Arch Argent Pediatr. 2026;e202611073

Anemia and iron deficiency in healthy, exclusively breastfed infants: Prevalence and association with sex and birth weight

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ABSTRACT

Introduction. Anemia and iron deficiency (ID) negatively affect health and neurocognitive development. There is controversy over whether breast milk meets iron requirements between four and six months of age, especially in populations with a high prevalence of anemia.

Objective. To determine the prevalence of anemia, ID, and iron-deficiency anemia (IDA) in healthy infants who are exclusively breastfed (EBF), and to analyze their association with sex and birth weight.

Population and methods. A retrospective, observational, cross-sectional analytical study. Data were analyzed from healthy infants who received exclusive breastfeeding and were seen at a pediatric center in La Plata from 2013 to 2016. Sex and birth weight (classified as lower birth weight if 2500–2999 g) were recorded. At six months of age, the following were assessed: anemia (hemoglobin <10.5 g/dL), ID (serum ferritin <12 µg/L), and IDA (meeting both criteria). Associations were evaluated, and the odds ratio (95% CI) was estimated using logistic regression (adjusted for sex).

Results. A total of 254 infants were included. The prevalence rates of anemia, ID, and IDA were 42.5%, 26.7%, and 13.3%, respectively. The geometric mean ferritin level was lower in boys (p = 0.002). An association was observed between ID and lower birth weight (adjusted OR = 4.45 [95% CI 2.08–9.67]).

Conclusion. The prevalence of anemia, ID, and IDA was high. Ferritin levels were lower in male infants. Infants with lower birth weight were more likely to develop DH.

Keywords: *anemia; iron deficiency; breastfeeding; infant.*

doi: http://dx.doi.org/10.5546/aap.2026-11073.eng

To cite: Varea A, Disalvo L, Sala M, Matamoros N, Fasano MV, González HF. Anemia and iron deficiency in healthy, exclusively breastfed infants: Prevalence and association with sex and birth weight. *Arch Argent Pediatr.* 2026;e202611073. Online ahead of print 30-JUL-2026.

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Funding*: This research was conducted with the support of the Prof. Dr. Fernando E. Viteri Institute for Pediatric Development and Research at the Hospital Interzonal de Agudos Especializado en Pediatría Sor María Ludovica.*

Conflict of interest*: None.*

Received*: 2-23-2026*
Accepted*: 5-19-2026*

INTRODUCTION

Anemia is a major global public health problem, affecting approximately 24.3% of the population; iron deficiency (ID) accounts for about half of these cases.[1] In Latin America, its persistence in early childhood reflects both structural inequalities and limitations in the implementation and effectiveness of preventive strategies.[2] Children under five years of age, especially during their first year of life, represent one of the most vulnerable groups.[3]

Iron deficiency anemia (IDA) in its early stages has significant consequences beyond hematological effects, as it affects key processes of neurodevelopment. IDA has been associated with abnormalities in myelination, neurotransmission, and synaptic function, potentially affecting cognitive, motor, and behavioral development, even in the long term.[4,5]

In full-term newborns, iron stores are usually sufficient to meet requirements during the first few months of life. However, starting in the fourth month, maintaining adequate iron status increasingly depends on exogenous intake.[6,7] Although breast milk is the optimal form of nutrition due to its many benefits, there is controversy regarding whether, under conditions of exclusive breastfeeding (EBF), it adequately meets iron requirements.[8,9] Studies conducted in various settings, including high-income countries, have reported high prevalences of anemia and ID in exclusively breastfed infants.[10-14]

In Argentina, anemia remains a public health problem.[15] The National Nutrition and Health Survey (ENNyS, by its Spanish acronym), conducted between 2004 and 2005, showed a high prevalence among children under two years of age, particularly during the second half of their first year of life.[16,17] However, evidence regarding exclusively breastfed infants is very limited. Among the few published reports, a study conducted more than three decades ago found that 44% of infants had anemia.[18] In this context, a significant knowledge gap persists in our setting, particularly during a critical period for the prevention of ID, when the depletion of neonatal iron stores coincides with the transition to complementary feeding. Given this lack of information, the objective was to determine the prevalence of anemia, ID, and IDA among exclusively breastfed infants and to analyze their associations with sex and birth weight.

