practised among participants as only a few urban mothers (10%) neither expressed nor stored breast milk compared to rural mothers ($p>0.05$). The findings of this study revealed that the nutritional constituent of human breast milk varies at different ages,*

environment, maternal diets and stages of lactation of the nursing mothers with bacterial contamination observed for most collected samples. Nursing mothers are advised to always practise good personal hygiene in order to minimise possible contamination of expressed breast milk.

Keywords: Breast milk; Bacterial growth; Contamination; Lactation; Microbial techniques; Infants

INTRODUCTION

The majority of the breast consists of glandular (milk producing) and fatty tissues. However, the ratio of the glandular to fatty tissue varies among individuals. The sex hormone estrogen heavily influences the breast. As menopause approaches, the levels of estrogen declines which also decreases the glandular tissues (Rivard *et al*., 2024). The pectoralis major muscle forms the base of the breast, which extends from the second to sixth rib early in life but may extend to below the sixth rib as the breast matures and sags. The breast is anchored to the pectoralis major fascia by the Cooper ligaments. However, these ligaments are flexible and allow for movements in the breast. In most women, the Cooper ligaments become stretched with time and age, eventually resulting in a ptotic breast. Because of gravity, the lower pole of the breast is fuller than the upper pole. At the lateral edges of the breast, the tail of Spence extends in the axilla (Rivard *et al*., 2024). The human breast milk is a dynamic physiological fluid, which contains many different kinds of live cells including the biological cells, epithelial cells and immune cells that contains immunoactive components which influences late and long lasting effects such as tolerance to infection on the neonates (Hawkes et al; 2002, Garafalo et al; 1999). The period of maternity leave in Nigeria lasts for only three (3) months and at the same time crèches are not provided in the workplaces to promote exclusive breastfeeding. Mothers are thus encouraged to express breast milk and store it in containers (Eteng *et.al.,* 2001). The nutritional values of the milk are expected to be conserved and infection free. It has to be stored within appropriate temperature range (Ezz *et al.,* 2004). The length of time expressed breast milk can be stored at room temperature has thus been a cause of concern by mothers in this part of the world especially in developing regions like Africa (Abosede and Esanabo, 1986). Most research on breast milk storage globally are concerned with optimal conditions for storing breast milk (Zinn B. 2000, Rectham *et al.,* 2006). Thus, to allay the fears of many lactating mothers, this study assessed the microbial agents responsible for breast milk spoilage, under two storage conditions at different time interval.

MATERIALS AND METHODS

Study Area: Abia state, in the southern-eastern part of Nigeria.

Study Population: Lactating mothers as seen in post-natal clinic of Federal Medical Centre, Post-natal clinic Abia state specialist hospital, Immunization clinic at World Bank Health Centre, Immunization clinic of health centre, Ojike Street, all in Umuahia, as shown in the geographical map in the appendix.

Study Designs: A cross-sectional descriptive approach was used in this study.
Sample Size: A total of sixty (60) mothers consisting of mother-infants pairs participated in this study.

Exclusion Criteria: Lactating mothers who are on antibiotics were excluded from this study; also, Non-consenting mothers were also excluded.

Inclusion Criteria: Consenting mothers and mothers who were not on antibiotics.

Ethical Considerations: Ethical approval was obtained from the Health Research Ethics Committee of Federal Medical Centre, Umuahia, Abia State.
Informed consent was obtained from all the participants. The consent form contained the details of the nature and benefits of the study.

Verbal consent was obtained from lactating mothers for the collection of their breast milk specimens as well as administration of questionnaires. Permission was sought and got from administrative heads of the various healthcare institutions in which study participants received post-natal care.

Pilot study was carried out which focused on the entire workflow practise. To this effect, lactating mothers seen at the post-natal clinic of Ikwuano Health Centre (a different population that is smaller but similar to the study population) were studied.

Research assistance were drawn from each centre and trained on Data collection. The Nurses volunteered to assist in mobilizing, organizing and following through with the Protocol. Nurses were also trained to supervise the hygienic expression of breast milk samples by the participants.

Data Collection: Two methods of data collection were adopted.

Questionnaires: were semi- structured and interviewer- administered questionnaire. Fields covered were socio-demographic, health status, and behavioural characteristics of participants.

