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# High-Resolution Micro-CT Mapping of Craniofacial Suture Gap Architecture in Vitamin D Deficiency: A Quantitative Anatomical Landmark Approach

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## Abstract

Craniofacial sutures are specialized fibrous joints whose gap architecture reflects complex interactions among skeletal growth and bone remodeling, making accurate 3D characterization essential for developmental and clinical investigations. However, conventional macroscopic and 2D imaging techniques are limited by insufficient spatial resolution and poor reproducibility. This study aims to establish and validate a high-resolution micro-CT-based quantitative of craniofacial suture gap architecture, with specific emphasis on differences associated with vitamin D deficiency. Using experimental models, were analyzed in vitamin D-sufficient and deficient groups. Micro-CT imaging was performed using a Bruker SkyScan-1173 system, followed by 3D reconstruction and morphometric analysis with DataViewer and CTVox software. Standardized anatomical landmarks were applied to ensure consistent spatial registration and objective measurement of suture gap width. The results demonstrated in mean suture gap width in the vitamin D-deficient group (1.037 mm) compared with the sufficient group (1.329 mm). The strong concordance

between individuals of the micro-CT–based methodology. These findings validate micro-CT as a high-impact, non-destructive imaging modality capable of resolving subtle 3D variations in craniofacial suture morphology. The proposed offers a strong foundation for combining advanced morphometric analysis and digital diagnostic tools, thereby increasing the practical value of micro-CT in craniofacial research and clinical digital diagnostics.

## Keywords
Craniofacial Suture, Micro-CT, Suture Gap Architecture, Vitamin D Deficiency, Bone Morphometry.

## 1. Introduction
Vitamin D plays a critical role in skeletal development and bone homeostasis, influencing mineralization, osteoblast differentiation, and remodeling processes throughout life. Beyond its classical function in calcium–phosphate metabolism, adequate vitamin D status is essential for maintaining bone microarchitecture and structural integrity (Garrahan et al. 2022). Vitamin D deficiency is a major global health issue, with high prevalence reported across diverse populations, including craniofacial trauma patients, suggesting broader implications for bone health beyond long bones (Varman et al. 2022)

The craniofacial skeleton is unique in its developmental biology, with sutures serving as essential growth centers that accommodate rapid brain expansion and facial morphogenesis. These fibrous articulations undergo highly coordinated processes of intramembranous ossification and remodeling, which are sensitive to nutritional and hormonal regulation. Disruption of these processes may underlie congenital and acquired craniofacial anomalies, yet the microstructural changes within sutural gaps under metabolic derangements such as vitamin D deficiency remain poorly characterized. Although previous studies have demonstrated associations between vitamin D deficiency and altered mandibular bone structure using radiological and morphometric indices (Zihni Korkmaz et al. 2023), quantitative assessment of sutural gap architecture using high-resolution imaging has received limited attention. Modern non-destructive imaging modalities, such as micro-computed tomography (micro-CT), provide three-dimensional insights into bone density, trabecular morphology, and suture microarchitecture that cannot be captured by conventional two-dimensional radiography (Parmasari et al. 2025).

Given the increasing recognition that subclinical and clinical vitamin D deficiency are linked to compromised bone microarchitecture (e.g., reduced bone quality and strength) across skeletal sites and life stages, and the paucity of data on craniofacial sutures specifically, there is a pressing need to quantitatively map how vitamin D deficiency affects sutural gap architecture. A deeper understanding of this relationship is critical, not only for elucidating basic mechanisms of craniofacial bone growth and remodeling, but also for improving clinical outcomes in conditions where altered craniofacial development or repair is implicated (Parmasari et al. 2025).

### 1.1 Objectives
The objectives of this study are: (1) to establish a high-resolution micro-CT-based quantitative methodology for craniofacial suture gap measurement using standardized anatomical landmarks; (2) to compare suture gap architecture between vitamin D-sufficient and vitamin D-deficient rat fetus groups; and (3) to validate the micro-CT methodology through statistical analysis and comparison with existing published research on craniofacial bone morphometry and vitamin D deficiency effects.

## 2. Literature Review
Micro-computed tomography (micro-CT) has emerged as a transformative imaging modality in skeletal research due to its ability to provide high-resolution, three-dimensional visualization and quantification of mineralized tissues in a non-destructive manner. Traditionally applied to long bone analysis, micro-CT has expanded into studies of complex craniofacial structures, enabling detailed assessment of microarchitecture, bone quality, and developmental changes that are not possible with conventional two-dimensional imaging (Cox 2020).