POPULATION AND METHODS

A retrospective, observational, cross-sectional analytical study was conducted using data from healthy infants aged six months (180 ± 15 days) who underwent routine checkups at the Health Observatory of the Institute for Pediatric Development and Research (IDIP, by its Spanish acronym) at the Hospital Interzonal de Agudos Especializado en Pediatría Sor María Ludovica (HIAEP, by its Spanish acronym) in La Plata, Argentina, between January 2013 and June 2016.

Exclusive breastfeeding was defined as the practice in which the infant received only breast milk, without the introduction of other foods, water, or formula.

The study included full-term infants (≥37 weeks' gestational age) with a birth weight ≥2500 g, a normal fetal and neonatal history, documented umbilical cord clamping (>30 seconds after birth), EBF, and no iron supplementation. Infants for whom hemoglobin (Hb) levels were not measured and those who were mixed-fed (breast milk and formula) or formula-fed exclusively were excluded.

Sample size

It was calculated to estimate the prevalence of anemia with a 95% confidence level and a margin of error of 0.07, based on the rate reported in a previous study of infants younger than six months (32.8%).[19] The minimum required sample size was 173 infants.

Biochemical, anthropometric, and sociodemographic variables

The presence of anemia and ID was determined based on complete blood count data. Anemia was defined as Hb <10.5 g/dL, ID as ferritin <12 µg/L, and IDA as the simultaneous occurrence of both criteria. Because ferritin is an acute-phase reactant, different cutoff values were applied to define iron deficiency: ferritin <12 µg/L when the white blood cell count was ≤15 000/mm³ and ferritin <30 µg/L when it was >15 000/mm³.[20,21]

Nutritional status was assessed by measuring weight and height at birth and at 6 months, and weight-for-age and height-for-age indicators were calculated using the World Health Organization's child growth standards (WHO).[22] Lower birth weight was defined as a birth weight between 2500 g and 2999 g.[10]

Sociodemographic information included the infant's age and sex, the parents' employment status and educational level, and unmet basic needs (UBN). UBN were defined according to the methodology of the National Institute of Statistics and Censuses (INDEC, by its Spanish acronym).[23]

Data sources and measurements

Blood samples were collected at the IDIP laboratory, and biochemical tests were performed at the Central Laboratory of the HIAEP Sor María Ludovica. The complete blood count was performed using a hematology analyzer (ABX Pentra 60™, Montpellier, France), and ferritin levels were measured by chemiluminescence (Access Beckman Coulter™, Brea, CA).

Anthropometric measurements were performed by trained pediatricians using standardized techniques. Weight was measured using a digital electronic scale (Tanita UM-061™; accuracy 0.1 g), and height was measured using a portable height rod (accuracy 0.5 cm).[24]

Sociodemographic information was obtained from clinical records.

Statistical analysis

The data were analyzed using the R statistical package (v. 4.0.3). The normality of the variables was assessed using the Kolmogorov-Smirnov test. Variables with a normal distribution were expressed as mean ± standard deviation, and those with a non-normal distribution as median and interquartile range (P25; P75). Given its log-normal distribution, ferritin concentration was expressed as the geometric mean (GM) and 95% confidence interval (95% CI).

Qualitative variables were reported as frequencies and percentages. To compare quantitative variables by sex and lower birth weight, the Student's t-test or the Mann-Whitney U test was used, as appropriate. The association between anemia, ID, IDA, and other qualitative variables was assessed using the chi-square test or Fisher's exact test. The odds ratio (OR) with 95% CI was estimated using logistic regression. A p-value <0.05 was considered statistically significant.

Ethical considerations

The study was approved by the Committee for Institutional Review of Research Protocols (CIRPI, by its Spanish acronym) of the HIAEP Sor María Ludovica in La Plata, dated August 23, 2016 (Code: 23082016).

RESULTS

Sample characteristics

The final sample consisted of 254 infants with EBF who met the inclusion criteria and had Hb levels recorded in their medical records. Serum ferritin levels were also available for 187 of these infants (*Figure 1*).

The sociodemographic characteristics and anthropometric data for the infants at birth and at six months (180 ± 15 days) are presented in *Table 1*.

Prevalence of anemia, iron deficiency, and iron-deficiency anemia

At six months, 108 (42.5%) infants had anemia. Among the 187 for whom ferritin levels were measured, 50 (26.7%) had ID and 30 (13.3%) had IDA.

