Maternal Micronutrient Supplementation Modulates Foetal Brain Development and Neuronal Architecture in Male Wistar Rats

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Abstract

Maternal micronutrient status plays a critical role in foetal brain development. This study examines the effects of maternal supplementation with iron, copper, and selenium on foetal brain development in male Wistar rats. Eighteen female and six male rats were assigned to six groups of 3 dams per group: control, chaff-fed, copper-supplemented (8 mg/kg), iron-supplemented (40 mg/kg), selenium-supplemented (0.2 mg/kg), and a combined supplementation group. Experimental diets were administered from gestational day 5 to 19. On gestational day 20, foetuses were harvested for morphometric measurements, brain weight assessment, trace element analysis using atomic absorption spectrometry, and histological examination. Foetuses from the chaff-fed group exhibited smaller crown-rump lengths, lower foetal weights, shorter tail lengths, and reduced brain weights compared with the other groups at $p < 0.001$. The iron-supplemented group showed increases in both foetal and brain weights, whereas the selenium group experienced an increase in crown-rump length and brain selenium concentration at $p < 0.001$. Copper supplementation led to elevated brain copper levels compared with both the control and chaff-fed groups at $p < 0.05$. The combined supplementation group showed better morphometric outcomes than the malnourished group. Histological examinations revealed decreased cortical density and disrupted neuronal structure within the chaff-fed group, whereas the supplemented groups exhibited enhanced cortical organisation and less vacuolation. These results imply that maternal micronutrient supplementation affects foetal development, brain elements, and cortical structure in Wistar rats.

Keywords: Maternal nutrition, Micronutrients, Brain development, Foetal neurobiology, Selenium, Iron, Copper.

Introduction

Adequate maternal nutrition plays a pivotal role in determining foetal growth, organogenesis, and postnatal health outcomes. Micronutrients, vitamins, and trace minerals such as iron (Fe), copper (Cu), and selenium (Se), though required in minute quantities, are indispensable for enzymatic processes, redox balance, and cellular development (Allen, 2005; Tulchinsky, 2017). During gestation, these micronutrients profoundly influence foetal neurodevelopment, particularly the proliferation, differentiation, and maturation of neural cells. Despite their importance, global malnutrition remains a significant public health concern, with undernutrition contributing to impaired brain development in millions of children each year (Pritchard *et al.*, 2016; Mattei and Pietrobelli, 2019).

The foetal brain is susceptible to both nutrient availability and environmental insults during critical developmental windows. Nutritional inadequacies can disrupt synaptogenesis, myelination, neurotransmitter synthesis, and glial differentiation, leading to long-term deficits in cognition, memory, and behaviour (Cortés-Albornoz *et al.*, 2021; Georgieff, 2023). Among the trace elements, iron is indispensable for brain energy metabolism, dendritic arborisation, and neurotransmitter function (Cheatham, 2019). Iron deficiency during gestation has been associated with altered hippocampal structure, delayed neural processing, and behavioural impairments in offspring (McCann and Ames, 2007; Georgieff, 2023).

Selenium, an integral component of antioxidant selenoproteins such as glutathione peroxidase, protects neural tissues against oxidative stress and regulates neuronal signalling (Arnal *et al.*, 2011; Ajmone-Cat *et al.*, 2022). Deficiency in maternal selenium levels is associated with disrupted cortical organisation, glial cell dysfunction, and neurodevelopmental impairments in offspring (Fruh *et al.*, 2021; Ojeda *et al.*, 2021). Furthermore, selenium's neuroprotective potential has been shown to improve cognitive performance and mitigate neurodegenerative risk in both humans and animal models (Guo *et al.*, 2024).

Copper is another essential micronutrient critical for mitochondrial respiration, myelin formation, and neurotransmitter metabolism (Cusick and Georgieff, 2016). Maternal copper deficiency impairs hippocampal development, alters oxidative balance, and may increase susceptibility to behavioural and neuropsychiatric disorders in offspring (Arnal *et al.*, 2010; Cortés-Albornoz *et al.*, 2021). Collectively, evidence suggests that maternal deficiencies in iron, copper, or selenium alone or in combination could lead to altered foetal brain cytoarchitecture, reduced neuronal viability, and long-term neurofunctional deficits. Given the high prevalence of micronutrient deficiencies in low- and middle-income regions and their potential transgenerational consequences, understanding the role of these nutrients in foetal neurodevelopment is essential (McGuire, 2015; Brion *et al.*, 2020).