Fundamentally, micro-CT leverages X-ray attenuation to reconstruct volumetric datasets that capture both cortical and trabecular bone microstructures at micrometer resolution. This allows for the quantification of key parameters such as bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), and bone mineral density (BMD), which serve as sensitive indicators of bone health, adaptation, and (El-Gizawy et al. 2023). ompared to histomorphometry and traditional radiography, micro-CT facilitates accurate visualization of internal structures

without physical sectioning, reducing potential measurement bias and preserving specimens for further analysis (Cox 2020).

In craniofacial research specifically, the application of micro-CT has enabled investigators to investigate skeletal development and morphological variation across species and life stages. For example Liao et al. (2023) used micro-CT to document age-related changes in bone mineral density within the craniofacial skeleton of zebrafish, revealing differential patterns of mineralization in specific bony elements that contribute to the understanding of craniofacial growth (Liao et al. 2023). Similarly, Tan et al. (2021) highlight specialized protocols for analyzing murine skull microarchitecture via micro-CT, acknowledging region-specific morphological variations that are critical to accurate quantitative assessment (Tan et al. 2021).

The complexity of craniofacial bone, which includes structures of varying embryonic origin, mineral content, and functional load, presents unique challenges for imaging and analysis. Micro-CT permits detailed assessment of these diverse tissues, enabling researchers to capture differences in bone geometry, density, and microstructural characteristics that would be obscured using lower resolution modalities (Cox 2020). In odontological and dentoalveolar research, emerging guidelines emphasize micro-CT's value in resolving complex mixed tissues such as enamel-dentin interfaces, cementum, and periodontal ligament interfaces, facilitating comprehensive evaluation of craniofacial or dental structures (Chavez et al. 2021).

Moreover, the quantitative micro-CT approach underpins comparative biomechanical and developmental studies, making it indispensable for research into bone disease, treatment responses, genetic models, and developmental biology. Micro-CT data can be integrated with computational models or advanced statistical analyses to predict biomechanical behavior, further enhancing its utility in translational research (El-Gizawy et al., 2023).

In other hand, Vitamin D plays a central role in bone mineralization, structural integrity, and skeletal homeostasis by regulating calcium and phosphate balance and influencing osteoblast and osteoclast function. When vitamin D levels are insufficient, the disruption in calcium absorption and metabolic regulation leads to impaired bone quality, increased bone turnover, and structural weakness (Weinert & Silveiro 2015). Recent clinical research demonstrates that vitamin D deficiency is prevalent among individuals with craniofacial pathology and may be associated with poorer bone quality in these regions. Varman et al. (2022) found a high prevalence of vitamin D deficiency among patients with craniofacial fractures, suggesting that inadequate vitamin D status may be linked to compromised bone strength or structural resilience in facial bones(Varman et al. 2022). Although direct causal mechanisms require further investigation. Although much of the research has focused broadly on skeletal health, an emerging body of evidence highlights similar effects in craniofacial bones, which have unique developmental dynamics and biomechanical demands.