LABORATORY DATA

Specimen collection: A minimum of 10ml breast-milk samples was aseptically obtained by manual expression into universal sterile containers from the participating lactating mothers. Nurses on duty assisted in the collection of samples aseptically, by washing of the hands and nipple of lactating mothers.
Specimens were transported to the analytical laboratory within 30-60minutes of collection.
Specimen analysis: Samples that were collected were analysed microbiologically.
The objective Microbiological analysis was firstly, to determine the shelf life when stored at room temperature (29.5^{o}C) and refrigeration temperatures (2-8^{o}C); and, secondly, to determine the patterns of Microbial organisms involved in breast milk spoilage.

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Microbial Analysis;
A minimum of 10ml of breast milk samples were collected aseptically each for this analysis, through manual expression into a sterile universal bottle. 5ml out of the 10ml was used for Microbial analysis, which was aseptically dispensed into sterile universal containers .This was to determine the onset of sample spoilage at different storage conditions, viz: room temperature (29.5^oC) and refrigeration ($2\text{-}8^0C$).
Sequential cultures of the samples were, therefore, carried out at different time interval of (0, 2, 4, 6, 8 and 10 hours) consecutively. The time at which cultural positivity was achieved for each sample was recorded.
The growth of fastidious Microorganisms. Preparation was according to the manufacturer's specification. Sterilization of media by autoclaving was done at 121^0C for 15minutes (Cheesbrough, 2004).
Cultural analysis; Specimens were aseptically cultured overnight, as plates were incubated at $35\text{-}37^0C$ for 24 hours aerobically on Blood Agar media (Cheesbrough, 2004).
Identification of Isolates; Isolates were identified by Gram staining and biochemical methods. This was done on distinct colonies according to (Cheesbrough, 2004).
Biochemical tests included Catalase tests, Oxidase tests, and Coagulase tests.

Statistical Analysis
Chi-square(X^2) test was used to analyse differences of the demographic factors. A p-value 0f ≤ 0.05 was considered statistically significant.

RESULTS

TABLE 1. The pattern of microbial changes in samples stored at different temperatures and different time intervals

Table 1shows the pattern of microbial changes in samples stored at different temperature and time interval. Different samples as observed started microbial proliferation at different time interval and storage conditions.

MICROBIOLOGICAL CULTURE AT ROOM TEMPERATURE (29.5^0C)/REFRIGERATION ($2\text{-}8^0$)											
RT Ohrs	**RT 2hrs**	**RT 4hrs**	**RT 6hrs**	**RT 8hrs**	**RT 10hrs**	**REF 0hrs**	**REF 2hrs**	**REF 4hrs**	**REF 6hrs**	**REF 8hrs**	**REF 10hrs**
+	+	+	+	+	+	-	-	-	-	-	-
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Microbial isolates involved in breast milk contamination. Microbial isolates include *Staphylococu.aureuss, 17(24%), Streptococcus.spp, 11(16%), Lactobacillus. spp, 11(16%), Micrococcus.spp. 6(9%), Enterococcus.spp. 7(10%) and E.coli, 18(25%)* presented in (Table 2)

TABLE 2: MICROBIAL ISOLATES INVOLVED IN BREAST MILK CONTAMINATION.

Onset of Microbial growth	**Frequency of occurrence**	**Percentage (%)**
Staphylococcus.aureus	**17**	**24**
Streptococcus.spp	**11**	**16**
Lactobacillus.spp	**11**	**16**
Micrococcus.spp	**6**	**9**
Enterococcus.spp	**7**	**10**
Escherichia.coli	**18**	**25**
Total	**70**	**100**

Onset of microbial growth in samples stored at room temperature. From the results gotten, at (0hour/22%), onset of microbial growth was observed as presented in (Figure 1). The highest growth was observed to occur at (2hours/40%), and at (10hours/0%) no growth was found.

