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The importance of iron and folic acid supplementation in pregnancy and strategies to improve outcomes

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ABSTRACT

Background: Iron and folic acid are essential micronutrients for reproductive health. While supplementation is common during pregnancy, excessive intake may disrupt hormonal balance and impair fertility. *Aim:* To evaluate the effects of iron and folic acid supplementation and overdose on fertility and reproductive hormone levels in female mice. *Methodology*: An experimental study was conducted on 36 female mice divided into six groups receiving different doses of folic acid, iron, or combinations of both for seven days. Reproductive hormone levels were measured, and pregnancy and fertility rates were assessed. Data were analyzed using ANOVA and chi-square tests. *Results*: Iron-treated mice showed a significant reduction in luteinizing hormone and follicle-stimulating hormone levels (p<0.001) and no pregnancies occurred in these groups. Low-dose folic acid improved pregnancy and fertility rates compared to high-dose folic acid (p=0.034). *Conclusion*: High-dose iron causes infertility in female mice by disrupting hormonal balance, while normal doses of folic acid enhance fertility. These findings highlight the need for cautious micronutrient supplementation and medical supervision during pregnancy.

KEYWORDS

folic acid, iron toxicity, reproductive hormones, fertility, pregnancy outcomes

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1. INTRODUCTION

Micronutrients like iron (Fe) and folic acid (FA) are vital for pregnancy and fetal development. Many women of reproductive age fail to meet the recommended intake levels and are at high risk of anemia, including gestational anemia, often caused by Fe and/or FA deficiencies. Globally, anemia affects 38.2% of pregnant women, with the highest rates in South-East Asia (48.7%), Africa (46.3%), and the Eastern Mediterranean Region (38.9%) [1]. Despite guidance from gynecologists and nutritionists, the effectiveness of FA and Fe supplementation is influenced by factors such as food

matrix, bioavailability, gut microbiota, and genetic variations. According to the 2020 Global Nutrition Report, none of the 194 countries are on track to achieve the 2025 goal of halving anemia rates among women of reproductive age. Infertility affects roughly 15% of couples trying to conceive, with female factors contributing to 35% of cases, male factors to 30%, and about 15% remaining unexplained. Research indicates that a healthy diet and adequate micronutrient intake play a key role in improving fertility rates [3]. FA is crucial for DNA and protein synthesis, cell division, and metabolic processes important for reproduction [4]. Fe is essential for mitochondrial respiration, cell growth, DNA synthesis, and oxygen transport as part of hemoglobin; however, excess iron can produce reactive oxygen species that disrupt hormonal balance and damage reproductive tissues [5].

The aim of this study was to investigate the effects of iron and folic acid supplementation and the impact of overdose on fertility and reproductive hormone levels in female mice.

2. METHODOLOGY

2.1. Study setting

The study was conducted at the Faculty of Veterinary Medicine, University of Tripoli. Environmental conditions were maintained at a temperature of 20 ± 5°C with a 12-h light-dark cycle. Mice had free access to food and water.

2.2. Animal model

Albino mice were used due to their common use in biomedical research. A total of 20 adult mice (15 females, 5 males) aged 12–14 weeks were initially used for mating purposes.

2.3. Pilot study

A pilot study was performed to identify toxic doses of folic acid (FA) and iron (Fe). Sixteen female mice were randomly assigned to two groups (n = 8 each) receiving different doses of FA or Fe for seven days. Feed intake and body weight were monitored. In the Fe group (30 mg/kg IP), 75% mortality occurred within 3–5 days, identifying this dose as lethal.

2.4. Study design

A completely randomized design (CRD) was applied. Thirty-six female mice (12–14 weeks old) were separated from males for 10 days to prevent early pregnancy. Mating was confirmed by weight monitoring. Gestation lasted 19–21 days.

2.5. Group allocation and treatment

Mice were randomly assigned to six groups of six mice each. Five groups received treatments while one served as the control. Two groups were administered folic acid (Prefolic® 50 mg/3 ml, ZAMBON Italia) orally at doses of 8 mg/kg/day and 4 mg/kg/day for seven consecutive days. One group received iron (Endofer® 100 mg/ml, FATRO S.P.A.) alone via intraperitoneal injection at a dose of 15 mg/kg every other day for one week. Two additional groups received combined treatments: one group received iron 15 mg/kg plus folic acid 4 mg/kg/day, and the other received iron 15 mg/kg plus folic acid 8 mg/kg/day. The control group received no treatment.

2.6. Blood collection and hormonal analysis

Blood was collected using a 3 mL syringe. Serum fertility hormone levels were measured using a biochemical analyzer (Mindray®).

2.7. Ethical approval

The study protocol received approval from the University of Tripoli Faculty of Veterinary Medicine Animal Ethics Committee.

2.8. Statistical analysis

Data were analyzed using SPSS version 30. One-way ANOVA and chi-square tests were used. *p*-values<0.05 were considered statistically significant.

3. RESULTS

The treatments significantly affected reproductive hormone levels and fertility outcomes. One-way ANOVA revealed a marked decrease in luteinizing hormone (LH) and follicle-stimulating hormone (FSH) among mice treated with Fe alone or Fe combined with FA compared to controls ($p < 0.001$).

The pregnancy rate was calculated as the ratio of birthed female mice per mated female and analyzed using a chi-square test. Pregnancy rates differed significantly between groups (chi-square, $p< 0.001$). Mice receiving a moderate dose of FA had higher pregnancy rates than those receiving high doses. No pregnancies occurred in mice receiving iron treatments (alone or with FA).

Fertility rates, expressed as number of offspring per mouse, were significantly higher in the low-dose folic acid group than in the high-dose group (ANOVA, p=0.034) as shown in Table 1.

Table 1. Comparison of fertility rates, pregnancy rates and female hormone levels across treatment groups.

Parameters	Control	FA 4mg/kg	FA 8mg/kg	Fe	FA 8mg/kg + Fe	FA 4mg/kg + Fe	*p*-value
Pregnancy Rate**	83.30%	100%	66.7%	0.0%	0.0%	0.0%	< 0.001
Fertility rate (offspring/ mouse)*	5 (2.68)	7.33 (1.03)	3.5 (2.74)	-	-	-	0.034
Hormones*							
FSH	6.900	11.817	10.350	0.375	0.1833	0.3617	< 0.001
LH	7.533	10.450	9.7333	0.270	0.3050	0.3200	< 0.001

*One-way ANOVA test, **Chi Square test

4. DISCUSSION

The significant reduction in LH and FSH in Fe-treated groups indicates that excess Fe disrupts hormonal balance. This supports previous findings on Fe toxicity in the anterior pituitary and its negative impact on ovarian function [6,7].

The complete absence of pregnancies in Fe-treated mice confirms the infertility effect of Fe overload. In contrast, the increased pregnancy and fertility rates with low-dose FA supplementation suggest a dose-dependent benefit of FA on fertility.

These findings align with earlier research showing FA's role in biosynthesis processes essential for reproductive function [8]. Other studies also support FA's positive effects on fertility, including improved embryo quality and pregnancy rates in in vitro fertility [9,10].

5. CONCLUSION

This study demonstrated that high doses of Fe significantly reduced reproductive hormone levels and caused infertility in female mice, while normal doses of folic acid improved pregnancy and fertility outcomes. The combination of iron and folic acid did not reverse the negative effects of iron overdose.

These findings highlight the risks of excessive iron supplementation without medical supervision and emphasize the importance of proper dosing of micronutrient supplements. Screening and individualized supplementation strategies should be implemented to avoid fertility impairment and ensure safe maternal health during pregnancy.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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