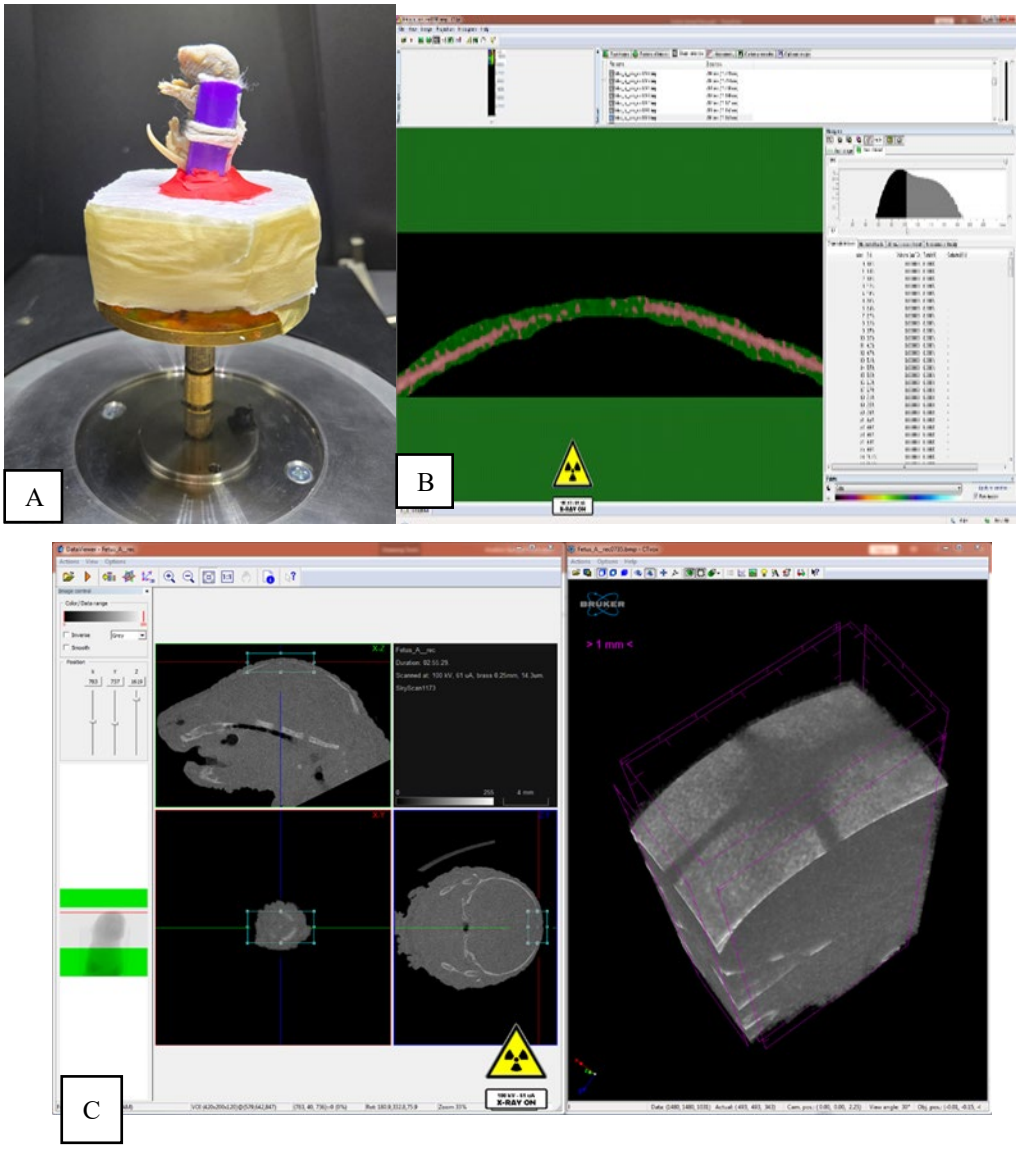
More direct evidence comes from morphometric imaging studies that examine mandibular bone integrity. In a retrospective radiological analysis using fractal and panoramic morphometry, individuals with vitamin D deficiency were found to have altered mandibular bone structure compared with vitamin D-sufficient controls, indicating that insufficiency can affect bone microarchitecture in craniofacial sites. Beyond regional imaging, studies of overall bone microarchitecture illustrate that low serum vitamin D is associated with poorer bone quality, as indicated by microarchitectural deterioration and reduced strength at weight-bearing skeletal sites. For example, lower 25-hydroxyvitamin D levels were associated with inferior bone microarchitecture and strength in a young adult cohort, suggesting that vitamin D deficiency broadly affects microstructural bone parameters that are likely relevant to craniofacial bone as well (Varman et al. 2022).

In addition, evidence from pediatric and developmental studies supports the importance of adequate vitamin D for proper bone formation. Wu et al., (2023), in a meta-analysis of clinical trials, reported that vitamin D supplementation improves bone mineral density in deficient children and adolescents, underscoring the role of vitamin D in building and maintaining bone quality during periods of growth. Taken together, these findings suggest that vitamin D deficiency detrimentally impacts bone quality at multiple anatomical sites, including the craniofacial skeleton. Although direct longitudinal studies on craniofacial sutures remain limited, the consistent association between low vitamin D and compromised bone structure an effect evident in mandibular morphology and general skeletal microarchitecture supports the biological plausibility that craniofacial bones are similarly affected by inadequate vitamin D status. Overall, micro-CT is a gold standard imaging tool for evaluating bone microarchitecture in both general skeletal and specialized craniofacial contexts, particularly where high resolution and volumetric quantification

are required to detect subtle changes in bone quality, morphology, and response to experimental conditions, especially for bone that deficiency vitamin D (Wu et al. 2023).