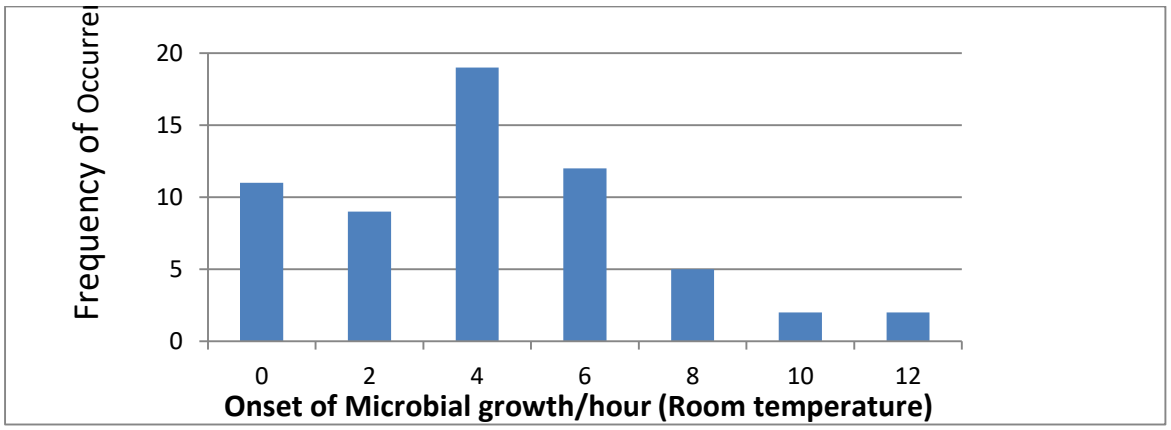

FIGURE 1: Graphical representation of onset of microbial frequency for room temperature

Onset of microbial growth in samples stored at refrigeration point. From (0hour/18%) growth was observed, and at (4hours/32%) growth was seen to be the highest. While at (12hours/0%), there were no growth observed as the time of incubation was extended due to the fact that at (10hours), very few samples had no growth as presented in (Table 3).

TABLE 3: ONSET OF MICROBIAL GROWTH IN SAMPLES STORED AT REFRIDGERATION POINT.

Onset of Microbial growth/hours	**Frequency of occurrence**	**Percentage (%)**
0	11	18
2	9	15
4	19	32
6	12	20
8	5	8
10	2	3
12	2	0
Total	60	100

Table 4. Shows the mean of both room temperature and refrigeration point. It shows that, at room temperature onset of microbial contamination occurred earlier than for refrigeration with a mean difference of (2.82 and 4.16) respectively for room and refrigeration storage condition

Table 4: Shows the mean of both room temperature and refrigeratio

Storage Method	**Mean (hours)**	**Median (hours)**	**Mode (hours)**
Room Temperature	2.86	2	2
Refrigeration Temperature	4.16	4	4

Table 5 Shows the onset of microbial growth at (≤ 4hours and > 4hours) against presumed association factors for room temperature. This table shows a summary of factors used to ascertain the onset of microbial growth based on the residence, educational, health status, marital status and ages of lactating mothers.

Table 5: Shows the onset of microbial growth against presumed associated factors for room temperature.

Factors	0-hour	2-hour	4-hour	6-hour	8-hour	10-hour
Residence						
Rural	5	7	4	3	1	0
Urban	8	17	8	3	4	0
Educational						
≤Primary	2	4	1	1	0	0
>Secondary	11	12	11	5	5	0
Health status						
Healthy	12	23	11	6	5	0
Unhealthy	0	1	0	0	0	0
Marital status						
Married	12	20	11	6	4	0
Single	1	3	1	0	0	0
Age						
15-25	3	8	2	2	0	0
25-35	8	12	7	2	4	0
35-45	2	3	3	2	1	0

Table 6: Association of Residence of lactating mothers with the onset of microbial growth for room temperature.
From the Statistical analyses, the association of the factors listed above in below table shows that (P>0.05) at ≤ 4hours and>4hours. There is no significant difference.

Factors	**≤ 4hours**	**>4hours**	**Total (%)**
Rural	16(80.0%)	4(20.0%)	20(33.3)
Urban	33(82.5%)	7(17.5%)	40(66.7)
Total	49(81.7%)	11(18.3%)	60(100)

Table 7: Association of Educational qualification of lactating mothers with the onset of microbial growth for room temperature.
From the Statistical analyses, the association of the factors listed above in below table shows that (P>0.05) at ≤ 4hours and>4hours. There is no significant difference.

Factors	**≤ 4hours**	**>4hours**	**Total (%)**
≤Primary	7(87.5%	1(12.5%)	8(15.4)
>Primary	34(77.3%)	10(22.7%)	44(84.6)
Total	41(78.8%)	11(21.2%)	52(100)

Table 8: Association of Health status of lactating mothers with the onset of microbial growth for room temperature.
From the Statistical analyses, the association of the factors listed above in below table shows that (P>0.05) at ≤ 4hours and>4hours. There is no significant difference.

