



Occult blood in stool in exclusively formula fed infants versus exclusively breast fed infants in the first six months of life[☆]



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Abstract *Background:* Since most of infant's formula are based on cow's milk to which allergy can occur, and considering the neurodevelopmental consequences of iron deficiency during infancy; we aimed to verify the occurrence of occult intestinal blood loss during the first 6 months of life in response to being fed cow's milk based formula versus breast milk. We also studied the iron status in order to assess prevalence of iron deficiency anemia.

Methods: Healthy full term infants from birth to 6 months who were either exclusively breast fed (BF) ($n = 50$) or formula fed (FF) ($n = 50$) were considered for enrollment. Detailed questionnaire describing perinatal period was taken from the mothers. Complete blood count, serum iron, total iron binding capacity (TIBC), serum transferrin and occult blood in stool were requested for each infant.

Results: We reported no significant differences in hemoglobin, hematocrit and MCV between both groups. FF infants had higher levels of TIBC. We found that 4/50 FF infants had positive occult blood in stool; while only 1/50 BF infants was positive. The prevalence of iron deficiency anemia was higher in the FF group (14%) rather than in the BF group (8%).

Conclusion: Although iron content in formula is higher than breast milk, BF infants attained better iron status than FF infants and the prevalence of anemia among the FF group was higher.

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[☆] The work was performed at outpatient neonatology clinic in the Pediatric Preventive Medicine Hospital of Cairo University, Cairo, Egypt.

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Introduction

Although the American Academy of Pediatrics and the American Academy of Family Physicians recommend breast milk for optimal infant nutrition, many parents still choose formula as an acceptable alternative.¹ Human milk is species-specific. The milk of each mammal species has adapted to supply its offspring with what is needed for optimal growth and survival. The feeding of cow's milk has adverse effects on iron (Fe) nutrition in infants and young children.²

Several different mechanisms have been identified that may act synergistically. Probably most important is the low iron content of cow's milk. It makes it difficult for the infant to obtain the amount of iron needed for growth. A second mechanism is the occult intestinal blood loss, which occurs in about 40% of normal infants during feeding of cow's milk. Loss of iron in the form of blood diminishes with age and ceases after 1 year of age.³ A third factor is calcium and casein provided by cow's milk in high amounts. Calcium and casein both inhibit the absorption of dietary non-heme iron.⁴

Iron deficiency is one of the most common nutritional problems around the world, both in developed and in developing countries.⁵ It is responsible for a long-lasting impairment of the neurological development in infants; Fe is also essential for immunity and growth.⁶

The breast milk contains about 0.5–1 mg/l; Fe. The absorption of this mineral is efficient at about 50% from breast milk versus 7% from formula milk.⁷

Almost all infant formulas are manufactured from pooled cow's milk. And because Fe absorption from cow's milk is low compared with breast milk,⁸ most of cow's milk based formulas are fortified. The main cow milk proteins are caseins, which bind to Fe. This strong binding keeps Fe soluble and prevents its release in a free form available for absorption by the duodenal mucosa.⁹ That explains why hydrolysis of whole caseins improves Fe absorption.¹⁰

Subjects and methods

Objectives

This study aimed to:

- Verify the occurrence of occult intestinal blood loss in Egyptian infants during the first 6 months of life in response to being fed cow's milk based formula versus breast milk.
- Study Fe status in order to identify the candidate for Fe supplementation.

Patients

A consecutive sample of 100 healthy term infants of both sex, in the first 6 months of life was selected from the outpatient neonatology clinic in the Pediatric Preventive Medicine Hospital of Cairo University. Studies of identical design were performed with *(50) exclusively breast fed infants (BF) and (50) formula fed infants (FF).

