RESULTS

Immunoglobulins assay in colostrum milk and serum samples of normal lactating women:

IgG:

Colostrum: The concentration of IgG on the first day of lactation was 89.5mg/dl (range 65-150mg/dl), then the concentration decreased rapidly during the first week postpartum to reach 18.4mg/dl (range 9.2-35mg/dl).

Milk: The concentration value of IgG during the first 6 months after the onset of lactation was low. It ranged from 1.9 to 2.4mg/dl. A slight elevation in the concentration of IgG was observed in most subjects during the second year of lactation, the level observed did not exceed 8.3-9.6mg/dl.

Serum: The level of IgG in serum samples was within the normal value of adult (normal serum value of adult is 900-1800mg/dl). The mean IgG value showed no

of the mean serum level.

IgG level in colostrum and milk and in serum samples during the different periods of lactation was observed in table (1) Figure (1),(4) & (5) demonstrated the relation between IgG concentration in milk and colostrum in relation to other immunoglobulins concentration (IgA, IgM).

IgM:

Colostrum: The mean concentration value of IgM was 127.8mg/dl (range 90-223mg/dl) on the first day of lactation, then the level dropped rapidly to reach 18mg/dl (range 10-30mg/dl) at the end of the first week postpartum.

Milk: The concentration of IgM was 2.8mg/dl (range 4-10mg/dl) in the first month postpartum. It showed no significant variation during the first 6 months of lactation. During the end of lactational period the level showed slight increase (mean 3.1mg/dl, range 3.8-7mg/dl).

Serum: IgM concentration was within the normal

250mg/dl), IgM levels in the serum samples during the whole periods of lactation were nearly similar (18 months).

The level of IgM in colostrum and milk and in serum samples of normal lactating women during the different periods of lactation was illustrated in table (2) Figure (2).

IgA:

The concentration of IgA showed a distinctive pattern, it decreased very rapidly over the first four days postpartum from the extremely high initial levels, almost 10 times the mean serum value, to a much low level in later samples.

Colostrum: IgA concentration was high in colostrum, the mean value was 1768mg/dl range (1150-2180mg/dl), then the concentration dropped immediately to reach 29.08mg/dl, (range 21-35mg/dl) in the fifth day postpartum.

Milk: The IgA concentration represented a constant value during different periods of lactation as noticed in Table (3) & Figure (3). Toward the end of

lactational period (12 months postpartum) the concentration of IgA increased slightly; its mean value was 14.04mg/dl (range 14.4-17.3mg/dl).

Serum: The level of IgA was within the normal level of adult, mean normal value of adult was 202mg/dl (range 100-315mg/dl).

The concentration of IgA in the colostrum and milk and in the serum samples of normal lactating women was demonstrated in Table (3) & Figure (3).

Effect of mastitis on immunoglobulins concentaration in milk:

The mean concentration value of immunoglobulins IgA, IgG & IgM in milk of mastitis women were 15.3mg/dl (range 7-29mg/dl), 4.3mg/dl (range 2.3-9.2mg/dl) and 5.5mg/dl (range 2.8-11.2mg/dl) respectively. IgG & IgM concentration were high in milk of mastitis women than of normal women.

Tables (4),(5),(6) and Figure (6) demonstrated the ratio of immunoglobulins IgA, IgG & IgM concentrations

Statistical analyses:

IgG concentration in colostrum of normal lactating women showed a significant correlation with its level in serum ($P < 0.05$, $r = 0.34$, $T = 0.4$)(r = correlation coefficient). No Correlation was reported between the concentration of IgG in serum and milk.

IgA and IgM concentration in colostrum and milk showed no correlation with their levels in serum.

IgG & IgM concentration in milk from mastitis women showed a significant elevation in relation to their levels in normal lactating women.

Leukocytes in human colostrum and milk:

The total number of cells found in colostrum and milk samples was calculated. It was found that, early in the colostrum (0-2 days), the mean total leukocytes counts was 4800 cells/mm^3 (range 2500-10600 cells/mm^3) then dropped to reach 2070 cells/mm^3 (range 500-3800 cells/mm^3) during the end of the first week postpartum.

Table (1) :- The mean concentration of IgG (mg/dl) in the samples of colostrum and milk of normal lactating women compared with its level in the serum during different periods after the onset of lactation

	Day (1)	Day (3)	Day (5)	month (1)	month (2)	month (4)	month (6)	month (12)	month (18)
No.	10	9	12	8	6	8	8	5	4
* Milk and colostrum									
Mean	89.5	55.4	18.4	2.2	2.05	2.4	1.9	6.26	5.75
S.D.	30.8	33.8	7.6	0.7	0.95	0.8	0.86	2.5	2.1
range	65.150	65-100	9.2-35	1.3-3.5	0.8-3.1	1.1-3.2	0.8-3.2	3.2-9.6	3.1-8.3
* Serum									
Mean	1208	1307.4	1490	1953.7	1376.6	1331.2	1233.7	1602	1463
S.D	80.8	2709	463.3	438.8	83.3	76.9	8	532	438.7
Range	1130.1370	1250-2110	1130.2340	1550-2650	1190-1430	1200-1400	1130-1360	1150.2230	1160.1310

SD = Standard deviation
No = numbers of samples within each lactational period

Table (2) :- The mean concentration of IgM (mg/dl) in the samples of colostrum and milk of normal lactating women compared with its level in the serum during different periods after the onset of lactation.