## 3. Methods

The micro-computed tomography (micro-CT) analysis was performed using the Bruker SkyScan-1173 High Energy Micro-CT system. Initially, the mouse fetus specimen was positioned on a preparation plate and securely immobilized to prevent movement artifacts during scanning. Stable fixation was ensured throughout the acquisition process to maintain image accuracy and structural integrity. Following image acquisition, quantitative and qualitative analyses were conducted using DataViewer for two-dimensional reconstruction and measurement of trabecular parameters, including bone volume fraction (BV/TV), trabecular thickness (Tb.Th), and grayscale value (GSV). Three-dimensional visualization and volumetric rendering were performed using CTVox. The reconstruction process applied a modified grayscale threshold range of 30–255 (within the full 0–255 color scale) to enhance hard tissue visualization. Lower-density soft tissues were excluded by adjusting the threshold to emphasize mineralized structures. Regions of interest (ROI) were systematically determined prior to defining volumes of interest (VOI), enabling precise measurement and simulation of suture gap morphology within the craniofacial region. Statistical analysis was performed using an independent samples t-test to compare mean suture gap widths between the control and treatment groups, with a significance threshold set at $p < 0.05$. Data normality was confirmed using the Shapiro–Wilk test prior to parametric testing. The complete workflow from specimen preparation through 3D suture gap simulation is illustrated in Figure 1.



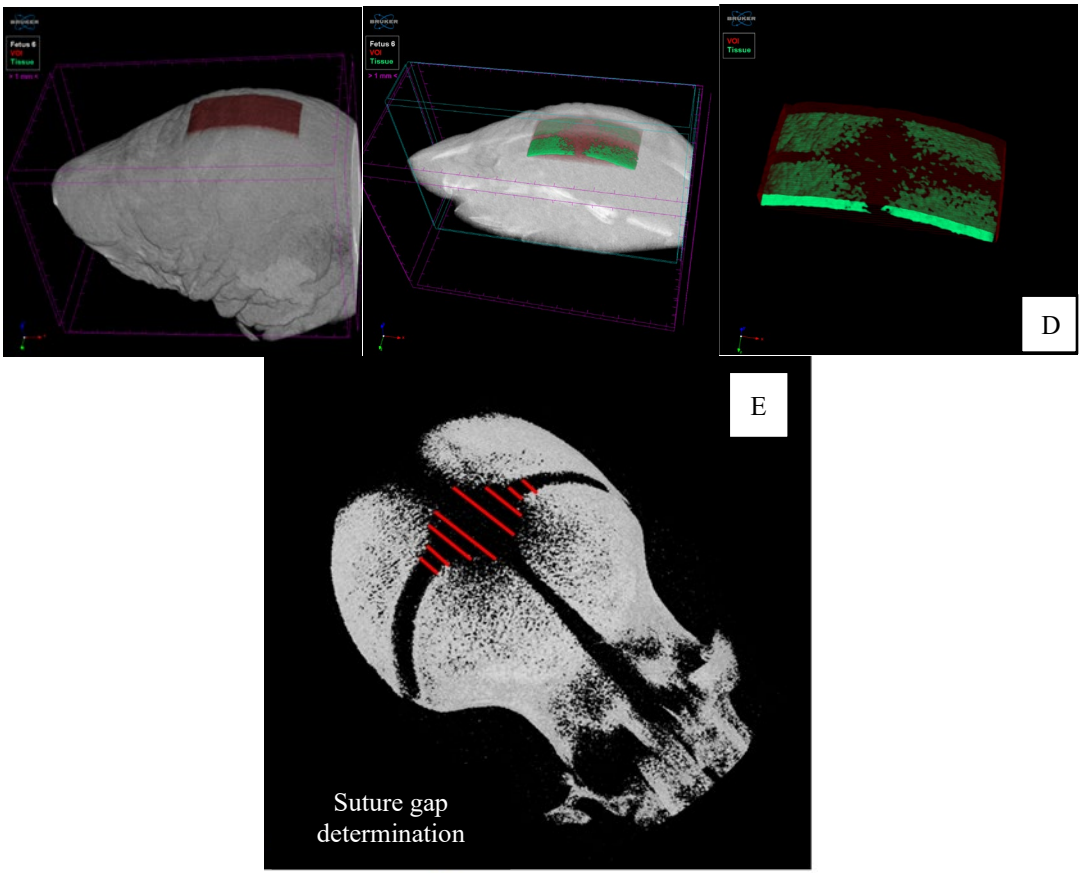



Figure 1. Micro CT scanning process: (A) Preparation of mouse fetus sample, (B) Reading and elimination of bone-tissue and non-bone tissue with software, (C) Measurement of suture gap using Micro CT software, (D) ROI determination stages focus on suture-gap, (E) Simulation of Sutura-gap measurement on sutures.