Factors	**≤ 4hours**	**>4hours**	**Total (%)**
Healthy	46(80.7%)	11(19.3%)	57((98)
Unhealthy	1(100%)	0(0%)	1(1.72)
Total	47(81%)	11(18.9%)	58(100)

Table 9: Association of marital status of lactating mothers with the onset of microbial growth for room temperature.

From the Statistical analyses, the association of the factors listed above in below table shows that (P>0.05) at ≤ 4hours and>4hours. There is no significant difference.

Factors	**≤ 4 hours**	**>4hours**	**Total (%)**
Married	43(81.1%)	10(18.9%)	53(91.4)
Single	5(100.0%)	0(0%)	5(8.6)
Total	48(82.8%)	10(17.2%)	58(100)

Table 10: Association of Ages of lactating mothers with the onset of microbial growth for room temperature.

From the Statistical analyses, the association of the factors listed above in below table shows that (P>0.05) at ≤ 4hours and>4hours. There is no significant difference.

Factors	**≤4 hours**	**>4 hours**	**Total (%)**
≤ 35 years	40(83.3%)	8(16.7%)	48(82.8)
>35 years	8(72.7%)	3(27.3%)	11(18.9)
Total	48(82.8%)	11(18.9%)	58(100)

Table 11: Shows the onset of microbial growth (≤ 4hours and > 4hours) against presumed associated factors for Refrigeration point. This table shows a summary of factors used to ascertain the onset of microbial growth based on the residence, educational, health status, marital status and ages of lactating mothers.

Factors	0-hour	2-hour	4-hour	6-hour	8-hour	10-hour
Residence						
Rural	5	4	3	7	1	0
Urban	6	7	15	7	3	0
Educational						
≤ Primary	3	0	5	2	0	0
>Primary	8	11	13	12	4	0
Health status						
Healthy	11	11	18	14	4	0
Unhealthy	1	0	0	0	0	0
Marital status						
Married	10	9	17	13	4	0
Single	1	2	1	1	0	0
Age						
15-25	3	3	4	4	0	0
25-35	6	8	10	5	2	0
35-45	2	0	4	3	2	0

Table 12: Association of Residence of lactating mothers with the onset of microbial growth for refrigeration point. From the Statistical analyses, the association of the factors listed above in below table shows that (P>0.05) at ≤ 4hours and>4hours. There is no significant difference.

Factors	**≤ 4hours**	**>4hours**	**Total (%)**
Rural	12(60.0%)	8(40.0%)	20(34.5)
Urban	28(73.7%)	10(26.3%)	38(65.5)
Total	40(68.9%)	18(31.0%)	58(100)

Table 13: Association of Educational qualification of lactating mothers with the onset of microbial growth for refrigeration point. From the Statistical analyses, the association of the factors listed above in below table shows that (P>0.05) at ≤ 4hours and>4hours. There is no significant difference.

Factors	**≤ 4hours**	**>4hours**	**Total (%)**
≤Primary	8(80.0%)	2(20.0%)	10(17.2)
>Primary	32(66.7%)	16(33.3%)	48(82.8)
Total	40(68.9%)	18(31.0%)	58(100)

Table 14: Association of Health status of lactating mothers with the onset of microbial growth for refrigeration point. From the Statistical analyses, the association of the factors listed above in below table shows that (P>0.05) at ≤ 4hours and>4hours. There is no significant difference.

Factors	**≤ 4hours**	**>4hours**	**Total (%)**
Healthy	40(69.0%)	18(31.0%)	48(81.4)
Unhealthy	1(100.0%)	0(%)	1(1.8)
Total	41(69.5%)	18(30.5%)	59(100)

Table 15: Association of Marital status of lactating mothers with the onset of microbial growth for refrigeration point. From the Statistical analyses, the association of the factors listed above in below table shows that (P>0.05) at ≤ 4hours and>4hours. There is no significant difference.

Factors	**≤ 4hours**	**>4hours**	**Total (%)**
Married	36(67.9%)	17(32.1%)	53(91.4)
Single	4(80.0%)	1(20.0%)	5(8.6)
Total	40(68.9%)	18(31.0%)	58(100.0)

Table 16: Association of Ages of lactating mothers with the onset of microbial growth for refrigeration point. From the Statistical analyses, the association of the factors listed in the table below shows that (P>0.05) at ≤ 4hours and>4hours. There is no significant difference.