	Day (1)	Day (3)	Day (5)	month (1)	month (2)	month (4)	month (6)	month (12)	month (18)
No	10	9	12	8	8	6	8	5	4
* Milk and colostrum									
mean	127.8	92.6	18	5.5	5.2	2.4	2.26	2.3	5.1
S.D.	42.1	30.5	6.1	2.8	0.7	0.7	0.5	0.6	1.35
range	90-223	60.150	10-30	4-10.5	4-6	1.8-4	1.6-2.8	1.6-3	3.8-7
*Serum									
Mean	174.9	176	184	172	165	181	174	173	168
S.D.	25.4	24.4	30.3	18.8	15.3	10.9	13.2	14.2	13.1
range	140.223	150-223	150-223	150-200	150-189	150-175	150-185	150-200	150-180

SD = standard deviation.No = number of samples in each group

ble (3) :- **The mean concentration of IgA (mg/dl) in the samples of colostrum and milk of normal lactating women compared with its level in the serum during different periods after the onset of lactation.**

	Day (1)	Day (3)	Day (5)	month (1)	month (2)	month (4)	month (6)	month (12)	month (18)
No.	10	9	12	8	6	8	8	5	4
Milk and colostrum									
mean	1768	554-1	79.08	12.4	10.1	9.9	10 — 6	14.05	23.5
S. D.	312.7	64.01	4.3	3.1	3	1.8	2 —	2.3	3.5
range	1700-2540	445-634	21-35	9.2-17.2	7.3-16.2	8.3-13.1	7.2- 1 5.2	11 .4-17.3	19.8-27.2
Serum									
mean	197.8	195.4	200.5	190.8	193	201.2	19 .2	194	192.5
S. D.	12.76	13.2	10.1	14.1	16.3	11.5	10 9	16.3	17.07
range	179-210	180-210	181-210	170-210	180-210	185-210	180— 10	175-210	170-210

= standard deviation.
= numbers of samples within each lactational period

Table (4) comparison of IgA concentrations in milk of mastitis patients and in milk of normal women.

	Mean mg/dl	S D	Range mg/dl
Normal milk (N = 39)	17.93	5.3	8-33
Mastitis milk (N = 15)	15.3	5.1	7-29

S D = standard deviation

T = 2.8 P <0.05 (significant)

Table (5) comparison of IgG concentrations (mg/dl) in milk of mastitis patients and in milk of normal women.

	Mean	S D	Range
Normal milk (N = 39)	2.98	2.08	0.8-9.4
Mastitis milk (N = 15)	4.3	1.69	2.3-9.2

S D = standard deviation

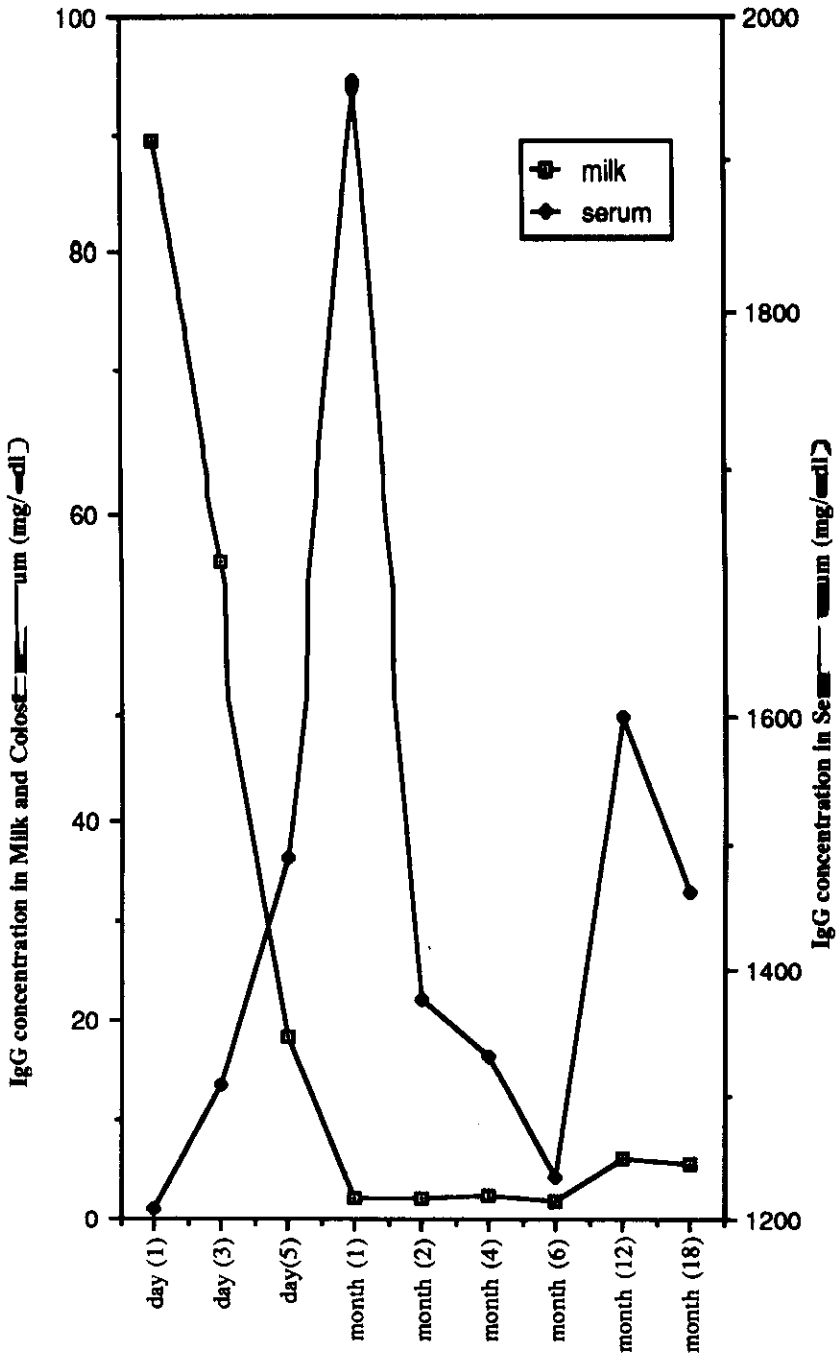
T = 2.2 P < 0.05 (significant)

Table (6) comparison of IgM concentrations (mg/dl) in milk of mastitis patients and in milk of normal women.