Inclusion criteria

- (a) Exclusively breast fed infant
- (b) Exclusively formula fed infant
- (c) Age is between 0 and 6 months
- (d) Only full terms were included in the study
- (e) Infants not taking any supplements

Exclusion criteria

- (a) Malnourished infants
- (b) Infants with major congenital anomalies
- (c) Infants with systemic diseases

Methods

For each infant, the following were assessed:

History taking

A questionnaire including:

- (a) Perinatal history
- (b) Developmental history
- (c) Vaccination history
- (d) Nutritional history

Clinical assessment

Thorough general and local examinations were done including anthropometric measurements (weight, length and head circumference). Results were expressed as mean (SD) over the Egyptian growth charts.

Laboratory investigations

Complete blood count, serum iron, total iron binding capacity (TIBC), transferrin saturation and occult blood in stool.

Specimen collection. Four milliliters (4 ml) venous blood were withdrawn under aseptic conditions, in two separate tubes (1 ml) for CBC collected in EDTA containing tube, and the other (3 ml) blood collected in serum separator tube and was left to clot formation, then was centrifuged and divided into 2 samples:

- (a) 0.5 ml serum to measure serum transferrin level (stored at -20°C).
- (b) 1.5 ml serum to measure serum iron and TIBC level.

On the light of the mentioned data regarding that the appropriate cutoffs for iron deficiency in infancy are subject to debate,^{11,12} and that the prevalence varies according to the cutoffs used, we defined microcytic anemia as age specific Hemoglobin and Mean Corpuscular Volume cut-off values < 2 standard deviation the reference mean value. Hemoglobin cut-off values were 100 g/l (1 mo), 90 g/l (2 mo), 95 g/l (3–6 mo) and 105 g/l (0.5–2 years). Corresponding values for MCV were 85 fl, 77 fl, 74 fl and 70 fl¹³ and we defined iron deficiency as 2 of 3 abnormal iron markers of the following: increased RDW $\geq 15\%$ ¹⁴ and transferrin saturation (TS) $< 10\%$ ¹² or serum iron $< 30 \mu\text{g/dl}$.¹⁵

Normal ranges

Serum iron	Serum TIBC
Newborn : 100–250 $\mu\text{g/dl}$	–
Infant : 40–100 $\mu\text{g/dl}$	100–400 $\mu\text{g/dl}$
Serum transferrin: 204–360 mg/dl	

Transferrin saturation (TS). It is the ratio of serum iron and total iron-binding capacity, multiplied by 100.

For infants

Transferrin saturation $> 10\%$ was considered within normal range.

Transferrin saturation <10% was identified as iron deficiency.¹²

Fecal Occult Blood (FOB) test device is rapid chromatographic immunoassay for the qualitative detection of human occult blood in feces. FOB can detect the levels of human occult blood as low as 50 ng/ml hemoglobin or 6 µg/g feces.

Statistical analysis

All statistical calculations were done using computer programs and SPSS [statistical package for the social science] version 9.01. Nominal data were expressed as frequency and percentage and were compared using Chi square test. Numerical data were expressed as mean, standard deviation and range and were compared using *T* test. Non-parametric data were compared using Mann Whitney test. Pearson's correlations were used to explore associations between numerical variables. *p* Values <0.05 were considered significant.

Results

Descriptive study was conducted on 100 healthy infants of both sex (54 males and 46 females) in the first 6 months of life. It was carried out in the neonatology clinic in the Pediatric Preventive Medicine Hospital of Cairo University.

They were divided into 2 groups:

- (1) Breast fed (BF) group: 50 exclusively breast fed infants (36 males and 14 females).
- (2) Formula fed (FF) group: 50 exclusively formula fed infants (18 males and 32 females).

Breast fed males (72%) were more than formula fed males (36%) due to Egyptian cultural issue concerned with preference to male gender.

We found low weight standard deviation (SD) more common among the FF group (16%) compared to the BF group (10%), *p* = 0.67 (Table 1).

There was no statistically significant difference in hematological indices between the BF group and FF group as shown in Table 2.