This study, therefore, investigates the effects of maternal supplementation with iron, copper, and selenium, individually and in combination, on foetal development in male Wistar rats. Using histological and biochemical analyses, the study evaluates the structural and compositional integrity of the foetal brains to elucidate how micronutrients modulate neurodevelopmental outcomes. This research hypothesises that a deficiency in maternal micronutrients negatively impacts the development and structure of the foetal brain. In contrast, fortifying the diet with either iron, copper, or selenium enhances both the structural and biochemical makeup of the foetal brain. Furthermore, the combination of these micronutrients yields better neurodevelopmental results than administering any single micronutrient alone.

The findings from this study aim to advance the understanding of micronutrient-dependent neurodevelopmental pathways and to contribute to evidence-based maternal nutrition strategies to prevent cognitive and structural impairments arising from prenatal malnutrition.

Materials and Methods

Laboratory Animals and Housing

A total of 24 Wistar albino rats (18 females and six males), weighing between 150 g and 200 g, were obtained from Jumorak Farm (JF), Iwo, Osun State, Nigeria. All animals were housed in the University of Lagos College of Medicine Animal House under standard laboratory conditions of a 12-hour light/dark cycle, 25°C ± 2°C, and 50%–60% relative humidity, with ad libitum access to standard chow and water. Animals were allowed to acclimatise for 2 weeks before experimentation. All procedures were conducted in accordance with the Institutional Animal Care and Use Committee (IACUC) guidelines and adhered to ARRIVE 2.0 reporting standards to ensure ethical and humane treatment of animals.

Ethical Approval

Ethical approval for this study was obtained from the College of Medicine, University of Lagos Health Research Ethics Committee (CMUL/ACUREC/03/26/2201).

Experimental Design and Breeding

Female rats were weighed, and their oestrous cycles were monitored by vaginal cytology to identify animals in proestrus (Ajayi and Akhigbe, 2020). For mating, three females were placed with one male in a breeding cage, maintaining a 3:1 female-to-male ratio (Zhang *et al.*, 2010). Mating was monitored daily between 6:00 AM and 7:00 AM, and the presence of a vaginal plug was used as confirmation of successful copulation. The day a plug was observed was designated as gestational day 0, and males were subsequently removed from the breeding cage to prevent repeated mating.

Grouping and Experimental Allocation

Pregnant females were randomly assigned to six experimental groups (n = 3 per group) using simple randomisation to minimise selection bias. Each group was housed separately to prevent stress and aggression and to maintain consistent environmental exposure. Grouping and treatment allocation were designed to evaluate the effects of maternal micronutrient supplementation on foetal brain development, in accordance with the study objectives.

Dietary preparation and feeding

Regular growers' mash was procured from Kay Farms Feeds, Akute, Ogun State, Nigeria. The feed was analysed at the Olukoya Central Research Laboratory, University of Lagos, to quantify the baseline content of iron (Fe), copper (Cu), and selenium (Se), providing a reference for designing the experimental diets. Experimental diets were formulated to evaluate the effects of individual and combined micronutrient supplementation on foetal brain development. Feeding commenced on gestational day 5 (GD5) and continued until gestational day 19 (GD19) (Corniola *et al.*, 2008). Pregnant rats were randomly allocated into six experimental groups (n = 3 per group) as follows:

a. Control Group: Standard growers' mash.
b. Chaff Diet Group: Rice bran and wheat offal mixture.
c. Copper-Supplemented Diet Group: Feed enriched with 8 mg Cu/kg.
d. Iron-Supplemented Diet Group: Feed enriched with 40 mg Fe/kg.
e. Selenium-Supplemented Diet Group: Feed enriched with 0.2 mg Se/kg.
f. Mixed Diet Group: Feed supplemented with 40 mg Fe/kg, 8 mg Cu/kg, and 0.2 mg Se/kg.