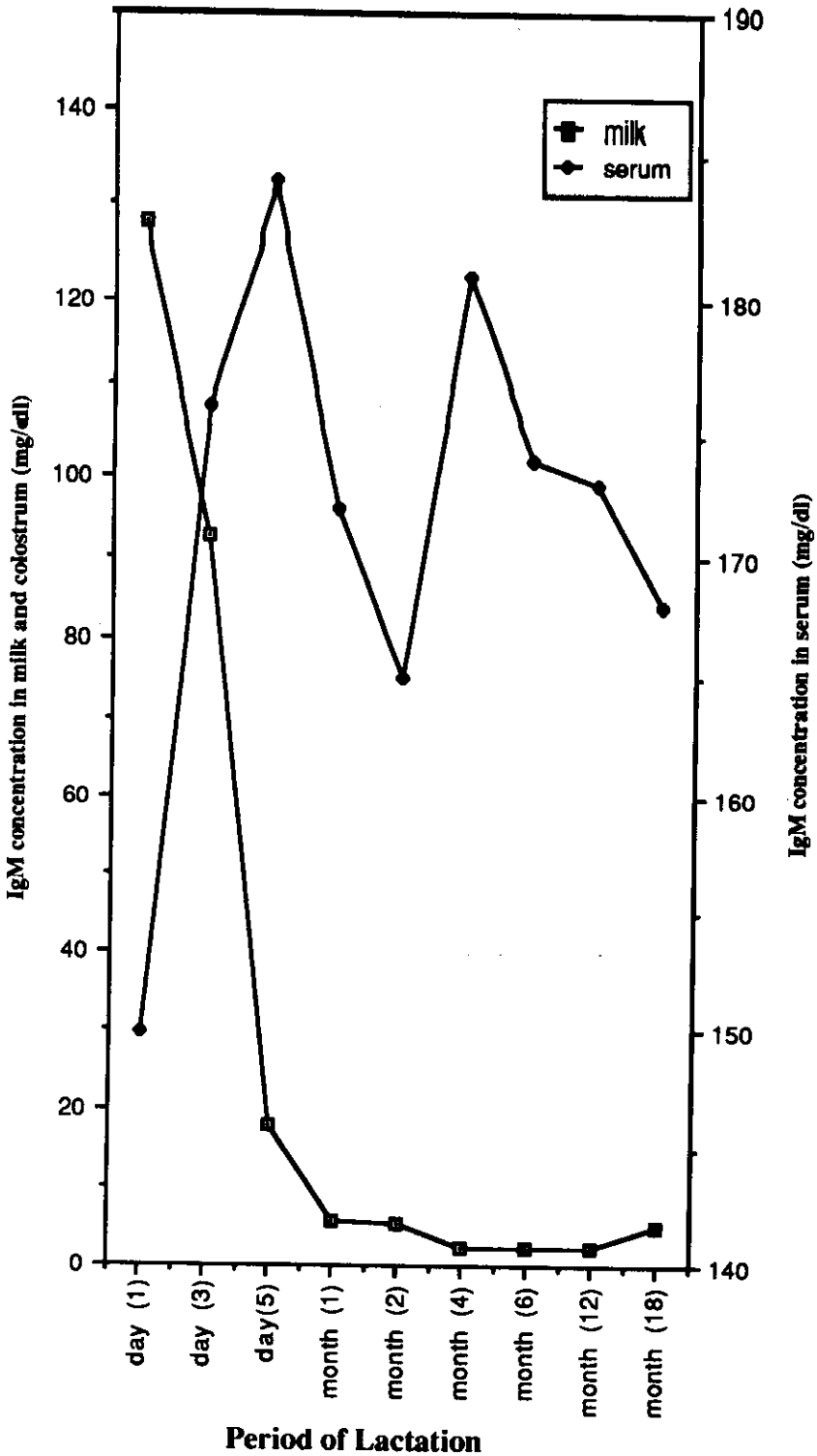
	Mean	S D	Range
Normal milk (No. = 39)	3.9	2.27	1.6-7
Mastitis milk (No. = 15)	5.5	3.1	2.8-11.2

S D = standard deviation

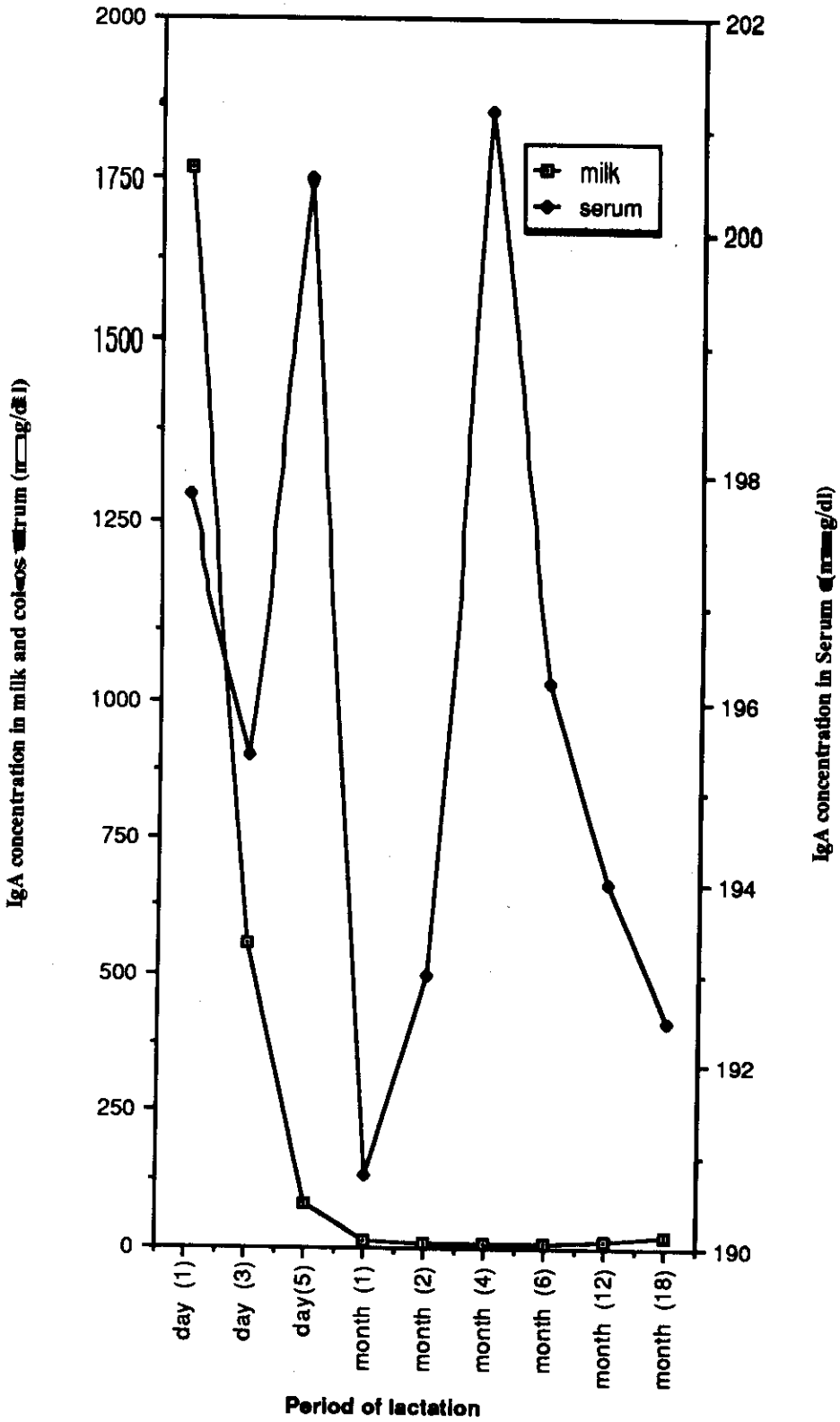
T = 2.2 P < 0.05 (significant)