When comparing both groups of the study regarding iron indices "serum iron, TIBC, transferrin saturation and serum transferrin":

The mean serum TIBC in the FF group was (387.74 ± 53.51) higher than that among the BF group (360.20 ± 70.81) µg/dl. This difference was statistically significant (*p* = 0.03) as shown in Table 3.

Positive occult blood in stool was more in the FF group (8%) than in the BF group (2%), *p* = 0.16.

Table 4 showed that in the FF group: cases with positive occult blood in stool had lower hematological indices (hemoglobin, hematocrit and mean corpuscular volume) when compared to cases with negative occult blood in stool (*p* = 0.00).

Table 5 showed that in the FF group: Infants with positive occult blood in stool had lower values of serum iron and transferrin saturation and significant higher values of serum transferrin than in negative cases (*p* = 0.00).

In the formula fed group, the prevalence of iron deficiency anemia was higher (14%) than in the breast fed group (8%), *p* = 0.33.

Table 1 Demographic data of the studied groups.

Comparison	Breast fed group	Formula fed group	<i>p</i> Value
Mean age (months)	(3.79 ± 1.14)	(4.0 ± 1.29)	0.39
Sex <i>n</i> (%)			
Female	14 (28%)	32 (64%)	0.00
Male	36 (72%)	18(36%)	
Mean weight (kg)	(6.38 ± 1.15)	(6.29 ± 1.33)	0.70
Low weight SD < -2 <i>n</i> (%)	5 (10%)	8 (16%)	0.67
High weight SD > +2 <i>n</i> (%)	2 (4%)	2 (4%)	-

SD: standard deviation.

We found that: maternal anemia and occult blood in stool were considered as contributing factors of iron deficiency anemia in infants during the first 6 months of life (*p* = 0.00) as shown in Table 6.

Discussion

The association between gastrointestinal blood loss, iron deficiency anemia and ingestion of cows' milk based formula was suggested by.²

In our study, we reported that the prevalence of low weight standard deviation was higher among formula fed infants. Castillo and his colleagues agreed with us.¹⁶ Another survey done by Lönnnerdal and Havel also reported that the breast-fed infants had significantly higher body weights than did the formula-fed infants at 1, 4, and 6 months of age.¹⁷ We referred to Brown and his colleagues for the explanation of our results. They reported that in developing countries, neglected mother education about bottle feeding and errors in formula concentration were aggravating factors. Moreover, the incidence and prevalence rates of diarrhea were less among those who were exclusively breast-fed and doubled in those who were formula fed. They ascribed increased risk of diarrheal infections due to improper sterilization and hygiene.¹⁸

While, Isomura and his colleagues in a Japanese study reported that breast-fed infants demonstrated similar growth patterns compared with partially breast-fed or formula-fed infants.¹⁹

Other researchers reported that; formula fed children grow faster than those who are breast fed,²⁰ and others ascribed the cause possibly due to the higher protein content of formula versus breast milk.²¹ They also suggested that infants who are formula-fed weigh more and have greater risk for later obesity.^{22,23}

Our results regarding hematologic and iron indices were different than most other studies. Findings of Lönnnerdal and Hernell, partially agreed with ours as they compared hematologic indices and iron-status indicators in infants who were either breast-fed or formula fed from age 4 ± 2 weeks to 6 months, they found no significant differences in hematology or iron status were observed between groups at 4 and 6 months of age.²⁴ The same observation in another study was made later in 2008 by Lönnnerdal and Hernell; in which infants were fed cow's milk-based formulas containing 4 mg of iron/l from 1.5 to 6 months of age and their hematological status was compared to infants receiving the same formula but with 7 mg of iron/l and with breast-fed infants. They found no significant

Table 2 Comparison of hematological indices between the breastfed group and the formula fed group.

