Study Protocol

OFFICIAL TITLE
Effectiveness of weekly and daily iron supplementation for the prevention of iron-deficiency anemia in infants. Impact on genomic stability.

NCT ID not yet assigned

03/01/2017
Iron deficiency anemia, iron supplementation and genomic stability in infants.

Iron deficiency is the most prevalent nutritional deficiency and the main cause of anemia. Globally, 43% of pre-school children have anemia. In Argentina, the National Nutrition and Health Survey reported that 34.5% of children less than two years of age and 50.8% of 6 to 9-month-old infants were anemic. Although daily iron supplementation is supported as a preventive strategy, adherence is low in our country due to adverse effects and low prescription rates. Likewise, excess iron may cause genomic instability and thereby structural and functional alterations in proteins, lipids and DNA. Such damage may be permanent and lead to the development of neoplastic processes, diabetes and cardiovascular disease. Weekly iron supplementation is proposed as an effective alternative for older children and pregnant women, but evidence of such an effect in infants is scarce. The aim of this study is to compare the effectiveness of weekly and daily (current prescription) iron supplementation to prevent anemia in infants and to analyze its impact on genomic stability. We will perform a randomized, controlled clinical trial in infants that attend IDIP’s Health Observatory for periodic health controls. Infants with exclusively breastfeeding (EBF) will be randomized into three groups: daily and weekly iron supplementation and no supplementation. Infants receiving both breastfeeding and infant formula i.e. mixed feeding (MF) will be randomized into two groups: daily and weekly iron supplementation. We will study anemia, iron status and genomic stability before supplementation (at 3 months) and at 6 months. Weekly iron supplementation is expected to be more effective than daily supplementation for the prevention of infant iron-deficiency anemia due to a higher adherence and a lower incidence of adverse effects. Further, genomic stability is also expected to be lower. We expect that our results will allow to revise the current supplementation schedules (start, frequency, dose, EBF) on scientific grounds and improve the economic resources devoted to anemia prevention (lowering medication expenses).

Research problem and clinical-sanitary relevance
Iron deficiency (ID) is the most prevalent nutritional deficiency and the main cause of anemia worldwide (WHO 2001), infants less than 2 years and pregnant women being the most vulnerable groups. Iron deficiency anemia (IRA) may impact at early stages of life by causing growth retardation, lower cognitive abilities, lethargy and poor attention (Clark 2008, Beard 2008, Carter 2010, Lozoff 2007). This public health concern has stimulated the research for the development of alternative preventive solutions. The overall prevalence of anemia among preschool children is 43% (Stevens 2013). In Argentina, the National Nutrition and Health Survey (ENNyS by its Spanish acronym) reported that such prevalence was 34.5% in children less than 2 years of age and 50.8% in 6-9-month-old infants (ENNyS 2007). A study performed at the Health Observatory of IDIP (La Plata Children’s Hospital) showed that one out of three clinically healthy infants less than 6 months of age had anemia, regardless of their being exclusively breastfed or receiving mixed feeding (MF) (Iannicelli 2012). According to a survey performed with clinical record data of the period 2013-2015, anemia affects more than half of infants that attend the Health Observatory (unpublished data).

Iron supplementation is recommended as a priority strategy and has been endorsed by the World Health Organization (WHO) when ID prevalence is high in a specific population. In our country, the Argentine Pediatrics Society recommends daily ferrous sulfate supplementation in infants less than 4 months of age receiving MF and from 6 months onwards in EBF (SAP 2009). Despite this recommendation and the free supply of ferrous sulfate through the health program RemediAR, adherence is low, mainly due to gastrointestinal adverse effects, mothers’ neglect and low rate of prescription by healthcare team members (Christensen 2013, Bernztein 2008). Thus, since the nineties, weekly ferrous sulfate supplementation has been proposed as an alternative preventive treatment for IDA. However, most studies are focused on pregnant women and children older than 1 year (WHO 2011, De Regil 2011, Peña-
Iron is involved in intracellular redox reactions, where reactive-oxygen species (ROS) are formed. As iron is not easily eliminated from the body, it may adversely affect proteins, lipids and DNA. Such damage may be permanent and increase the risk of developing neoplastic processes, diabetes and cardiovascular and neurological diseases (Evans 2004, Liu 2009, Toxqui 2010). Furthermore, reports in the literature suggest that the iron dose used for the treatment of anemia in children may affect genomic stability (Aksu 2010). However, such effect has not been demonstrated in infants. The advantages of weekly iron supplementation regarding adherence and the probably lower damage on the genome could support the need of reconsidering the current IDA preventive guidelines on time and manner of administration and further design health promotion and disease prevention policies.