Factors	**≤ 4hours**	**>4hours**	**Total (%)**
≤ 35years	34(75.6%)	11(24.4%)	45(80.4)
>35 years	6(54.5%)	5(45.5%)	11(19.6)
Total	40(71.4%)	16(28.6%)	56(100)

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DISCUSSION

The human breast milk has many advantages over infant formula in the prevention of neonatal infections. (Chen and Rogan, 2004). During breast-feeding, infants suckle directly from the mother's breast and are supposed to be free from contamination. However, the use of expressed breast milk has been noted to provide both nutritional and immunological benefits, especially if its nutritional values can be conserved (Ezz *et al.,* 2004). This becomes very necessary when babies may be separated from the mother due to maternal employment or schooling. In Nigeria, where the mother is entitled to 3-4months maternity leave due to maternal employment or schooling, mothers are thus left with no other choice to make provisions for where their babies can be kept, while their mothers go to work. A study showed that expressed breast milk stored at different conditions and time interval as supported by WHO *(*WHO, 2002), confirms the incidence of microbial growth. However, expressed breast milk cannot be said to be entirely sterile or free from bacterial contamination due to the practises of the nursing mothers (Moulin *et al.,* 1998; Doedhar and Joshi. 1999). It was observed from this study, isolates of microbial growth include*:*

Staphylococcus.aureus,(17/24%)Micrococcus.spp.,6/9%),Lactobacillus. spp.(11/16%), Enterococcus. Spp, (7/10%), E.coli,(18/25%)and Streptococcus. spp, (11/16%). Microbial growth occurred at both storage conditions of room temperatures and refrigeration point and at the different time interval of storage (0-10 hours) (Olowe *et al.,* 1987). Similarly, another study isolated pathogenic microorganisms, such as *Enterococcus. feacalis, E. coli and Staphylococcus. Aureus,* which shows that these organisms are bacterial isolates from the normal flora of human *(*Nwankwo *et al.,* 1988).

E. coli and Staphylococcus. aureus occurred higher than other organisms because they make up the micro floras found on the skin. At the different time intervals the onset of microbial growth were observed for room temperature as, (0hour, 13/22%), there was already an onset of microbial growth at this hour; it became more pronounced at (2hour/40%) for room temperature. At (4, 6, 8 and 10 hours), onset of microbial growth was recorded as (12/20%, 6/10%, 5/8% and 0/ 0%) respectively (Deodhar and Joshi, 1991). While that of refrigeration, microbial growth was observed to be (0hour, 11/18%), microbial growth was observed to be more pronounced at (4hour, 19/32%). Other microbial onset at (2, 6, 8 &10 hours), occurred at (9/15%, 12/20%, 5/8% & 2/3%) respectively. Some samples stored at refrigeration showed no growth, but started growth at the (12hour). This shows that breast milk stored at refrigeration point preserves breast milk longer than at room temperature. But there was a reduction in microbial growth as the time progresses. This shows that the nutritive component has been used up by microbes and thus causes deterioration of breast milk. (Eteng *et.al.,* 2001). This shows that for nursing mothers, the way and manner the process of breast milk storage is been handled from its expression to the storage container and mode of storage is very important. Socio-demographic factors were used to establish a relationship between microbial growths and time of onset of growth for different time (≤ 4hours & >4hours) and condition (room temperature & refrigeration point). Studies shows that, socio-demographic factors such as Residence (urban and rural dwellers), educational qualification (educated and uneducated),

health status, marital status (married and single mothers) and ages (≤35 &>35 years) of lactating mothers were not dependable on the microbial onset since statistical analysis showed that there was no significant difference as P (P>0.05) at ≤ 4hours & >4hours. Other studies by (Pardou *et.al.*, 1994) observed that human breast milk contained fewer bacteria at the time it was expressed, developed other bacteria growth during storage. Other several factors considered to be a major influence of microbial growth of expressed breast milk if stored at room temperature and refrigeration point are; Improper hand washing and sanitation, improper cleaning of the breast teats (i.e. Nipple), continuous use of dirty or damp braziers that can harbour microbes, thereby facilitating breast milk contamination, and improper washing and sterilisation of breast milk storage containers. This is because bacteria can enter through equipment used; human breast milk is a potential source of transmission of microbial infections. All the above listed factors can implicate high risk of breast milk contamination. (Novak *et.al*. 2008). Other studies showed that Poor hygiene and improper hand washing could be possible reasons for contamination (Hamosh. *et. al* 1996).