All diets were prepared fresh and stored under controlled conditions to maintain nutrient stability. Daily monitoring ensured that rats consumed the diets without restriction, and any spillage or feed refusal was recorded to maintain accurate intake data. The composition and administration of each diet were designed to mimic maternal nutritional supplementation while allowing for direct comparisons with standard and malnourished (chaff-only) diets.

Brain tissue collection

On gestational day 19 (GD19), pregnant rats were anaesthetised with sodium pentobarbital (100 mg/kg, intraperitoneally) to ensure deep surgical anaesthesia and minimise pain or distress. Following anaesthesia, rats were transcardially perfused with ice-cold normal saline to clear blood from the vasculature, then fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.4). The foetuses were delivered via Caesarean section, and foetuses from dams in each experimental group were screened for morphometric assessment and detailed physical examination (three foetuses were randomly selected from each dam (n = 9 per group).

The following foetal parameters were measured: Crown-rump length (CRL); Tail length; Distance from the implantation site to the dam's uterus wall, and foetal body weight. After morphometric measurements, foetal brains were carefully excised. Wet brain weights were recorded using an MS Weighing Scale (Knoxe Electronics, Majiwada, Mumbai, India). Brains were then fixed in 4% paraformaldehyde for 24 hours at 4°C in preparation for histological analysis.

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Metal Analysis of Foetal Brain Tissue

Foetal brain samples were analysed to determine iron (Fe), copper (Cu), and selenium (Se) concentrations. Twenty-four foetal brains were selected for digestion, with four brains from four different dams within each experimental group, ensuring representative sampling. The digestion was performed using the ashing method. Briefly, each brain was placed in a ceramic crucible and ashed in a Goodman gas furnace (Goodman Global, INC., Houston, Texas, USA, 2015) at high temperature for 3-5 hours until complete combustion of organic material was achieved. After cooling, the resulting ash was dissolved in 10 ml of 1 M nitric acid (HNO_3) (Akinyele and Shokunbi, 2015). To standardise the volume for analysis, 40 ml of deionised water was added, yielding a final volume of 50 ml per sample. The digested samples were analysed using Atomic Absorption Spectrometry (Thermo AAS 301, K Chandra Equipment and Services, Nangloi, Delhi, India) according to the manufacturer's protocols. Calibration curves for Fe, Cu, and Se were prepared using certified standard solutions, and all measurements were performed in triplicate to ensure reproducibility and accuracy. Metal concentrations were expressed in µg/g of wet tissue.

Histology

For histological analysis, five foetal brains from each experimental group were selected. Brains were initially fixed by immersion in 4% paraformaldehyde for 24 hours at room temperature, followed by three successive washes in 70% ethanol to remove excess fixative. The tissues were subsequently dehydrated through an ascending series of ethanol concentrations, cleared in xylene (xylol), and embedded in molten paraffin. Paraffin blocks were sectioned at 5 µm thickness using a rotary microtome (Leica RM 2125, Germany). Sections were mounted on albumin-coated glass slides and stored until staining. Histological staining was performed using Ehrlich's hematoxylin, followed by counterstaining with aqueous eosin to highlight cellular structures. From each brain, five representative slides were prepared to ensure coverage of multiple brain regions and reproducibility. Microscopic examination was conducted using an Olympus BX41 light microscope, and photomicrographs were captured to document cellular morphology and cytoarchitecture. All analyses were performed blinded to group allocation to minimise observational bias.

Statistical Analysis

GraphPad Prism 6 (version 5.01) was used for the statistical analyses. All data sets were expressed as the mean ± Standard error of the mean. SEM and analysis were conducted using a one-way ANOVA to compare groups, followed by a Tukey's post hoc test, or a two-way ANOVA, with statistical significance set at $p<0.05$ and 95% confidence interval.