Comparison	Breast fed group	Formula fed group	<i>p</i> Value
Hemoglobin (g/dl)	(10.83 ± 0.90)	(10.69 ± 1.26)	0.50
Hematocrit %	(31.89 ± 2.61)	(31.90 ± 3.43)	0.99
Mean corpuscular volume (fl)	(77.24 ± 5.92)	(78.17 ± 7.42)	0.49

Table 3 Comparison of iron indices values between the breast fed group and the formula fed group.

Comparison	Breast fed group	Formula fed group	<i>p</i> Value
Serum iron (µg/dl)	(50.08 ± 24.71)	(45.94 ± 18.84)	0.34
Serum total iron binding capacity (µg/dl)	(360.20 ± 70.81)	(387.74 ± 53.51)	0.03
Transferrin saturation %	(12.16 ± 6.14)	(12.64 ± 7.23)	0.72
Serum transferrin (mg/dl)	(269.24 ± 102.88)	(279.20 ± 94.83)	0.61

Transferrin saturation = (serum iron/TIBC) 100.

Table 4 Comparison between positive and negative occult blood in stool cases among the formula fed group as regards hematological indices.

Comparison	Occult blood in stool (-ve) <i>n</i> = 46	Occult blood in stool (+ve) <i>n</i> = 4	<i>p</i> Value
Hemoglobin (g/dl)	(10.90 ± 1.04)	(8.28 ± 1.10)	0.00
Hematocrit %	(32.45 ± 2.83)	(25.65 ± 3.93)	0.00
Mean corpuscular volume (fl)	(79.35 ± 6.33)	(64.55 ± 5.27)	0.00

Table 5 Comparison between positive and negative occult blood in stool cases among the formula fed group as regards iron indices.

Comparison	Occult Blood in Stool (-ve) <i>n</i> = 46	Occult Blood in Stool (+ve) <i>n</i> = 4	<i>p</i> Value
Serum iron µg/dl	(52.65 ± 24.09)	(20.50 ± 1.73)	0.00
Serum TIBC µg/dl	(384.65 ± 53.25)	(423.25 ± 49.01)	0.16
Transferrin saturation %	(13.35 ± 7.11)	(4.50 ± 0.58)	0.00
Serum transferrin mg/dl	(269.15 ± 92.17)	(394.75 ± 9.98)	0.00

Table 6 Comparison between anemic and non-anemic infants as regards possible contributing factors of anemia.

Comparison		Non anemic <i>n</i> (%)	Anemic <i>n</i> (%)	<i>p</i> Value
Maternal anemia	Negative	38 (43)	1 (9)	0.03
	Positive	51 (57)	10 (91)	
Pregnancy	Single	76 (85)	9 (82)	0.75
	Twins	13 (15)	2 (18)	
Mode of delivery	CS	43 (48)	5 (45.5)	0.89
	NVD	41 (46)	5 (45.5)	
	NVD	5 (6)	1 (9)	
	+			
Occult blood in stool	Cephalhematoma			0.00
	Negative	84 (94)	6 (54.5)	
Nutrition	Positive	5 (6)	5 (45.5)	0.33
	Breast feeding	46 (52)	4 (36)	
	Formula feeding	43 (48)	7 (64)	

CS: Cesarean section, NVD: normal vaginal delivery. *p* values in bold means significant (*p* < 0.05).

differences in hematological indices among the groups at 6 months of age; all infants had satisfactory iron status.²⁵

In our study, occult intestinal blood loss was 8% in the FF group and 2% in the BF group; it occurs in about 40% of normal infants during feeding of cow's milk secondary to cow's milk protein-induced colitis.²

Repucci reported that the presence of occult blood in stool in exclusively breast fed infants may indicate that cow milk protein must have passed into the breast milk. Prenatal sensitization probably occurred.²⁶

Fernandes and his colleagues agreed with us as they reported that in infants fed with cow milk formula, occult fecal blood loss in stool is an aggravating factor of iron deficiency.²⁷

In assessing the prevalence of iron deficiency anemia in breast fed infants compared to formula fed in the 1st 6 months of life but we faced a problem which is the absence of good cut-off values for infants < 6 months old. Cut-off values have been established for groups of different ages and sex, starting from the age of 6 months.²⁸