CONCLUSION

It was observed that breast milk in storage both at room temperature and refrigeration point, showed minimal bacterial growth and may possibly have come from contamination from skin micro flora of the mother.

REFERENCES

Abosede O.A and Esanbodo .D. (1986). Health education needs fathers on breastfeeding: A study in Lagos, Nigeria. *Nigerian Journal Nutrition Science*, 8, 1-5.

Cheesbroug. M. (2004).District laboratory practice in tropical countries, Part 2. Cambridge University press. United kingdom.434pp.

Chen, A. and Rogan, W.J (2004). Breast feeding and the risk of neonatal death in the united states.*Peadiatrics.* 113, e435-e439.

Deodhar. G and Josh. S. (1991). Microbiological Study of breast milk in special reference to its storage in milk bank. *Journal Medical*. 37**,** 14-6.

Eteng M.U, Ebong P.E, Eyong E.U and Ettarh R.R. (2001), Storage beyond three hours at ambient temperature alters the Biochemical and nutritional qualities of breast milk. *African Journal Reproductive Health*.**5**:130-134.

Eteng MU, Ebong PE, Eyong EU, Ettarh RR. (2001). Storage beyond three hours at ambient temperature alters the biochemical and nutritional qualities of breast milk. *Afr J Reprod Health*, 5(2),130-4.

Ezz E.L, Din Z.M. Ghaffer S.A, Garby E.K, Fahmi W.A and Bedair R.F. (2004). Is storage and expressed breast milk an alternative for Egyptian mothers. *Eastern Mediterranean Health Journal*.10(6), 815-821.

Garofalo, R.P. and Goldman A.S. (1999). Expression of functional immunomodulatory and anti-inflammation in human milk. *ClinicalPerinnatol*.26, 361-377.

Hamosh M, Ellis LA, Pollock DR, Henderson TR, Hamosh P. (1996). Breastfeeding and the working mother: effect of time and temperature of short-term storage on proteolysis, lipolysis, and bacterial growth in milk. *Pediatrics*, 97(4), 492-8.

Hawkes, J., Dixon, D.-L., and Gibson, R. (2002). Cytokine production by human milk cells and peripheral blood mononuclear cells from the same mothers. *Journal of Clinical Immunology*, *22*(6), 338-344.

Moulin, Z. S., Lamounier, J. A., Vieira, M. B., Baêta, M., Silva, M. A., and Araújo, R. S. (1998). Contaminação bacteriana do leite humano coletado por expressão manual e estocado à temperatura ambiente [Bacterial contamination of breast milk collected through manual expression and stored at room temperature]. *Jornal de Pediatria*, 74(5), 376-382.

Nwankwo, M. U., Offor, E., Okolo, A. A., and Omene, J. A. (1988). Bacterial growth in expressed breast milk. *Annals of Tropical Paediatrics*, *8*(2), 92-95.

Olowe, S. A., Ahmed, I., Lawal, S. F., and Ransome-Kuti, S. (1987). Bacteriological quality of raw human milk: effect of storage in a refrigerator. *Annals of Tropical Paediatrics*, *7*(4), 233–237.

Pardou A, Serruys E, Mascart-Lemone F, Dramaix M, Vis HL. (1994). Human milk banking: influence of storage processes and of bacterial contamination on some milk constituents. *Biol Neonate*, 65(5), 302-9.

Rectham D.J, Lee M.L and Berg.H. (2006). Effects of environmental conditions on unpasteurized donor human milk. *Breastfeeding Medicine*. 1**,** 24-26.

Rivard, A. B., Galarza-Paez, L., and Peterson, D. C. (2023). Anatomy, thorax, breast. In *StatPearls* [Internet]. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK519575

WHO. (2002). Infant and young child nutrition. Global strategy on infant and young child feeding.55th World health assembly. 4 55/15. Geneva.

Zinn .B. (2000). Supporting the employed breastfeeding mothers Journal Midwifery Women's Health. 45**,** 216-226.