Results and Discussion

Compared with all other groups, foetuses from the chaff diet group exhibited significantly reduced crown-rump length at $p<0.001$. Additionally, the mixed diet showed a significant increase compared with all other diet groups, except the chaff group, which showed a significant reduction ($p < 0.001$). Furthermore, the control group was significantly lower than all other diets except the iron diet, which differed remarkably from the control ($p<0.001$). Compared with all other groups, foetuses from chaff-diet groups demonstrated significantly lower birth weight ($p<0.001$). There was a statistically significant increase in the mixed diet compared with all other diet groups, except the chaff groups, which showed a significant reduction ($p<0.001$). Also, there was a statistically significant increase when comparing the control diet with other diets, except for the chaff groups (which decreased significantly) and the selenium group, which showed a difference that was not statistically significant.

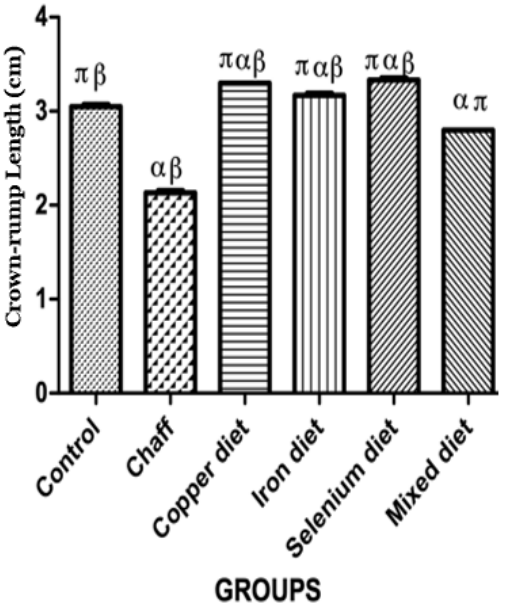

Fig 3.01: Crown-rump length (cm) of the foetuses in each group. Values are expressed in mean ± SEM (n=9), α = *p<0.001* when compared with control π = *p<0.001* when compared with chaff, β = *p<0.001* when compared with mixed diet.

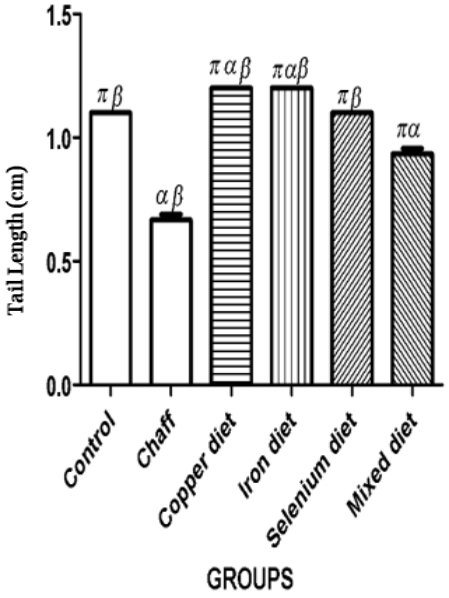

Fig 3.02: Tail length (cm) of the foetuses of the groups. Values are expressed in mean ± SEM (n=9), π=*p<0.0001* when compared with chaff, α=*p<0.001* when compared with control, β=*p<0.001* when compared with mixed diet.

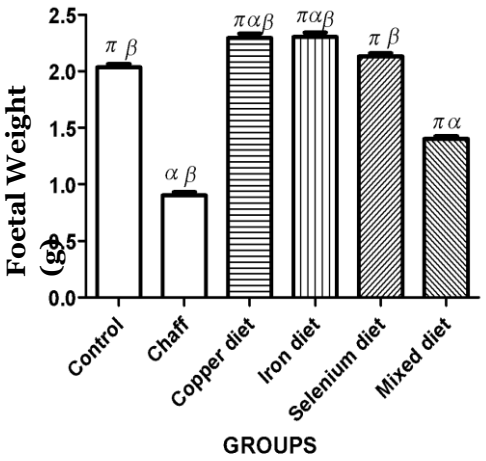

Fig 3.03: Foetal weight (g) of the groups. Values are expressed in mean ± SEM (n= 9), π = *p<0.001* when compared with chaff, α = *p<0.001* when compared with control, β = *p<0.001* when compared with mixed diet.