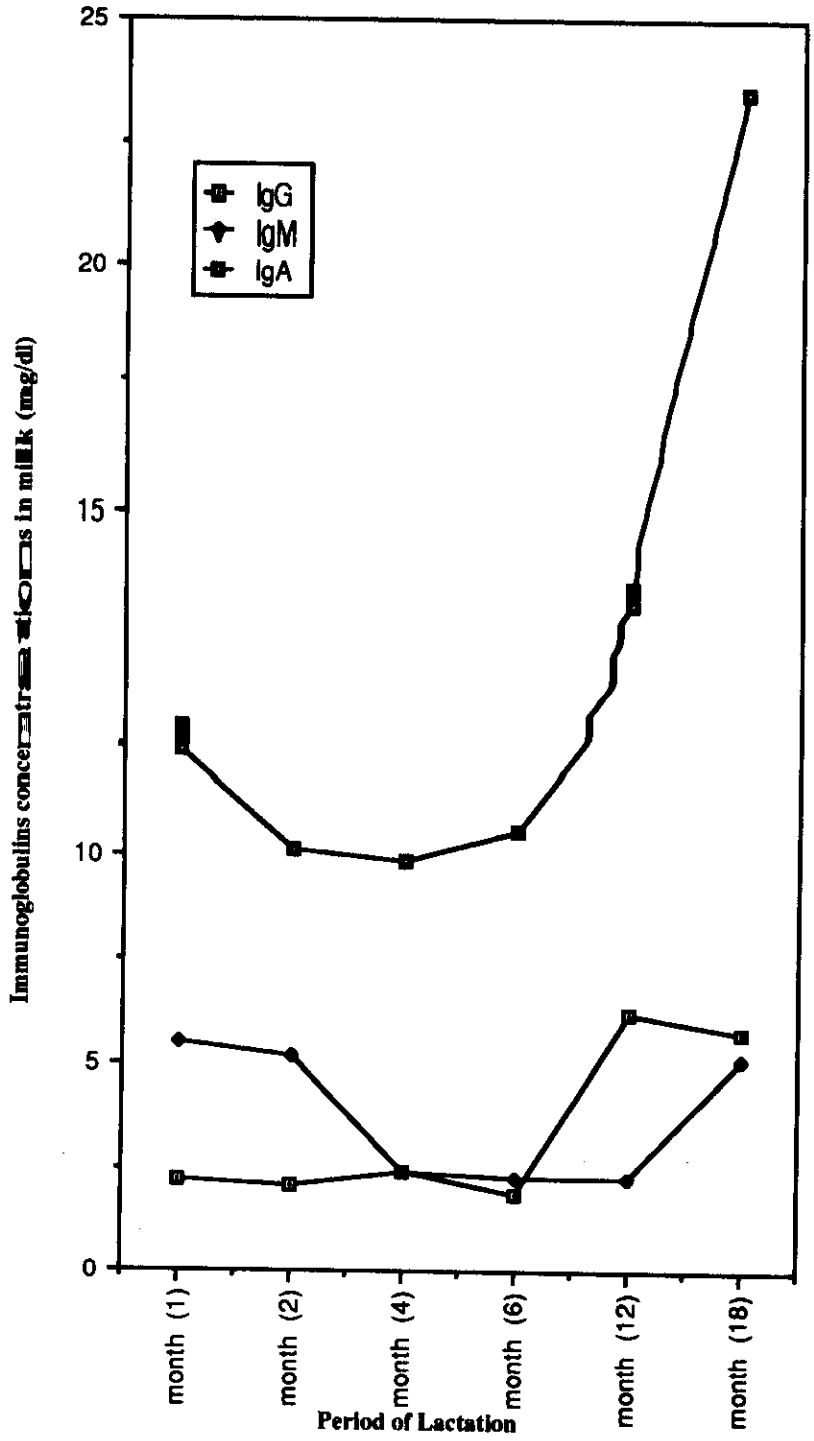
Fig(1) Geometric mean levels of IgG immunoglobulin in the serum and in colostrum and milk of 90 female subjects at various intervals after the onset of lactation.