**Theoretical framework and empirical evidence**

ID is the most prevalent nutritional deficiency and the main cause of anemia worldwide. Infants less than 2 years and pregnant women are among the most vulnerable groups. In developing countries, ID coexists with other conditions such as protein-energy malnutrition, vitamin A and folic acid deficiency, and infectious diseases (Olivares, 1999). In children, the most common cause of ID is an increased iron requirement, which is related to higher growth development rates (Clark, 2008). In 2009, the prevalence of anemia reported by the WHO was 47.4% in pre-school children (McLean, 2009). More recently, the global estimation of such prevalence was 43% (Stevens 2013). In Argentina, the ENNyS reported that anemia affected 34.1% of infants between 6 and 23 months of age; this figure was higher in households with unsatisfied basic needs (Durán, 2009).

Iron reserves in babies with normal gestational age and birth weight from well-nourished mothers and delayed umbilical cord clamping (> 2 min) should be enough to meet iron requirements until 6 months of age (Dewey, 2007). However, different studies report a high prevalence of anemia at early ages. In Latin America, around 60% of infants less than 6 months have anemia: 71.4% in Bolivia, 63.5% in Honduras and 59.4% in Peru (Lutter, 2008). Likewise, studies performed in other countries have also reported a high prevalence of anemia in EBF infants (Yang, 2006; Torres, 2006; Marques, 2014). A study performed in Argentina showed that the prevalence of anemia in EBF infants was comparable to that found in formula-fed infants, affecting 28.9% of infants less than 6 months assisted at the public healthcare sector (Ianicelli, 2012). Iron-deficiency anemia (IDA) is a pathologic condition characterized by the shortage of iron for the synthesis of iron-containing proteins and enzymes such as hemoglobin, myoglobin, cytochromes, catalases and various peroxidases (Archer, 2015; Dallman, 1986). Besides the classical symptoms, other non-hematological manifestations have been described, namely, decreased capacity for physical work and spontaneous motor activity, impaired cell immunity and bactericidal capacity of neutrophils, decreased thermogenesis, functional and histological impairment of the gastrointestinal tract, defective mobilization of liver vitamin A, decreased growth development rate, behavioral, cognitive and motor impairment, lower sensory, auditory and visual conduction velocity, and reduced muscle tone.

During brain development, ID causes the reduction of brain iron content, which is irreversible when treatment is not timely (Algarin, 2003; Beard, 2003 y 2008; Carter, 2010; Lozoff, 2011). At early life stages, IDA impacts strongly on public health. Therefore, research on alternative preventive solutions has been encouraged. The WHO endorses the general agreement that infant and pregnant women iron supplementation is a priority strategy when IDA prevalence in a particular group of individuals is higher than 40% (WHO, 2001). In Argentina, the Argentine Pediatrics Society recommends daily ferrous sulfate supplementation in infants less than 4 months of age receiving MF and from 6 months onwards in infants with EBF (SAP 2009). Despite ferrous sulfate is prescribed and freely supplied through the health program Remediarte, adherence is low due to gastrointestinal adverse effects, mothers’ neglect and low rate of prescription by healthcare team members (Christensen 2013, Bernztein 2008). On the other hand, it has been pointed out that daily oral iron supplementation reduces iron absorption a few days after treatment initiation, whereas intermittent doses maximize absorption because iron
provision is concomitant with intestinal cell turnover (Anderson 2005; Frazer 2003a; Frazer 2003b). It is postulated that daily iron doses saturate the transport mechanisms, and that this correlates with intolerance symptoms, whilst weekly doses optimize absorption through transport mechanisms, decreasing intolerance symptoms (Viteri 1997, Srigiridhar 2001). On this basis, weekly iron supplementation is proposed as an effective IDA preventive alternative for older children and pregnant women (WHO 2011, De Regil 2011, Peña- Rosas 2012). Although different studies report the use of weekly iron supplementation, age-groups are dissimilar and include anemic patients; therefore, treatment instead of prophylactic doses are used (Coutinho 2008, Lima 2006). Meanwhile, evidence of weekly preventive supplementation in infants less than 6 months is scarce (Yurdakov 2004).