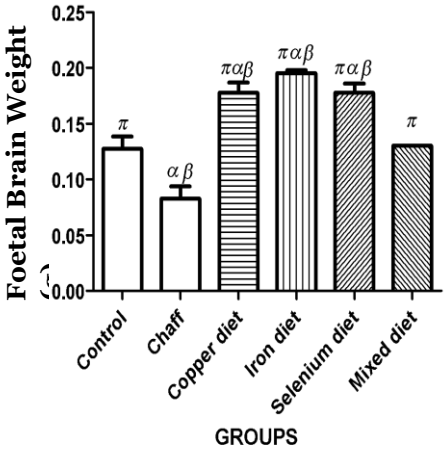

Fig 3.04: Foetal brain weight (g) of the groups. Values are expressed in mean ± SEM (n= 9), π = *p<0.001* when compared with chaff, α = *p<0.001* when compared with control, β = *p<0.001* when compared with mixed diet.

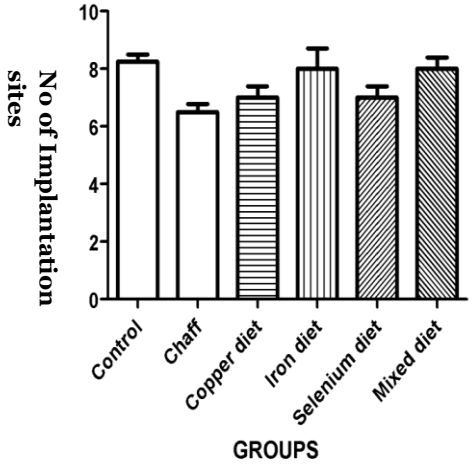

Fig 3.05: Number of implantation sites in various groups. Values are expressed in mean ± SEM (n= 3).

Also, the tail length of the foetuses from the chaff-diet groups was significantly decreased ($p<0.001$) compared with all other diet groups. There was a significant difference when comparing the tail length of foetuses in the mixed diet with all other diet groups ($p<0.001$). The tail length of the control group differed significantly from that of all other groups, except the selenium diet ($p<0.001$). Furthermore, the chaff group exhibited the lowest foetal brain weight ($p<0.001$) among all diet groups, including the control. The foetuses' brain weights in all other diets were significantly ($p<0.001$) different from the control group, except for the selenium-rich diet ($p<0.05$). The iron diet group showed the highest brain weight, although there was no statistically significant difference from the copper- and selenium-rich diet groups. There were differences in implantation sites (dam) between groups, but they were not statistically significant ($p<0.05$). Moreover, the number of viable Foetuses per litter did not differ significantly among groups.

Metal Analysis Results

The concentration of copper in the copper diet group was significantly increased when compared to other diets, except for the iron diet ($p<0.0001$). While there was a slight increase in copper concentrations in the copper and iron diets, it was not statistically significant. The concentration of copper in the control diet was significantly lower when compared with the copper and iron diet group ($p<0.05$). The chaff diet group demonstrated a significantly lower concentration of copper when compared with the iron and copper diet group ($p<0.001$).

Iron concentration was statistically significantly higher in the iron-rich diet group compared to all other groups ($p<0.0001$). In particular, the iron concentrations in the control group differ from those in the copper- and iron-rich diet groups ($p<0.05$). However, they are similar to those in the

Table 3.1.1 shows the effects of diet on the levels of copper, iron, and selenium in Foetal brains.