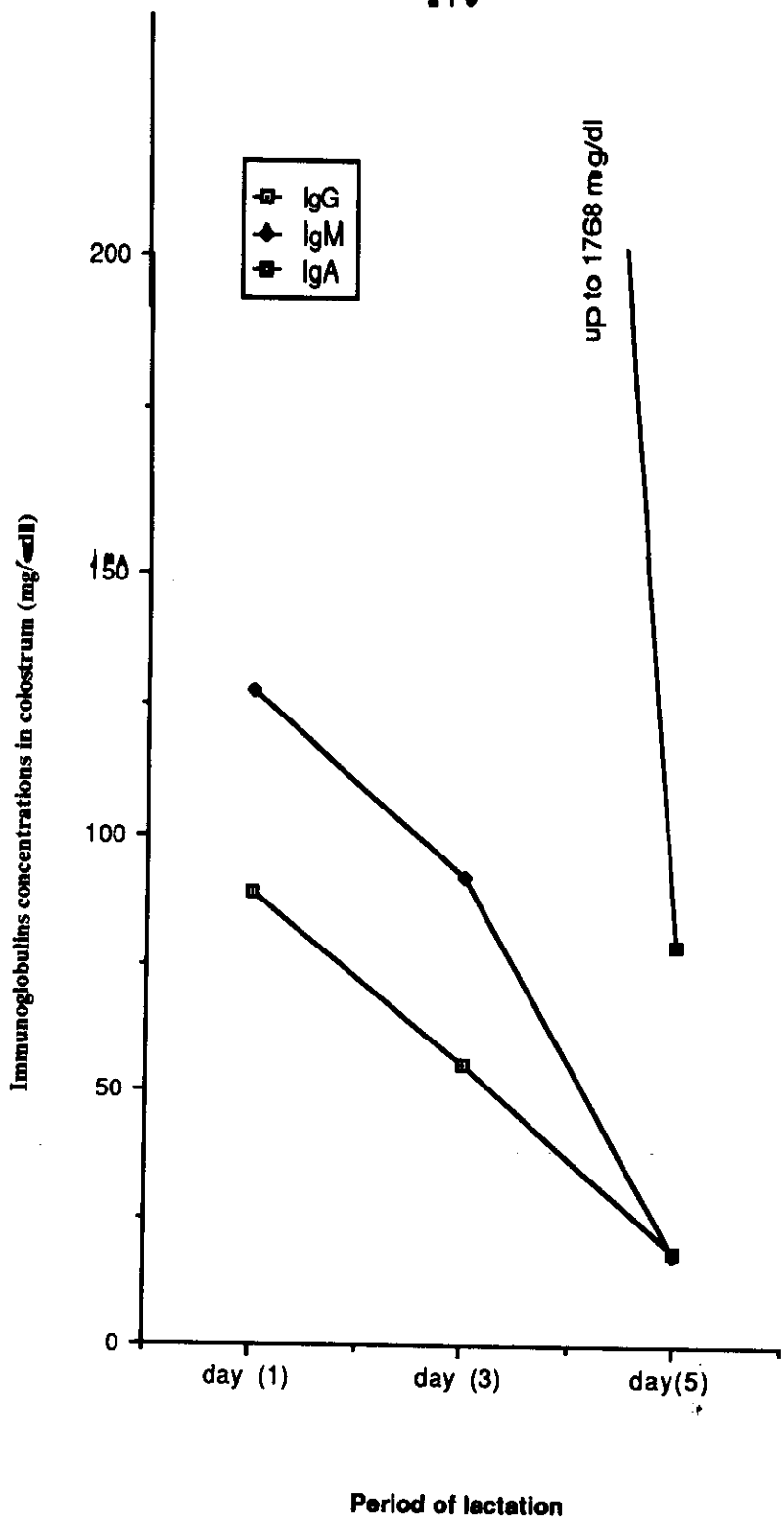
Fig(2) Geometric mean levels of IgM immunoglobulin in the serum and in colostrum and milk of 90 female subjects at various intervals after the onset of lactation.



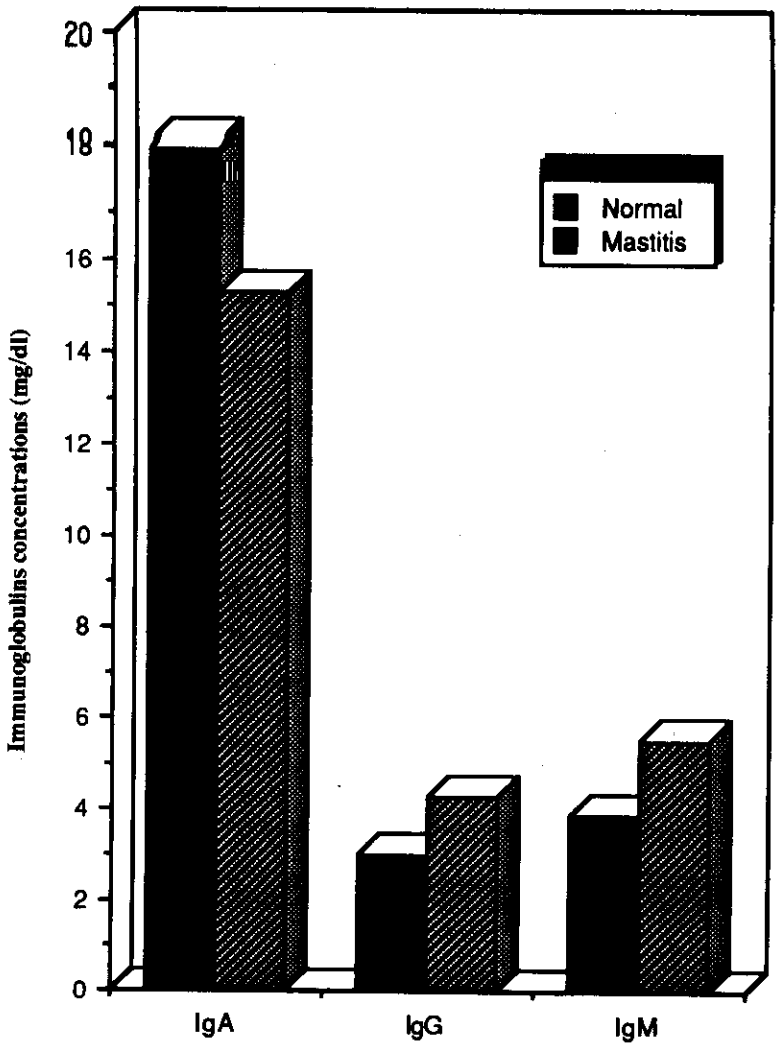
Fig(3) Geometric mean levels of IgA immunoglobulin in the serum and in colostrum and milk of 90 female subjects at various intervals after the onset of lactation.



Fig(4) Geometric means levels of IgA, IgM and IgG immunoglobulins in milk of 39 female subjects at various intervals after the onset of lactation.



Fig(5) Geometric means levels of IgA, IgM and IgG immunoglobulins in colostrum of 31 female subjects during the first week of lactation



Fig(6) The mean concentrations of Immunoglobulins IgA, IgG and IgM in milk samples of normal and lactational mastitis women

The total cells counts continued to drop during the second week of lactation (reach 155 cells/mm^3 (range 336-55 cells/mm^3) at the end of the first month, and to 57 cells/mm^3 range from 2-213 cells/mm^3 during the last period of lactation).

The total cell count in the colostrum and milk samples of normal lactating women at various intervals after the onset of lactation was illustrated in Table (7).