The mean Hb level was 10.7 ± 0.9 g/dL. Among infants with anemia, 79.6% had mild anemia (Hb 9.5-10.4 g/dL), and the rest had moderate anemia (Hb 7.0-9.4 g/dL). No cases of severe anemia (Hb <7.0 g/dL) were reported.

The mean ferritin concentration in the total sample was 20.46 µg/L (17.91-23.36). In anemic infants, ferritin concentrations were significantly lower, and the prevalence of iron deficiency was higher (*Table 2*).

Association of anemia, ID, and IDA with sex

No significant differences were observed in the prevalence of anemia, ID, and IDA by infant sex (*Table 3*). However, the mean ferritin level was significantly lower in male infants (p = 0.002).

Association between anemia, ID, and IDA with birth weight

Hb levels at six months did not differ significantly between infants with lower birth weight and those with normal birth weight. In contrast, ferritin concentrations were significantly lower in infants with lower birth weight: 11.59 µg/L (9.19-14.61) versus 23.76 µg/L (20.50-27.54) (p <0.001).

When analyzed by sex, ferritin levels were significantly lower in infants with lower birth weight, in both males and females (*Figure 2*).

In the bivariate analysis, a significant association was observed only between lower birth weight and the presence of ID. The prevalence of ID was higher in infants with lower birth weight than in those with normal birth weight: 51.3% vs. 20.3% (p <0.001; OR 4.10; 95%CI 1.83-9.30). This association remained significant after adjusting for sex (OR 4.45; 95% CI 2.08-9.67).

Figure 1. Flowchart

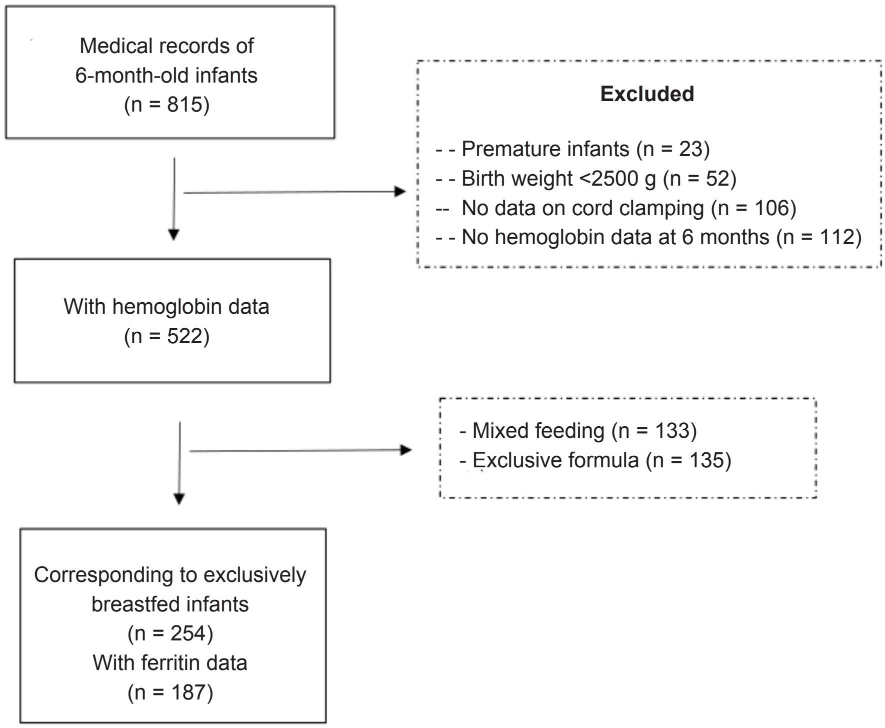


Table 1. Sociodemographic characteristics of families and healthy, exclusively breastfed infants (n = 254)

Variables	Frequency (%)	Median (IQR)
Sociodemographic characteristics		
Age (years)		
Mother		28 (22-34)
Father		25 (20-30)
Level of education (≥12 years)+		
Mother (n = 247)	92 (37.2)	
Father (n = 232)	78 (33.6)	
Employment status* (employed)		
Mother (n = 248)	38 (15.3)	
Father (n = 239)	233 (97.5)	
UBN	165 (65.0)	
Perinatal characteristics		
Sex (female)	117 (46.1)	
Type of delivery (vaginal)	178 (70.1)	
Gestational age (weeks)		39 (38-40)
Birth weight (g)		3345 (3.052-3684)
Length at birth (cm)		48.5 (48-51)
Characteristics of infants at six months		
Weight (g)		8100 (7450-8848)
Height (cm)		67 (65-68.5)
Z-score for height-for-age*		0.8 (1.06)
Z-score for weight-for-age*		-0.03 (1.07)
Z-score for weight/height*		0.74 (1.10)

UNB: unmet basic needs; IQR: interquartile range.