GROUPS (DIET)	CONCENTRATION OF COPPER (PPM)	CONCENTRATION OF IRON (PPM)	CONCENTRATION OF SELENIUM (PPM)
CONTROL	0.0851 ± 0.0219 ϒ	0.0772 ± 0.0043 ï	0.1181 ± 0.0058 ⁿ
CHAFF	0.0627 ± 0.0027 ϒ	0.01275 ± 0.0009 ï	0.0000 ± 0.0000 ᵅᵝ¥
COPPER	0.1368 ± 0.0021 ⁿᵅᵝ	1.725 ± 0.0371 ⁿᵅᵝ	0.07745 ± 0.0042 ⁿᵝ¥
IRON	0.1323 ± 0.0029 ⁿᵅᵝ	3.164 ± 0.4334 ⁿᵅᵝ	0.0870 ± 0.0245 ⁿᵝ
SELENIUM	0.0629 ± 0.0076 ϒ	0.2092 ± 0.0029 ï	0.1543 ± 0.0035 ⁿ
MIX	0.0448 ± 0.0073 ϒ	0.0459 ± 0.0062 ï	0.1714 ± 0.0295 ⁿ

Values are expressed in mean ± SEM (n=4), n=p<0.001 when compared with chaff, α=p<0.05 when compared with control, β=p<0.001 when compared with mixed diet, Y=p<0.0001 when compared with copper diet, ï = p<0.0001 when compared with iron diet, ¥ = p<0.05 when compared with selenium diet.

mixed-diet and chaff-and-selenium diet groups (p<0.05). The chaff diet group exhibited a low iron concentration and differed significantly from the copper- and iron-rich diet groups (p<0.001). In addition, there was a significant difference comparing the iron concentrations in the mixed diet group with copper- and iron-rich diet groups (p<0.001).

Except for the chaff diet group, all other groups presented similar selenium concentrations (p<0.05). However, the selenium concentration in a mixed diet differed significantly from that in the iron, chaff, and copper diet groups (p<0.001). Overall, the selenium-rich diet group had the highest selenium concentrations compared to the chaff and copper diet (p<0.001).

Histology Results

The foetal cerebral cortex in the control group showed a well-structured, cell-dense cortical plate (CP), containing young, ovoid, dividing migratory neurons, with their projections traversing the cortical plate radially and tangentially. The neuropil appeared homogeneous, with no vacuolation (Fig. 3.2a & b). The cortical plates of foetuses fed with chaff have a less dense cortical plate, with intermediate zones showing a segregated stream of neuronal cells with ablated projections, giving a distorted networking among cells, which could pose a disruption in developing neuronal cell communication, in their course compared with the control group (Fig. 3.2c & d). On the other hand, the developing foetal cerebral cortex from the selenium group (3.2e & f) showed a densely packed cortical plate with cells with homogeneous neuropil, vertically migrating with radial glial processes.

In contrast, the iron group showed rapidly dividing cells with darkly stained neuropil, and the copper groups (3.2i & j) showed intensely stained cells with vacuolated neuropil. Radial glial scaffolds appeared with no clear demarcation between the IZ and the SVZ, suggesting that interconnections at this level might be greater than in the control group. Compared with the control group, the mixed group (treated with a combination of Se, Fe, and Cu) demonstrated a relatively preserved cortical architecture with mild disorganisation. There was minimal vacuolation, and the cortical plate retained a high density of granular cells.

Only occasional pyknotic neurons were observed, and neuronal projections appeared more intact relative to the single-element treatment groups, suggesting a possible compensatory protective effect among the administered elements (Fig. 3.2k & l).

This study demonstrates the critical influence of maternal dietary composition, particularly copper, iron, and selenium intake, on foetal growth, morphometric indices, brain development, and cortical neuroarchitecture. Our findings highlight the adverse effects of nutrient deficiencies and reveal the benefits of a balanced micronutrient intake during gestation for optimal foetal neurodevelopment.

The chaff diet group, which received a nutrient-deficient diet, exhibited significant reductions in crown-rump length, foetal weight, tail length, and brain weight. These findings strongly indicate that inadequate maternal nutrition induces intrauterine growth restriction (IUGR), consistent with previous reports showing that maternal undernutrition impairs foetal linear growth and body mass accumulation (Hubbard *et al.*, 2013; Georgieff, 2023). Beyond foetal size, poor maternal nutrition compromises placental efficiency, disrupting the transplacental transport of growth factors, hormones, and essential trace elements necessary for neurodevelopment (Schweizer and Fradejas-Villar, 2016).