## 4. Data Collection

The micro-CT datasets obtained from the scanning procedure were systematically processed using dedicated Bruker software applications to ensure standardized reconstruction, quantitative evaluation, and three-dimensional visualization. Re-orientation and cropping of the raw projection data were performed using DataViewer (Bruker, Kontich, Belgium) to achieve consistent anatomical alignment and accurate selection of the region of interest before analysis. Quantitative morphometric assessment was subsequently conducted using CTAn (Bruker, Kontich, Belgium), enabling the calculation of structural parameters based on defined thresholding and segmentation protocols. For qualitative assessment and volumetric rendering, three-dimensional visualization of the reconstructed datasets was carried out using CTvox (Bruker, Kontich, Belgium), which facilitated comprehensive evaluation of craniofacial structures. Figure 2 presents representative 3D micro-CT reconstructions from the control group (K), showing well-mineralized cranial sutures with wider gap dimensions across all six specimens. Figure 3 shows the corresponding reconstructions from the treatment group (P), where narrower suture gaps and reduced bone mineralization density are visually apparent compared to the control group. This integrated analytical workflow ensured precise spatial orientation, reliable morphometric quantification, and high-resolution 3D representation of the micro-architectural features across both experimental groups.

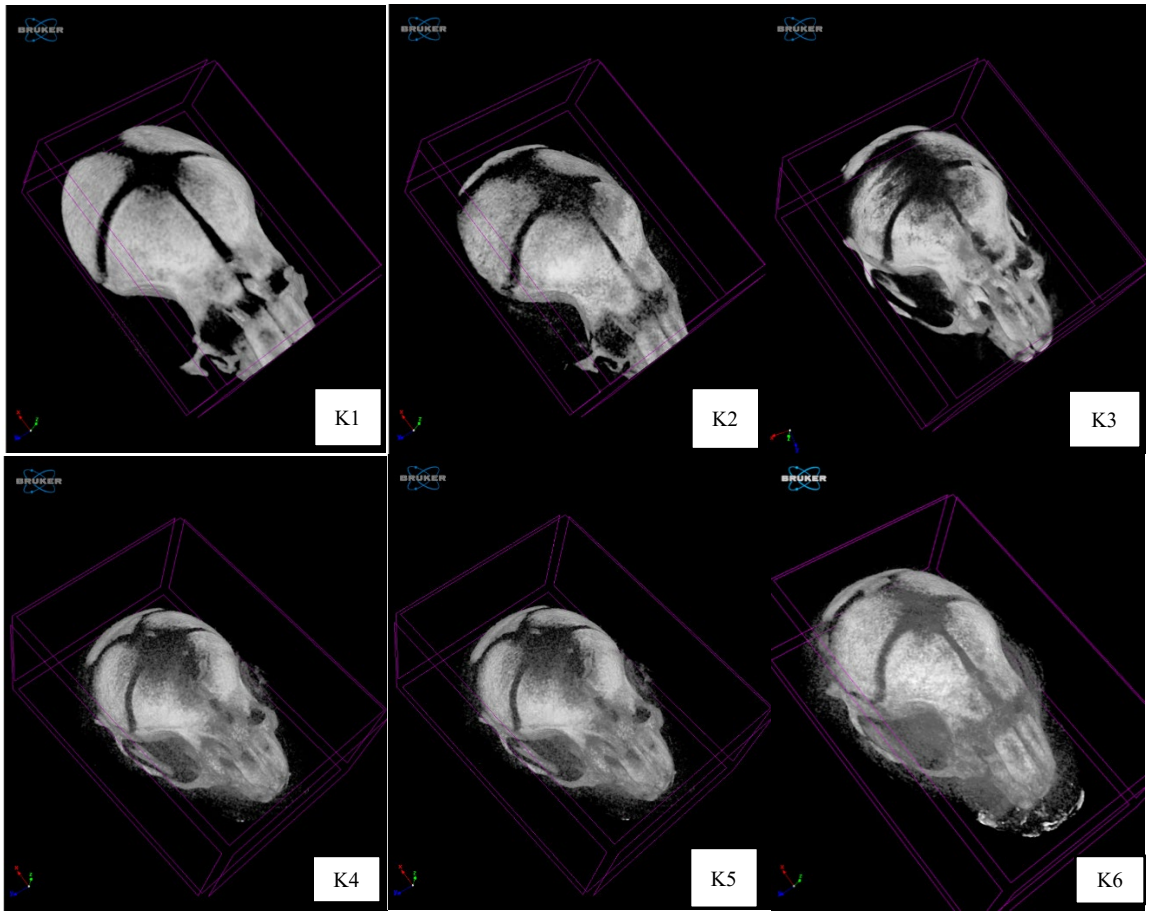
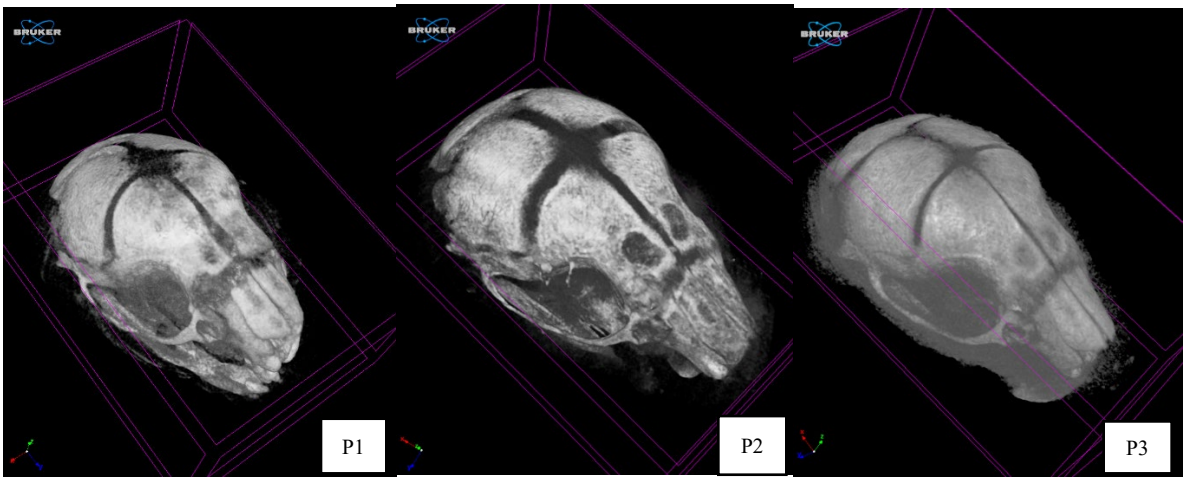