Finally, micronutrient deficiencies and excess may cause genomic damage, which could be in the same range of magnitude, or even higher, than genomic damage caused by exposure to high doses of environmental genotoxic agents such as carcinogenic agents, ultraviolet radiation and ionizing radiation (Fenech, 2010). Furthermore, since iron is not easily eliminated from the body and participates in the cell redox reactions, where ROS generate through Fenton reactions, increased iron concentrations could damage proteins, lipids and DNA permanently, causing structural and functional impairments. High plasma iron concentrations may amplify the effect of free radicals (Aksu, 2010); although cells have developed different mechanisms to reduce iron toxicity, including the regulation of iron transport through cell membranes and the production of ROS-eliminating enzymes. An imbalance of these systems caused by excess iron could increase oxidative damage susceptibility that could cause an increase in the mutation rate. These could increase the risk of developing neoplastic processes, diabetes and cardiovascular and neurological disease (Evans, 2004; Liu, 2009; Toxqui, 2010).

The effect of treatment with iron on DNA oxidation has been investigated in children with IDA, observing a significant increase of DNA strand breaks and baseline oxidative DNA damage as compared with children not receiving treatment (Aksu, 2010). However, we have not found evidence in the literature about the effect of preventive doses of iron supplementation on DNA. Therefore, we propose to compare the effectiveness of weekly and daily supplementation for the prevention of anemia in infants less than six months of life and to analyze their impact on genomic stability.

**Objectives**

**General:** To compare the effectiveness of weekly and daily iron supplementation for the prevention of anemia in infants.

**Specific:** To compare:
- To compare the effectiveness of weekly, daily and no iron supplementation at six months of age in EBF infants.
- To compare the effectiveness of weekly and daily iron supplementation in six-month-old infants receiving MF.
- To compare the incidence of adverse effects for weekly and daily iron supplementation at six months of age.
- To compare the adherence to treatment of weekly and daily iron supplementation at six months of age.
- To compare the concentration of oxidative stress biomarkers for both types of iron supplementation.
- To compare the genomic damage in both types of iron supplementation.

**Study hypothesis**

Weekly ferrous sulfate supplementation will be more effective in the prevention of IDA in infants than daily supplementation due to a higher adherence and a lower incidence of adverse effects. Weekly iron supplementation will cause less genomic instability as compared with daily iron supplementation.

**Study design**
Randomized controlled clinical trial.

Study sample
Eligibility
Infants performing monthly health controls at the Pediatric Health Observatory of IDIP, La Plata Children’s Hospital, from birth to six months of age will be included in the study.

Inclusion, exclusion and elimination criteria
Inclusion criteria: Three-month-old clinically healthy breastfeeding infants with birth age > 37 weeks, birth weight between 2500 and 4000 g, a normal fetal and neonatal history, that attend IDIP’s Health Observatory and whose parents/guardians express the will to participate in the study and sign a written informed consent form.

Exclusion criteria: Anemic and/or iron deficient infants or with chronic diseases or acute infections or under antibiotic/vitamin treatment within 15 days prior to the study.

Elimination criteria: infants with severe intolerance to iron preventive supplementation, who will receive the alternative preventive treatment with ferrous fumarate or iron polymaltose complex.

Unit of analysis: Three to six month-old infants.

Sample size and selection
According to ENNyS, the prevalence of anemia in 6-9 months old infants is 50.8%. Our controlled interventions would reduce such prevalence to 30%. Sample size was estimated with 0.95 significance level and 0.80 power, resulting in 174 infants (n= 87 for each supplementation group). This number was adjusted considering a 15% dropout, thus enrolling 204 infants.

Methods
Operational definition of variables
Main variables

- **Anemia** is defined as the “decrease in the total amount of blood cells or a blood hemoglobin (Hb) concentration more than 2 standard deviations below the mean of an age- and sex-matched reference range”. We will define the presence of anemia when Hb < 9.5 g/dL at 3 months of age and Hb <11.0 g/dL at six months of age (CDC, 2007).

- **Iron deficiency** is defined as insufficient iron in the body. It will be considered when blood ferritin <12 ng/ml (WHO 2001). Since ferritin is an acute phase reactant, we will measure C-reactive protein (CRP) to assess an eventual inflammatory state. When CRP is ≥5 mg/L, ID will be defined by a blood ferritin <30 ng/ml. The soluble transferrin receptor (sTfR) which is not influenced by inflammation will also be used (cut-off value, sTfR 8.3 mg/L) (Grant 2012).

Secondary variables

- **Adverse effects**: presence (or absence) and frequency of one or more of the following effects during ferrous sulfate supplementation (rejection, constipation, vomiting, diarrhea, abdominal pain).