The cells types in colostrum and milk were differentiated by staining with Giemsa stain. It was noticed that, the proportion of the macrophages and neutrophils was variable. The mean proportion of macrophages was 52.2% (range 30-81%) on the first two days postpartum increased to 67.6% (range 60-76%) during the end of the first week postpartum. The mean proportion of polymorphonuclear leukocytes showed reciprocal fall in counts from 41% (range 17-64%) on the first two days, to 23.9% (range 11-37%) during the end of the first week postpartum. The mean percentage of lymphocyte was fairly constant during the first week postpartum (range from 2-13%).

Table (8) & Figure (7) demonstrated the differential leukocytes counts during the first week postpartum. Morphology of

macrophage, neutrophils and lymphocyte in colostrum was demonstrated in Figures (8), (9) & (10).

Epithelial cells and cytoplasmic fragments in mature milk were present in high counts. Epithelial cells performed more than 75% from the total cells counts, while the leukocytes represented in a very low count.

Neutrophil and macrophage function assay revealed that, colostrum and milk leukocytes phagocytize more Staph aureus than peripheral blood leukocytes as demonstrated in Table (9). Colostrum and milk macrophage was more active than colostrum and milk neutrophil for phagocytizing Staph aureus as demonstrated in Table (10). The phagocytic activity of blood neutrophils was nearly similar to the phagocytic activity of colostrum and milk neutrophil as demonstrated in Table (11). Figure (11) illustrated the positive phagocytic cells (macrophages) in the colostrum and early milk. Figure (12) showed the positive phagocytic cells (neutrophil) in the peripheral blood.

leukocytes showed significant difference from the phagocytic index of blood leukocytes ($P < 0.05$; $T = 5.7$). There was no significant difference in the phagocytic index, between neutrophils of blood and milk ($P > 0.05$; $T = 1.57$).

In milk and colostrum, the phagocytic index of macrophage showed significant elevation from that of polymorphonuclear leukocytes ($P<0.05$; $T=4.66$).

Table (3) :- **The mean number of cells/mm^3 in colostrum and milk samples of normal lactating woman during the different periods after the onset of lactation.**

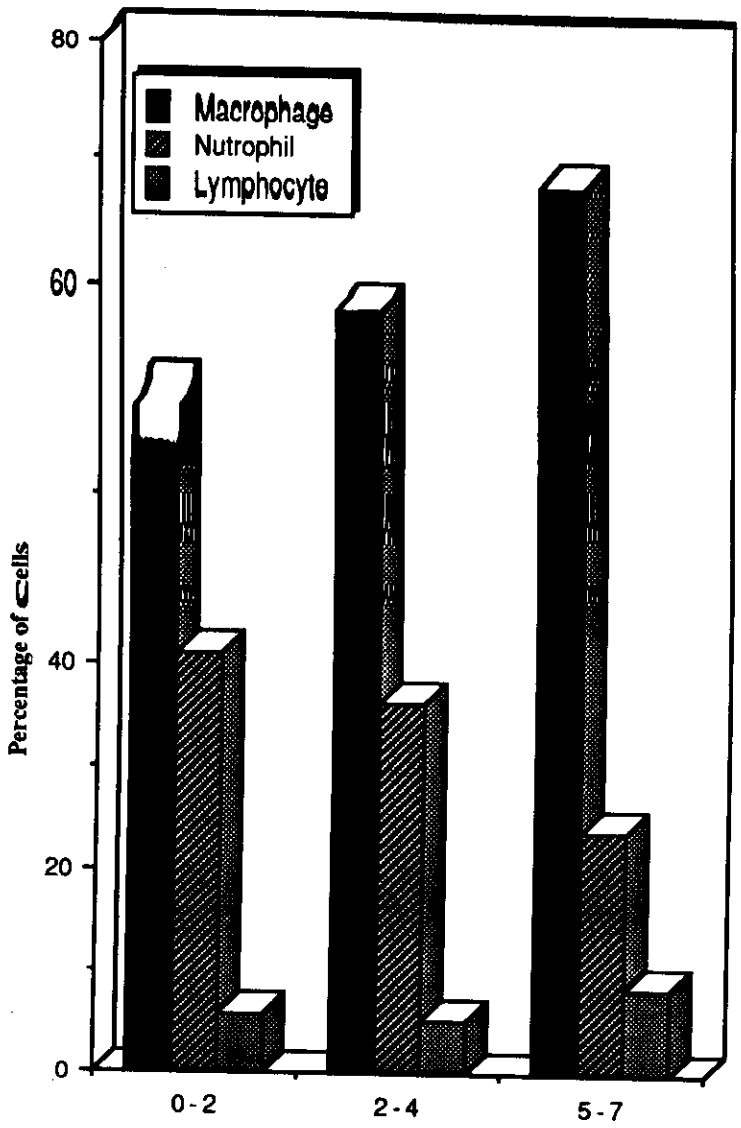
	Day (1)	Day (3)	Day (5)	month (1)	month (2)	month (4)	month (6)	month (12)	month (18)
Mean	4800	2100	2070	155	136	134	23	55	57
Range	2500-10600	900-6100	500-3800	336-55	420-3	363-15	50-2	1-69	2-213
No.	12	17	7	8	8	6	8	4	5

No = number of samples within each lactational period.

Table (8) The mean total and differential leukocytes counts in samples of postpartum colostrum and milk during the first week of lactation

Period of lactation	Total cells mean cells/mm^3	Macrophage mean%	Neutrophil mean%	Lymphocyte mean%
0-2 days	4800	52.2	41	5.9
No = 12	(2500-20600)	(30-81)	(17-64)	(2-11)
2-4 days	2100	58.1	36.3	5.2
No = 17	(900-6100)	(36-85)	(4-52)	(1-11)
5-7 days	2070	67.6	23.9	8.3
No = 7	(500-3800)	(60-76)	(11-37)	(3-13)

No= Number of samples



Fig(7) The means percentages of nutrophils, macrophages and lymphocytes counts in colostrum and milk samples of normal subjects during the first week of lactation

Table (9) Illustrates the mean phagocytic indices of leukocytes in blood and colostrum samples of normal lactating women.

Cell type	Mean	Range	S D
Blood leukocytes (No. = 15)	1.84	1.5-2.1	0.153
Colostrum leukocytes* (No. = 36)	2.3	1.4-3.2	0.387

* combined index

S D = standard deviation

T = 5.7

P < 0.05 (significant)

Table (10) demonstrates the mean phagocytic indices of macrophage and neutrophil in colostrum samples of normal lactating women.