Figure 2. Results of micro-ct research of rat fetus of the Control Group (K)



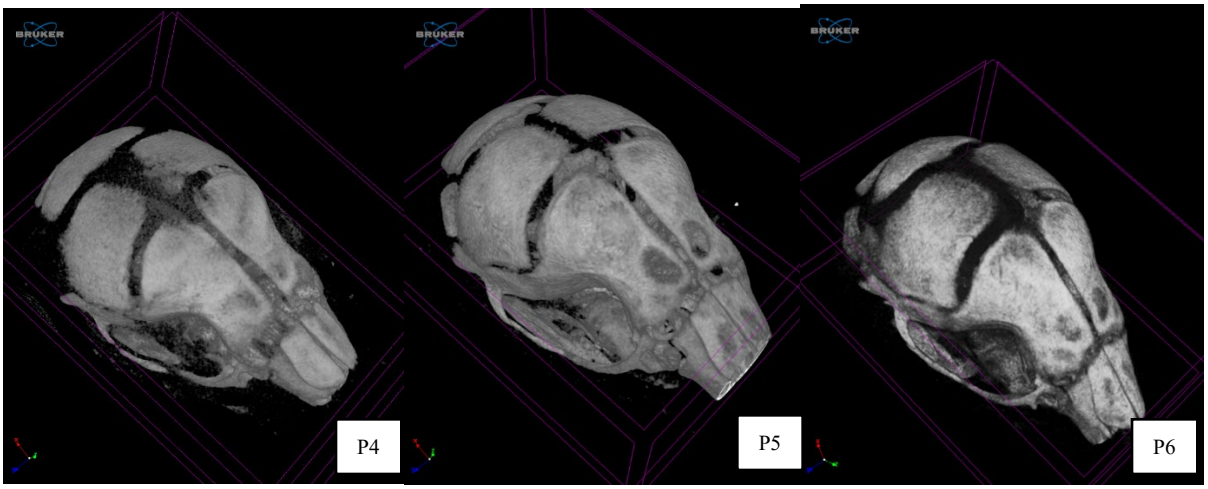



Figure 3. Results of micro-ct research of rat fetus of the Treatment Group (P).