** Mean (standard deviation).*

+ Educational level (≥12 years corresponds to completion of high school).

Table 2. Ferritin levels and prevalence of iron deficiency in exclusively breastfed infants according to the presence of anemia at six months of age (n = 187)

Variables	Anemia		*p*-value
	No (n =108)	Yes (n = 79)	
Ferritin (µg/L)*	27.80 (22.20-34.81)	17.37 (14.81-20.37)	<0.001
Prevalence of ID, n (%)	20 (18.5)	30 (38.0)	0.004

ID: iron deficiency.
** Geometric mean (95%CI).*

Table 3. Hemoglobin and ferritin levels, and prevalence of anemia, iron deficiency, and iron-deficiency anemia by sex in healthy, exclusively breastfed infants at six months of age

Variables	Female (%)	Male (%)	*p*-value
Anemia	47 (40.2)	61 (44.5)	0.525
Hemoglobin* (g/dL)	10.76 ± 0.87	10.57 ± 0.92	0.164
ID	16 (20.0)	34 (31.8)	0.102
Ferritin** (µg/L)	26.21 (21.32-32.24)	16.99 (14.37-20.09)	0.002
IDA	10 (12.5)	20 (18.7)	0.237

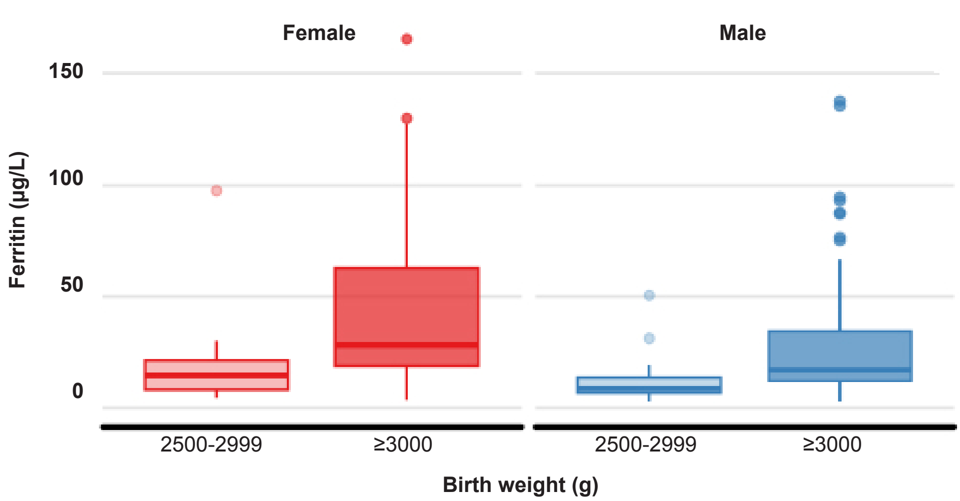
ID: iron deficiency, IDA: iron deficiency anemia.
** Mean ± standard deviation.*
*** Geometric mean (95%CI).*
A total of 254 hemoglobin measurements were performed (female: n = 117; male: n = 137) and 187 ferritin measurements (female: n = 80; male: n = 107).

DISCUSSION

In this study, the prevalence of anemia, ID, and IDA among healthy infants exclusively breastfed at 6 months of age was high: 42.5% had anemia, 26.7% had ID, and 13.3% had IDA. These findings are particularly relevant, given that they pertain to a population of healthy, full-term infants who are exclusively breastfed, a group considered at low risk for ID. In this context, anemia remains a serious public health problem according to the WHO classification.[25]