- **Adherence to treatment**: is the rate of adherence to medication intake, dietary plan or lifestyle changes according to recommendations agreed on with healthcare professionals (OMS, 2003).

Adherence will be measured with the formula:

\[
\frac{(\text{ml SO}_4\text{Fe received} - \text{ml SO}_4\text{Fe remaining})/\text{days between the medication vial delivery and its return} \times 100}{\text{where ml SO}_4\text{Fe received is the volume of ferrous sulfate delivered in a vial, sufficient to cover the prescription until the infants' next control (1 month), and ml SO}_4\text{Fe remaining is the residual volume in the vial at the moment of the health control.}}
\]

We will consider low adherence the consumption of less than 50% of the amount of medication
prescribed for the study period, and less than 50% of intakes prescribed for the study period. We will consider high adherence consumption of more than 80% of the amount prescribed and more than 80% of the intakes prescribed.

All combinations not covered by low and high adherence will be considered as moderate adherence.

- **Genomic instability**: Chromosome, DNA and cell damage caused by certain agents. We will evaluate oxidative stress and genomic damage at cytomolecular level and at DNA base level.
- **Oxidative stress**: imbalance between reactive oxygen species (ROS) production and accumulation and the counteracting enzymatic/non-enzymatic antioxidant mechanisms. We will measure catalase and superoxide-dismutase enzymatic activity and evaluate cell damage by lipid peroxidation through thiobarbituric acid-reactive substances (TBARS) assessment.
- **Genomic damage**: DNA damage assessed by cytomolecular techniques using the Comet assay (damage index assessment in 200 cells, quantifying damage by Tail moment and Olive Tail moment). The frequency of oxidized DNA bases will be determined by competitive 8-oxo-dG Elisa.

Other variables:
- Sex, weeks of gestation, umbilical cord clamping.
- Infant nutritional condition will be assessed by anthropometry (weight, height, head circumference) at three and six months.
- Sociodemographic data of infants and relatives, including place of residence, unsatisfied basic needs, maternal education.
- Maternal nutritional condition will be evaluated with anthropometry and iron status.
- Environmental exposure to genotoxic agents (antibiotics, radiation (X-rays) and chemical substances like pesticides, solvents and industrial waste) will be assessed by questionnaire.

**Data collection**

- **Patient enrolment**
  During health control visits to IDIP Health Observatory on the first and second month of life, parents and tutors will be invited to participate in the study; the study's protocol, its aims and benefits will be explained in a plain language. They will be asked to sign a written informed consent form in case they accept their children be incorporated to the study. Infants meeting the inclusion criteria with exclusively breastfeeding (EBF) will be randomly divided into three groups: weekly, daily and no supplementation. Those with mixed feeding (breastfeeding and formula; MF) will be randomly divided into two groups: weekly and daily supplementation.

- **Intervention**
  Weekly supplementation (EBF or MF): infants will receive a weekly dose of ferrous sulfate (4mg/kg/week).
  Daily supplementation (EBF or MF): infants will receive a daily dose of ferrous sulfate (1 mg/kg/day).
  Without intervention (only EBF): infants will receive no supplementation, following the current SAP guidelines. Ferrous sulfate will be supplied by pediatricians.

- **Randomization**
  Randomization will be performed by computer-generated random sequences of numbers in blocks of variable length. Randomization will be stratified by type of intake (EBF or MF).
  Treatment assignments will be placed in sequentially numbered, opaque and sealed envelopes which will be consecutively put in a box and later removed one at a time. The person in charge of the random allocation process will not take part in the enrollment process.

- **Blinding**
  Pediatricians in charge of supplementation will know the assigned intervention only when the envelopes are open. Professionals in charge of performing laboratory tests will not know intervention assignments.

- **Sample collection, Surveys**
  Before supplementation, 3-ml blood samples from infants will be drawn to assess anemia, ID, and genomic stability. All non-anemic/iron deficient infants will be recruited and will be supplemented
according to the group assigned during 12 weeks (three months). At the end of this period (six months of age), blood samples will be drawn again to measure the study variables. Infant and family sociodemographic data will be surveyed at the first visit (three months of age). Anthropometric measurements will be taken at three and six months. Nutritional assessment of the mothers will be performed at the beginning and at the end of the study, measuring weight and height and drawing blood samples for anemia and ID assessment.