De Pee and his colleagues ascribed the cause of lack of prevalence data on anemia and iron deficiency in this particular age group to two reasons the first is that; there were no good cut-off values for infants < 6 months old. And for infants > 6 months old, the cut-off value (110 g/l) is sometimes applied. However, this is not appropriate.²⁹

The second reason; is that during the first month of life, hemoglobin declines from the very high level at birth to its lowest level at 6–10 weeks of age known as the “physiologic anemia of the newborn.” Therefore, until 6–10 weeks of age, hemoglobin does not reflect iron storage or supply. After hemoglobin has reached its lowest level at ~2 months of age, it slowly increases again and becomes more or less stable at 6–9 months of age unless depleted. Another reason; is that it has generally been assumed that infants born at term and with an adequate birth weight have adequate iron stores for the first 4–6 months of life.³⁰

Kumar and his colleagues agreed with us regarding the risk of infant's iron deficiency conferred by maternal iron deficiency. They reported that, breast milk iron was significantly reduced in mothers with severe anemia but appeared to be preserved in mothers with mild-to-moderate anemia.³¹

Conclusion

In the light of this study we found that (11%) of the whole study group (100 infants) were anemic (iron deficiency anemia). We reported that occult blood in stool is an aggravating factor of iron deficiency anemia among formula fed infants. We also reported an association between infant's anemia and maternal anemia.

Conflict of interest

The authors have declared no conflict of interest.

References

- O'Connor NR. Infant formula. *Am Fam Physician* 2009;**79**(7):565–70.
- Ziegler EE. Consumption of cow's milk as a cause of iron deficiency in infants and toddlers. *Nutr Rev* 2011;**69**(1):320–50.
- Fomon SJ, Ziegler EE, Nelson SE, et al. Cow milk feeding in infancy: gastrointestinal blood loss and iron nutritional status. *J Pediatr* 1981;**98**:540–5.
- Bernos E, Girardet JM, Humbert G, et al. Role of the O-phosphoserine clusters in the interaction of the bovine milk α s1-, β -, κ -caseins and the PP3 component with immobilized iron (III) ions. *Biochim Biophys Acta* 1997;**1337**:149–59.
- Bouglé D, Laroche D, Bureau F. Zinc and iron status and growth in healthy infants. *Eur J Clin Nutr* 2000;**54**:764–7.
- Castillo-Duran C, Cassorla F. Trace minerals in human growth and development. *J Pediatr Endocrinol Metab* 1999;**12**:589–601.
- Griffin IJ, Abrams SA. Iron and breastfeeding. *Pediatr Clin North Am* 2001;**48**(2):401–13.
- Butte NF, Lopez-Alarcon MG, Garza C. Nutrient adequacy of exclusively breastfeeding for the treatment during the first six months of life. Department of nutrition for health and development, Department of child and adolescent for health and development. Geneva, World Health Organization; 2002.
- Kibangou IB, Bouhallab S, Henry G, et al. Milk proteins and iron absorption: contrasting effects of different caseino-phospho-peptides. *Pediatr Res* 2005;**58**(4):731–4.
- Chong DK, Roberts W, Arakawa T, Illes K, Bagi G, Slattery CW, et al. Expression of the human milk protein beta-casein in transgenic potato plants. *Transgenic Res* 1997;**6**:289–96.
- Aggett PJ, Agostini C, Axelsson I. Iron metabolism and needs in early childhood: do we know enough? A commentary by the ESPGHAN Committee on Nutrition. *J Pediatr Gastroenterol Nutr* 2002;**34**:337–45.
- Zetterström R. Iron deficiency and iron deficiency anaemia during infancy and childhood. *Acta Paediatr* 2004;**93**:436–9 [Stockholm. ISSN: 0803–5253].
- Willows ND, Dewailly E, Gray-Donald K. Anemia and iron status in Inuit infants from Northern Quebec. *Canad J Public Health* 2000;**91**:407–10.
- Gill DG, Vincent S, Segal DS. Follow-on formula in the prevention of iron deficiency: a multicentre study. *Acta Paediatr* 1997;**86**:683–9.
- Oski FA. Iron deficiency in infancy and childhood. *N Engl J Med* 1993;**329**:190–3.
- Castillo C, Atalah E, Riumalló J, Castro R. Breast-feeding and the nutritional status of nursing children in Chile. *Bull Pan Am Health Organ* 1996;**30**(2):125–33.
- Lönnnerdal B, Havel PJ. Serum leptin concentrations in infants: effects of diet, sex, and adiposity. *Am J Clin Nutr* 2000;**72**(2):484–9.
- Brown KH, Black RE, Lopez de Romaña G, Creed de Kanashiro H. Infant-feeding practices and their relationship with diarrheal and other diseases in Huascar (Lima), Peru. *Pediatrics* 1989;**83**(1):31–40.
- Isomura H, Takimoto H, Miura F, Kitazawa S, Takeuchi T, Itabashi K, et al. Type of milk feeding affects hematological parameters and serum lipid profile in Japanese infants. *Pediatr Int* 2011;**53**(6):807–13.
- Kramer MS, Guo T, Platt RW, Vanilovich I, Sevkovskaya Z, Dzikovich I, et al. For the promotion of breastfeeding intervention trial study group feeding effects on growth during infancy. *J Pediatr* 2004;**145**:600–5.
- Koletzko B, Baker S, Cleghorn G, Neto UF, Gopalan S, Hernell O, et al. Global standard for the composition of infant formula: recommendations of an ESPGHAN coordinated international expert group. *J Pediatr Gastroenterol Nutr* 2005;**41**:584–99.
- Grummer-Strawn LM, Mei Z. Does breastfeeding protect against pediatric overweight? Analysis of longitudinal data from the centers for disease control and prevention pediatric nutrition surveillance system. *Pediatrics* 2004;**113**(2):43–50.
- Burke V, Beilin LJ, Simmer K. Breastfeeding and overweight: longitudinal analysis in an Australian birth cohort. *J Pediatr* 2005;**147**(1):56–61.