In contrast, the mixed diet group, which received iron, copper, and selenium concurrently, demonstrated optimal foetal growth and brain development, aligning with the principle of nutrient coaction, where balanced micronutrient intake enhances absorption and utilisation, whereas single-nutrient supplementation may result in antagonistic interactions (Cottin *et al.*, 2018). This is supported by the metal analysis (Table 3.1.1), which showed that the mixed group had the highest selenium concentration (0.1714 ± 0.0295 ppm), along with moderate iron (0.0459 ± 0.0062 ppm) and copper (0.0448 ± 0.0073 ppm). The iron-supplemented group exhibited increased foetal and brain weights (Fig. 3.03 and Fig. 3.04) and the highest brain iron concentration (3.164 ± 0.4334 ppm), highlighting iron's pivotal role in neurodevelopment.

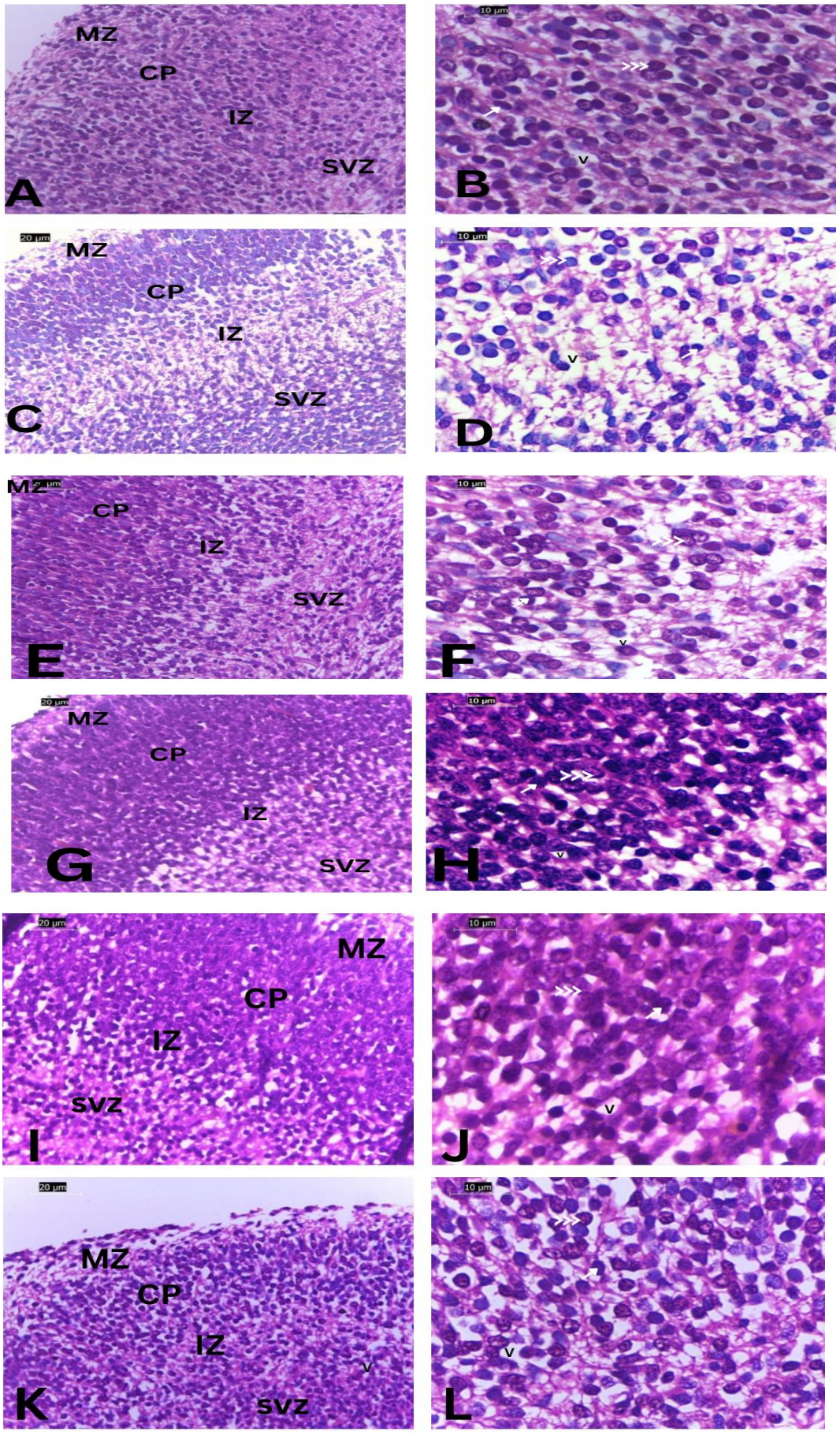

Fig. 3.2: Photomicrographs of H & E-stained coronal sections of foetal rat brain at e19. **(A-B)** control, **(C-D)** chaff, **(E-F)** selenium only, **(G-H)** iron only, **(I-J)** copper only and (**K-I)** mixed only showing cortical plate (CP), granular cells (Arrow), migrating and dividing cells (White Arrow), vacuoles (V), scale bar: (a, c, e, g, I, k)-0.02mm and (b, d, f, h, j, l) -0.01mm

Iron is essential for neurotransmitter synthesis, myelination, neuronal energy metabolism, and cell proliferation (Lozoff *et al.*, 2008; Rao *et al.*, 2011). Our data corroborate previous studies showing that maternal iron deficiency reduces foetal brain iron concentrations and disrupts neurogenesis, with long-lasting effects that cannot be fully reversed postnatally (Greminger *et al.*, 2014; Georgieff, 2020; Zych-Krekora *et al.*, 2025).

Similarly, selenium supplementation improved foetal growth parameters (Fig. 3.01) and resulted in elevated brain selenium levels (0.1543 ± 0.0035 ppm), consistent with its role in antioxidant defence and protection against oxidative stress during critical periods of cortical development (Ojeda *et al.*, 2021; Behl *et al.*, 2023). The copper-supplemented group demonstrated higher brain copper levels, increased foetal weight, and longer crown-rump lengths compared to the chaff diet group.