Cell type	Mean	Range	S D
Macrophage (No = 36)	2.5	1.5-3.5	0.463
Neutrophil (No = 36)	1.95	1.3-3.4	0.386

S D = standard deviation

T = 4.66 P < 0.05 (significant)

Table (11) demonstrates the mean phagocytic indices of neutrophil in blood and colostrum samples of normal lactating women.

Cell type	Mean	Range	S D
Blood neutrophil (No = 15)	1.84	1.5-2.1	0.159
Colostrum and milk neutrophil (No = 36)	1.95	1.3-3.4	0.389

S D = standard deviation

T = 1.57 P > 0.05 (insignificant)

Figure (8)

a- Small macrophages in postpartum colostrum. The nucleus is round oval or reniform and

b- Large macrophage in postpartum colostrum. The cytoplasmic vacuoles are numerous giving the cell its characteristic foamy appearance (Giemsa stain, X1500).

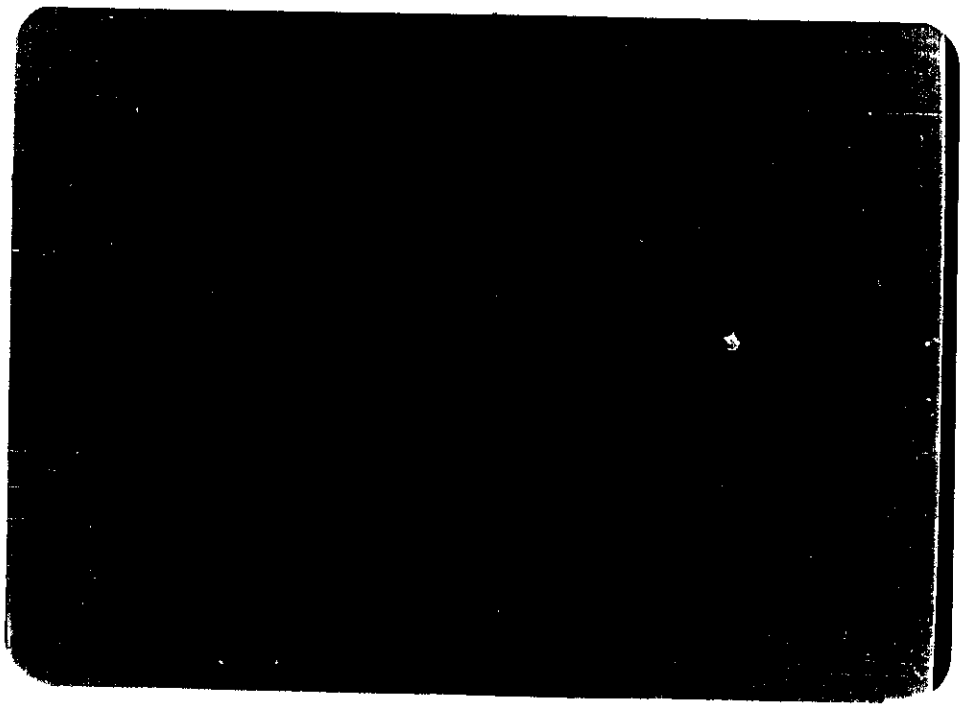


Figure (9) polymorphes (neutrophil) in postpartum colostrum (Giemsa stain X1500).

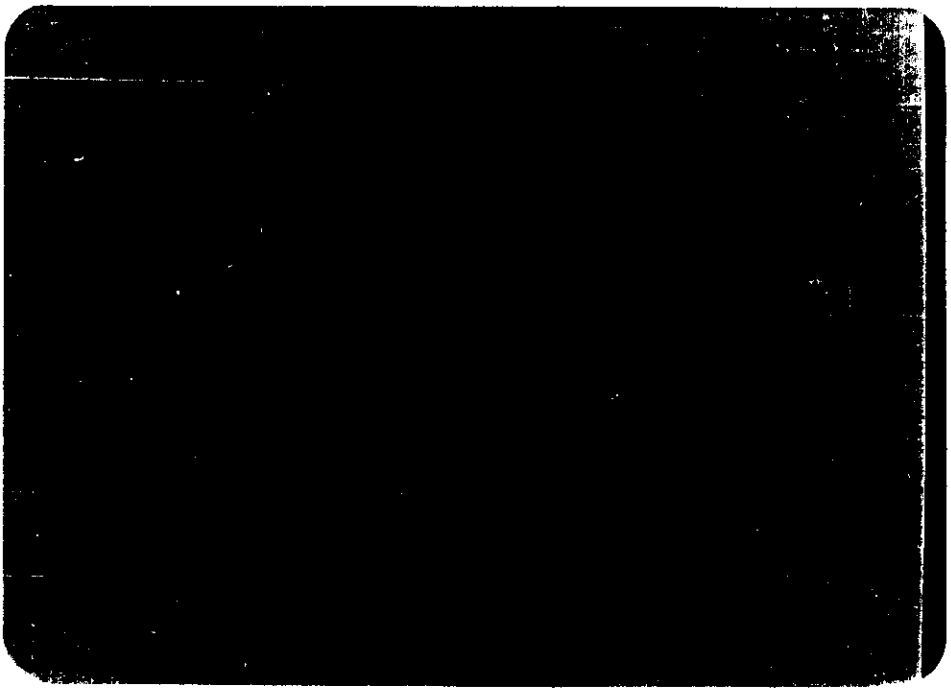


Figure (10) lymphocyte in postpartum colostrum.

(Giemsa stain X1500).