24. Lönnerdal B, Hernell O. Iron status of infants fed low-iron formula: no effect of added bovine lactoferrin or nucleotides. *Am J Clin Nutr* 2002;**76**:858–64.
25. Lönnerdal B, Hernell O. Iron, zinc, copper and selenium status of breast-fed infants and infants fed trace element fortified milk-based infant formula. *Acta Paediatr* 1994;**83**:367–73 [Article first published online: 14 May 2008].
26. Repucci A. Resolution of stool blood in breast-fed infants with maternal ingestion of pancreatic enzymes. *J Pediatr Gastroenterol Nutr* 1999;**29**(4):500.
27. Fernandes SM, de Morais MB, Amancio OM. Intestinal blood loss as an aggravating factor of iron deficiency in infants aged 9 to 12 months fed whole cow's milk. *J Clin Gastroenterol* 2008;**42**(2):152–6.
28. WHO/UNICEF/UNU. Iron deficiency anaemia: assessment, prevention, and control. A guide for programme managers. WHO, 2001; United Nations Children's Fund; United Nations University; World Health Organization.
29. De Pee S, Bloem MW, Sari M, Kiess L, Yip R, Kosen S. The high prevalence of low hemoglobin concentration among Indonesian infants aged 3–5 months is related to maternal anemia. *J Nutr* 2002;**132**:2215–21.
30. Calvo EB, Galindo AC, Aspres NB. Iron status in exclusively breast-fed infants. *Pediatrics* 1992;**90**(3):375–9.
31. Kumar A, Rai AK, Basu S. Cord blood and breast milk iron status in maternal anemia. *Pediatrics* 2008;**121**:e673.