Studies of breastfed infants conducted in

Figure 2. Ferritin levels by birth weight and sex among exclusively breastfed infants at 6 months of age



other countries show mixed results. In India, a study of infants aged three to five months reported prevalence rates of anemia and ID of 36.4% and 11.4%, respectively.[11] In Brazil, among infants aged one to six months, the prevalence rates of ID and IDA were 26.1% and 23.9%, respectively.[12] In comparison, another Brazilian cohort showed a prevalence of anemia of 37.5% at six months of age.[13] In Japan, despite being a developed country, the prevalence was 28.4%.[14] Furthermore, a review of six randomized clinical trials conducted in four countries showed prevalence rates of ID ranging from 6% (Sweden) to 37% (Ghana), and for IDA, from 2% to 16%.[10] This variability likely reflects methodological, age-related, and population-based differences that make direct comparisons between studies difficult. Nevertheless, the values found in the present study fall within the upper range of those reported, underscoring the significance of the problem in our context.

It is important to note that in most of the studies cited, an Hb level of <11 g/dL was used as the cutoff for determining the presence of anemia,[25] whereas in the present study, the WHO 2024 threshold of 10.5 g/dL was adopted.[20] Even with this lower cutoff, the prevalence of anemia in the study population was high. More than three decades after the study by Calvo et al.,[17] and despite the implementation of various prevention and control strategies in Argentina, our results show comparable anemia rates. In a previous study by our group on infants aged four to five months, using the Hb <10.3 g/dL cutoff proposed by Saarinen and Siimes,[26] 28.9% of infants on EBF had anemia.[19] Subsequently, in a more recent study that evaluated the effectiveness of daily versus weekly ferrous sulfate supplementation between three and six months of age, the prevalence of anemia (Hb <11 g/dL), ID, and IDA at six months in the group receiving exclusive breast milk without supplementation was 60.9%, 40.5%, and 33.3%, respectively.[27] Consistent with these results, most cases were mild anemia, and no cases of severe anemia were reported.

Several studies have indicated that sex may influence iron nutritional status during early childhood, suggesting that being male may be a risk factor for ID. Although the underlying mechanisms are not fully understood, they may involve hormonal differences that modulate erythropoiesis or physiological factors, such as greater lean mass gain and lower iron stores at birth in boys compared to girls.[28,29] In contrast to our previous findings, which showed a higher prevalence of anemia in boys,[19] the present study found no significant differences in the prevalence of anemia, ID and IDA by sex. However, the mean ferritin level was significantly lower in male infants, which is consistent with reports by other authors.[10,30-32]

Furthermore, iron stores at birth are strongly influenced by birth weight and gestational age.[9] Although premature infants and those with low birth weight (<2500 g) were excluded from this study, infants with lower birth weight were approximately four times more likely to develop ID. Similar results were reported by Yang et al., who observed a higher risk of ID in infants with lower birth weight, even after adjusting for study site and timing of umbilical cord clamping.[10]

This study has limitations inherent to its retrospective design, given that the data were obtained from medical records. In this regard, it was not possible to obtain information on relevant maternal factors, such as nutritional and hematological status during pregnancy. Likewise, information on umbilical cord clamping was limited; it could only be classified as ≤30 seconds or >30 seconds, without more precise data on early or late clamping. Furthermore, ferritin levels were measured in a subsample due to operational and technical limitations, which may have introduced selection bias, in that the infants included in the study may not be fully representative of the entire sample.

Despite the time elapsed, this study provides important evidence—which is scarce in our setting—on the prevalence of anemia and ID in healthy, exclusively breastfed infants at 6 months of age. The consistency of these findings with more recent studies[27] suggests that the magnitude of the problem has not changed substantially. It should be noted that, during the period evaluated, national recommendations focused on preventive iron supplementation for specific at-risk groups and did not systematically include healthy, full-term infants who were exclusively breastfed.[33] In this context, the absence of prophylactic supplementation in the study cohort reflects the practices recommended at that time. It suggests that even infants considered low-risk could develop ID. Furthermore, using updated cutoff points to determine the presence of anemia enables adequate comparability with contemporary studies. In this regard, characterizing iron status in the absence of supplementation provides a useful benchmark for understanding IDA in the first six months of life and for evaluating the impact of preventive strategies.

Given the potential impact of ID on neurodevelopment during a critical period of life, it is essential to strengthen prevention strategies, including raising awareness of the importance of preventive iron supplementation for exclusively breastfed infants.

CONCLUSION

In the study population, the prevalence of anemia, ID, and IDA was high. Ferritin levels were lower in male infants. Infants with lower birth weight were more likely to develop ID. ■

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