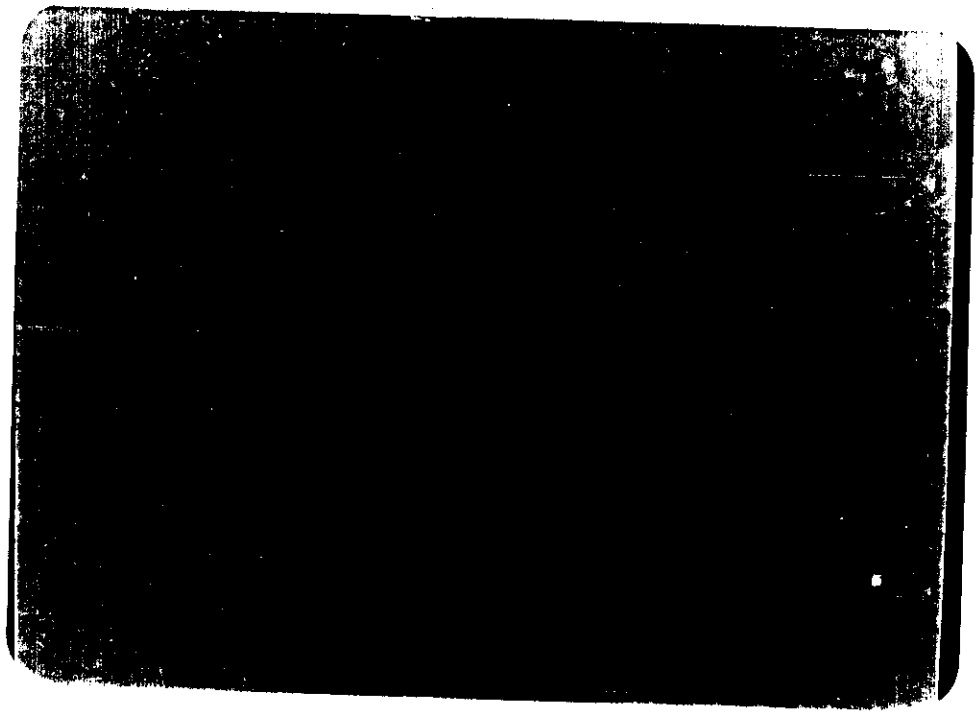


Figure (11) active phagocytic cell (macrophage) in colostrum of normal lactating women. (Giemsa stain X1500).

Figure (12) active phagocytic cell (neutrophil) in the blood of normal lactating female. (Giemsa stain X1500).

Bacteriology of human colostrum and milk:

Aerobic cultivation of colostrum and milk from normal lactating women revealed that, 17.9% were sterile and 82.1% were contaminated with different types of organisms.

Colony forming units (CFU) in normal milk was 8017.7 units/ml, (range 1000-18400 units/ml). It was similar to CFU in colostrum which was 7336.5 units/ml, (range 1000-21700 units/ml). Table (12) demonstrated the number of CFU in colostrum and milk.

The organisms isolated from milk and colostrum were nearly similar and mostly represent skin flora. The most common commensal organisms isolated from the samples were, coagulase negative Staphylococci, micrococcus, diphtheroid and neisseria. Potentially pathogenic organisms as Staph. aureus, gram negative bacilli. Streptococcus viridans and Streptococcus faecalis were also isolated from colostrum and milk.

Table (13) showed the different types of organisms isolated from colostrum and milk of normal lactating women.

Aerobic cultivation of milk from mastitis women revealed that, two samples were sterile (13.3%). In one sample Staph. aureus was isolated and the CFU was very high (more than 10^5) and there was excess number of pus cells. In the rest of the samples the CFU showed no significant different from CFU in milk from normal lactating women. Also, the isolated organisms were nearly similar to that isolated from normal milk as coagulase negative staphylococci, micrococcus and diphtheroid. Table (14) demonstrated the mean value of CFU in milk from mastitis and normal women which is of no significance. Table (15) demonstrated the distribution of different types of organisms in milk samples from mastitis women.

Antibiotic assay in colostrum and milk:

Detection of antibiotic Penicillin G, Cephradine and Ampicillin in the whey of milk and colostrum and in

the serum of 49 lactating women under the treatment with these antibiotics with a dose of 1000,000 units Penicillin G administered intra-muscular, or 500mg Ampicillin or 500mg Cephradine used orally, 2-3 hours before assay, revealed that, Cephradine was detected in 8 samples out of 27 samples in the blood, and not detected in any sample of milk or colostrum. Its level in blood was very low. Ampicillin was detected in 11 blood samples out of 15 samples,. the mean level was 0.823ug/ml, range 0.488-1.2ug/ml and was detected in 8 samples of milk, its mean level was 0.198ug/ml, range 0.19-0.789ug/ml. Trace amounts of Penicillin G. was detected in 4 colostrum samples out of 7 samples. Penicillin G. was detected in all the 7 samples of serum mean level was 7 unites/ml, ranged from 12-0.9 unites/ml.

Table (12) The mean number of viable colony forming units/ml in colostrum and milk samples of normal lactating women.

	Bacterial counts/ml		
	Mean	Range	S D
Colostrum (No. = 41)	7336.58	1000-21700	5028.15
Milk (No. = 46)	8017.39	1000-18400	4029

S D = standard deviation

T = 0.7 P > 0.05 (insignificant)

Table (14) The mean number of viable colony forming units/ml in milk from normal women and mastitis women.

	Bacterial counts/ml		
	Mean	Range	S D
Normal milk (No. = 46)	8017.39	1000-18400	4029
Mastitis milk (No. = 12)	7050	1400-10200	3143.6

S D = standard deviation

T = 0.89 P > 0.05

Table (13) Distribution of isolated organisms in 106 samples of colostrum and milk from normal lactating women

	Number of samples			
	Colostrum		milk	
	No.	%	No.	%
* Potential pathogen				
Staph. aureus	3	6.1	2	3.5
Gram negative bacilli	4	8.1	2	3.5
Strept. viridans	4	8.1	3	5.2
* Unlikely pathogen				
Coagulase negative Staphylococci	16	32.6	23	40.3
Micrococcus	3	6.1	6	10.5
Strept. faecalis	-		1	1.75
Diphtheroid	3	6.1	3	5.2
Neisseria	1	2.1	2	3.5
Staph. albus + Micrococcus	4	8.1	1	1.75
Staph. albus + Strept. viridans	1	2.1	-	
Staph. albus + Diphtheriod	2	4	3	5.2
Sterile	8	16.3	11	19.2
Total	49		57	

Table (15) Distribution of organisms in 15 samples of milk from patients of lactational mastitis.

Types of organisms	CFU/ml	No. of samples	%
Sterile	-	2	13.3
Staph. aureus	$>10^5$	1	6.6
Staph. albus	6450	6	40
Staph. albus + Diphtheroid	8470	4	26.6
Staph. albus + micrococcus	7000	1	6.6
Micrococcus	5000	1	6.6

CFU = colony forming units.