- Intervention progress monitoring

Pediatricians will prescribe iron doses and manner of administration. Parents and/or tutors will receive one or more droppers with ferrous sulfate sufficient to complete one-month supplementation, considering that all infants will perform monthly health controls. The Telegram program “LactaPlusIDIPBOT”, designed at IDIP, will be used to send reminders for iron supplement intake. They will be also contacted weekly (Facebook messenger, Whatsapp, Telegram). To monitor adherence, we will use a daily/weekly consumption record form that will be completed by parents/tutors and delivered at each health control. To determine consumption, we will control the excess volume in droppers during each monthly control.

- Record of adverse effects

Parents and/or tutors will receive a form to register adverse effects which will be presented at each monthly control. They will register intake rejection, constipation, vomiting, diarrhea, and abdominal pain. Infants with intolerance to iron supplementation will be excluded from the study but will continue on an alternative treatment (1 mg/kg/day fumarate or iron polymaltose complex).

- Laboratory tests

Blood samples (3 ml) from infants will be drawn at IDIP’s laboratory by venipuncture and divided into two vials: 1) an EDTA tube containing 1 ml blood for complete blood count, and 2) lyophilized lithium heparin tube that will be centrifuged to obtain plasma for the assessment of ferritin, CPR, sTfR, 8-oxodG and TBARS, and erythrocytes for the assessment of CAT and SOD determinations and for the Comet assay. The following methods will be used:

- Hemoglobin: Modified Drabkin method (ABX Pentra DX 120 Horiba).
- Ferritin and sTfR: enzymatic immunoassay using a chemiluminescence substrate (Access-Beckman Coulter, Fullerton, US).
- C-reactive protein: turbidimetry (Autoanalyser BT 3000 Plus Wiener).

All these tests will be performed at the Children’s Hospital Central Laboratory.

Cytomolecular genomic stability: alkaline version of the Comet assay (electrophoresis of a cell sample previously embedded in agarose, being migration proportional to the damage present (Sinh et al., 1988, Padula 2016).

ROS-mediated DNA damage: ELISA to quantify 8-oxodG, the most frequently oxidized nucleotide (Rossner et al 2006; Rossner & Sram 2012).

These tests will be performed at IGEVET.

CAT activity: spectrophotometry using Aebi’s method.

SOD activity: commercial kit (Ransod; RandoxLabs).

TBARS: spectrophotometry according to Okhawa.

These tests will be performed at IDIP and IGEVET.

Iron nutritional status (hemoglobin and ferritin) of mothers: blood samples taken at three and six months after delivery.

Data analysis

The effectiveness of the interventions will be evaluated at six months of age by comparing the incidence of anemia in both study groups as a relative risk (RR) and its corresponding 95% confidence interval (95% CI). For the EBF group, no supplementation will be the reference category, whilst for the MF group, the reference category will be daily iron supplementation.

Intention-to-treat analysis will be used to account for the possible changes in type of feeding and/or intervention arms. However, per-protocol analysis will be also performed and compared with intention-to-treat analysis in order to determine the result robustness.
Losses to follow-up will be censured since analyses will be performed in closed cohorts because we will not be able to estimate incidence density (we will only collect data on anemia and ID at three and six months).

We will also determine the incidence of adverse effects and the incidence of low treatment adherence at six months of age for weekly vs. daily iron supplementation in both EBF and MF and calculate RR (95% CI) at six months of age.

Means and differences of enzymatic SOD and CAT activity and TBARS concentration at three and six months will be compared in both study groups. The same will apply to the Comet and 8 oxodG assays.

Differences between groups will be determined by Anova or Kruskal-Wallis test for quantitative variables with post-hoc comparisons and Chi-square for categorical variables. Within group comparisons will be analyzed using Student’s or Wilcoxon test.

**Risk of bias assessment**

Selection bias could be present since parents/tutors willing to participate could show higher interest in the research project than those who do not participate. Considering that the population sample attends for monthly controls, a trust relationship may be established between parent/tutor and pediatrician, resulting in a higher degree of adherence to supplementation than in the general population.