Copper supports neuronal energy metabolism through enzymes such as cytochrome c oxidase, dopamine β-hydroxylase, and Cu/Zn-superoxide dismutase, and is essential for angiogenesis and astrocyte maturation (Cusick and Georgieff, 2016; Locatelli and Farina, 2024). The observed cortical improvements in this group suggest that adequate copper intake enhances glial scaffolding and supports axonal development.

Histological analysis revealed significant cortical alterations associated with maternal diet. Foetuses from the chaff diet group displayed sparse cortical plates, disrupted layering, and vacuolation in the intermediate and marginal zones, indicative of impaired neuronal migration and synaptogenesis. These findings align with prior reports showing that nutrient deprivation during gestation disrupts radial glial scaffolding, cortical lamination, and dendritic development (Cortés-Albornoz *et al.*, 2021; Stepien *et al.*, 2021). In contrast, the mixed diet group exhibited near-normal cortical organisation, with dense pyramidal and granular cell layers and minimal vacuolation, suggesting that balanced micronutrient intake preserves radial migration, neuronal differentiation, and cortical integrity.

Conclusion

This study provides evidence that maternal dietary micronutrients, iron, copper, and selenium, enhance foetal growth and cortical cytoarchitecture, while deficiencies compromise neurodevelopment. Integration of morphometric, metal analysis, and histological data demonstrates that combined nutrient supplementation is essential for neurodevelopment, and dietary interventions aimed at improving foetal brain health are warranted.

Limitations of Study

This research offers important insights into how maternal micronutrient supplementation affects foetal brain development. However, the mechanisms underlying neuronal development, neuroglial, and neuronal migration were not assessed in this study.

Recommendations

We recommend ensuring adequate maternal nutrition, particularly micronutrient sufficiency, before and during pregnancy to optimise foetal development. Future research should focus on the impact of maternal micronutrient supplementation on mechanisms of neuronal development, migration, and maturation. Postnatal assessment should be performed to investigate the long-term effects of maternal diet on offspring health.

Declaration of interest

The authors declare no conflict of interest.

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