## 5. Results and Discussion

### 5.1 Numerical Results

The Table 1 shows that the highest average suture gap value was in the control group, at 1.329 mm, and the lowest was in the treatment group, at 1.037 mm. The data indicate that cranial suture measurements were generally higher in the control group (K) than in the treatment group (P). The mean value in group K was 1.329 mm, compared to 1.037 mm in group P, reflecting an overall decrease in the treatment group. The values in group K ranged from 0.929 mm to 1.705 mm, while group P ranged from 0.798 mm to 1.310 mm. Although minor variations were observed in certain samples, the overall pattern demonstrates reduced suture dimensions in the treatment group relative to the control group.

Table 1. Suture Gap Value from the result of Micro-CT scanning for Control Group (K) and Treatment Group (P)

| Samples | Groups (mm) | |
|---|---|---|
| | K | P |
| 1 | 1,207 | 1,010 |
| 2 | 1,487 | 1,175 |
| 3 | 0,929 | 0,798 |
| 4 | 1,173 | 1,310 |
| 5 | 1,705 | 0,999 |
| 6 | 1,475 | 0,928 |
| average | 1,329 | 1,037 |
| Min | 0,929 | 0,798 |
| Max | 1,705 | 1,310 |

Source: Research results, 2025

### 5.2 Graphical Results

Figure 4 illustrates the boxplot analysis of suture gap measurements for both the control (K) and treatment (P) groups. The control group demonstrates generally higher median values, with samples such as K2, K4, and K6 displaying broader interquartile ranges, which reflect increased variability in suture width. A noticeable outlier is present in K1, indicating a value exceeding the upper quartile range. Conversely, the treatment group exhibits lower median

measurements and a more compact data distribution across most samples, suggesting narrower suture gaps and reduced dispersion. Although some variability and isolated outliers are still evident within the treatment group, the overall central tendency remains consistently lower than that observed in the control group. Collectively, the graphical representation reinforces the numerical results, indicating that the treatment condition is linked to a reduction in suture gap dimensions relative to the control group.