<table>
<thead>
<tr>
<th>Project Schedule</th>
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<tr>
<td><strong>Activity</strong></td>
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<tr>
<td>Enrolment according to inclusion/exclusion criteria</td>
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<tr>
<td>Intervention assignment</td>
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<tr>
<td>Sampling for hemoglobin, ferritin, CPR and sTfR assessment.</td>
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<tr>
<td>Oxidative stress and genomic damage assessment</td>
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<tr>
<td>Anthropometric nutritional assessment</td>
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<tr>
<td>Monitoring (registry of drug administration, adverse effects)</td>
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<tr>
<td>Data registry (data base entry)</td>
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<tr>
<td>Data analysis and interpretation of results</td>
</tr>
<tr>
<td>Conclusions. Final report</td>
</tr>
</tbody>
</table>
Patient enrolment at IDIP’s Health Observatory
One or Two-month-old healthy infants

Three-month-old (±10 days) healthy infants
Anemia, ID, genomic instability

EBF infants
Randomization

MF infants
Randomization

Anemic and/or ID infants are excluded and referred for treatment

Without supplementation
Daily supplementation
Weekly supplementation
Weekly supplementation

INTERVENTION
Daily: 1 mg/kg/day ferrous sulfate
Weekly: 4 mg/kg/week ferrous sulfate

Six-month-old (±10 days) healthy infants
Anemia, ID, genomic instability

Anemic and/or ID infants are referred for treatment
**Work plan**

**SPECIFIC OBJECTIVE 1:** Compare the effectiveness of weekly, daily and no iron supplementation in EBF infants.

**SPECIFIC OBJECTIVE 2:** Compare the effectiveness of weekly, daily and no iron supplementation in MF infants.

<table>
<thead>
<tr>
<th>ACTIVITY</th>
<th>RESPONSIBLE</th>
<th>EXPECTED RESULT</th>
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<tbody>
<tr>
<td>Enrollment of infants according to the inclusion criteria. Informed consent. Intervention assignment.</td>
<td>Pediatricians, Statistician</td>
<td>Incorporate infants who comply with inclusion criteria Random allocation</td>
</tr>
<tr>
<td>Blood sample collection.</td>
<td>Laboratory technician</td>
<td>Adequate specimen collection (enough volume, non clotted)</td>
</tr>
<tr>
<td>Hemoglobin, ferritin, PCR and sTfR determinations.</td>
<td>Biochemists</td>
<td>Assessment of the prevalence of anemia and iron deficiency</td>
</tr>
<tr>
<td>Indication for and monitoring of the intervention Text message reminders</td>
<td>Pediatricians</td>
<td>Complete registry of the intervention. Intervention adherence.</td>
</tr>
<tr>
<td>Weight and height measurement. Registry of data.</td>
<td>Pediatrician, Nutritionist, Data entry</td>
<td>Determine the anthropometric nutritional condition. Complete database.</td>
</tr>
<tr>
<td>Data analysis and interpretation of results.</td>
<td>Team, Statistician</td>
<td>Report of results and conclusions.</td>
</tr>
</tbody>
</table>

**SPECIFIC OBJECTIVE 3:** Compare the incidence of adverse effects on weekly vs. daily iron supplementation for the prevention of anemia

| Monitoring of the record of adverse effects. Participant follow-up. Control of data record forms Data analysis and interpretation of results. | Pediatricians | Adequate record of the presence and frequency of adverse effects in both types of supplementation. Early detection of adverse effects that may prevent intervention progression. Early detection of adverse effects. Report of results and conclusions. |

**SPECIFIC OBJECTIVE 4:** Compare the degree of treatment adherence to weekly and daily iron supplementation at six months of age.

| Monthly control of droppers to calculate adherence Monitoring of drug intake (registry form and phone calls) Data analysis and interpretation of results. | Biochemist, Pediatricians, Team, statistician | Correct administration of iron supplementation Adequate drug intake according to prescription. Report of results and conclusions. |

**SPECIFIC OBJECTIVE 5:** Compare oxidative stress indicators resulting from both forms of iron supplementation.

| Blood sample collection | Laboratory technician | Adequate specimen collection |
TBARS, CAT and SOD assessment
Data analysis and interpretation of results.

Biochemist, IGEVET researchers
Team, statistician

Evaluation of oxidative stress
Report of results and conclusions.

**SPECIFIC OBJECTIVE 6: Compare genomic damage with both forms of iron supplementation**

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<thead>
<tr>
<th>Task</th>
<th>Responsible</th>
<th>Description</th>
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<tbody>
<tr>
<td>Blood sample collection</td>
<td>Laboratory technician</td>
<td>Adequate specimen collection (enough volume, non clotted)</td>
</tr>
<tr>
<td>Comet assay</td>
<td>IGEVET researchers</td>
<td>Cytomolecular damage evaluation</td>
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<tr>
<td>8 oxo-dG assessment</td>
<td>IGEVET researchers</td>
<td>Quantification of oxidized bases</td>
</tr>
<tr>
<td>Data analysis and interpretation of results.</td>
<td>Team, statistician</td>
<td>Report of results and conclusions.</td>
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References
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