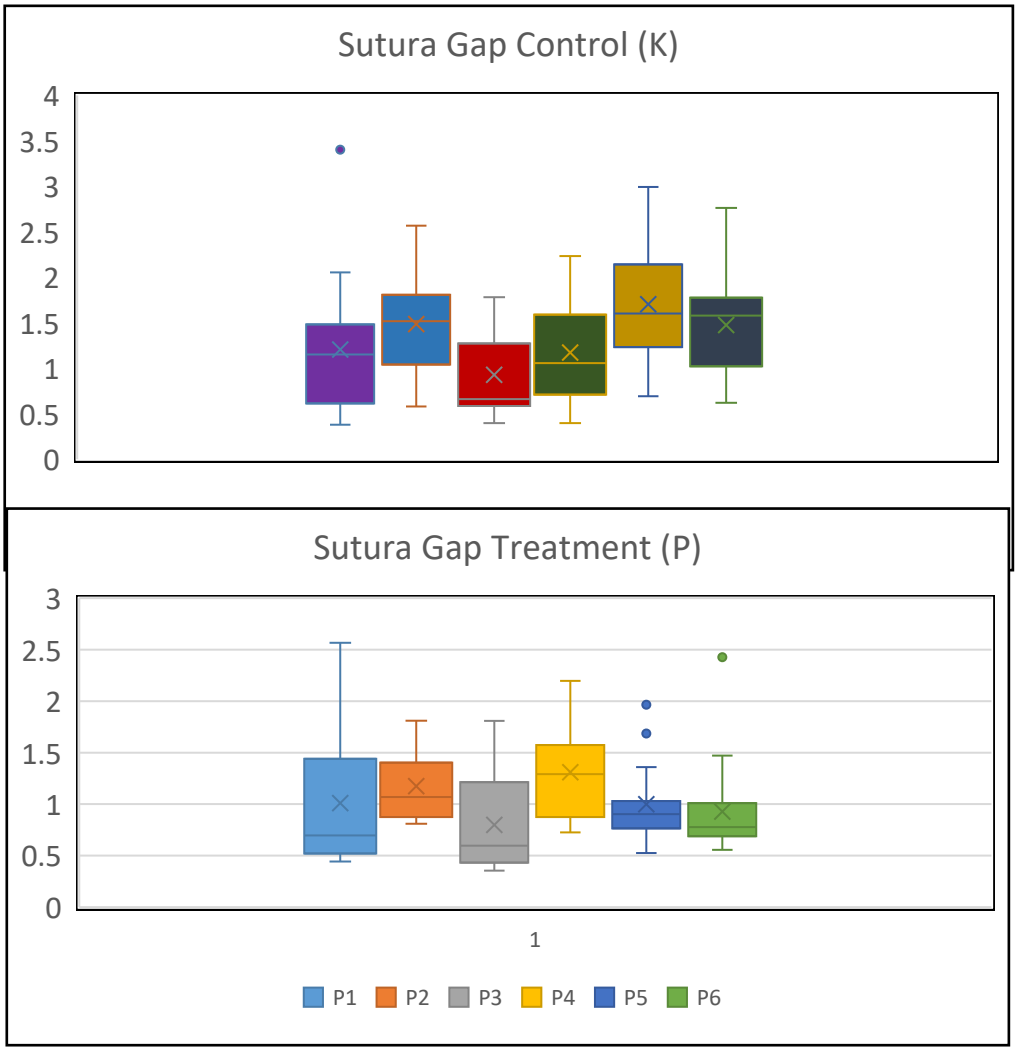



Figure 4. Box-Plot for Group Control (K) and Treatment Group (T)

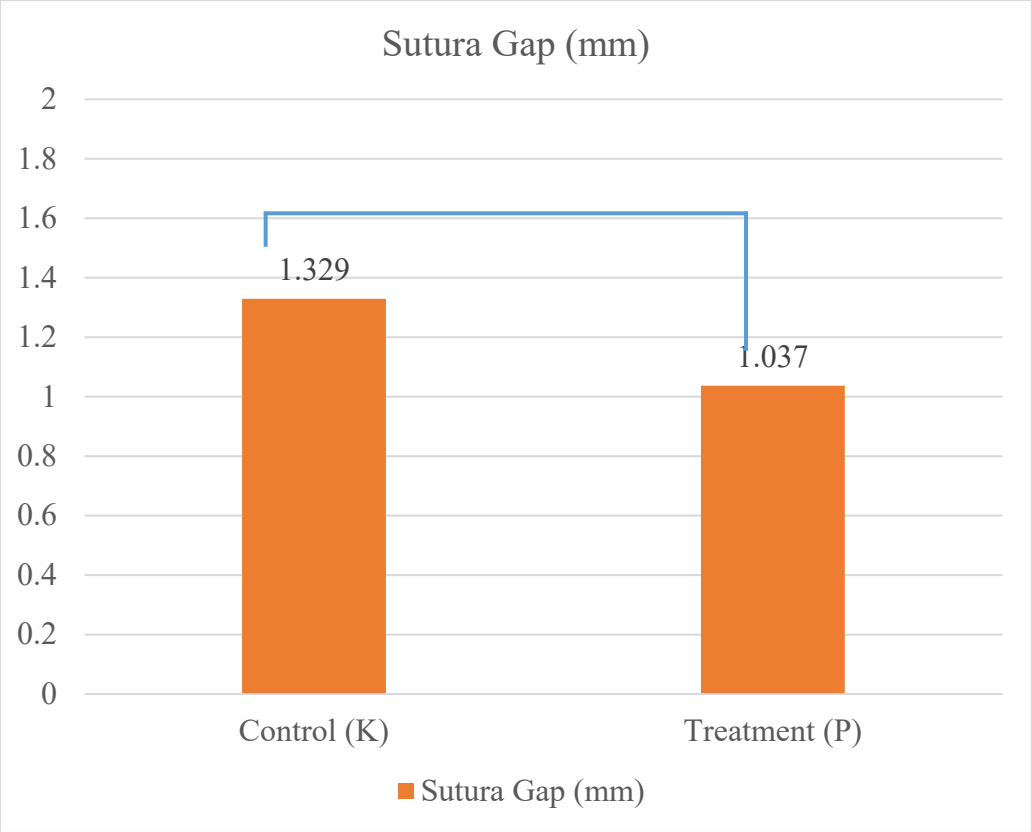



Figure 5. Bar chart of Micro-CT suture gap examination results of the rat fetus.

Figure 5 presents a bar chart comparing the mean suture gap measurements (mm) between the control (K) and treatment (P) groups based on Micro-CT evaluation of rat fetuses. The control group demonstrates a higher average suture gap value of 1.329 mm, whereas the treatment group shows a lower mean value of 1.037 mm. This difference indicates a reduction in suture gap dimension in the treatment group relative to the control group. The graphical comparison clearly highlights the disparity between groups, suggesting that the treatment condition is associated with decreased suture width. Overall, the bar chart visually reinforces the quantitative findings, demonstrating a measurable decline in suture gap measurements in the treatment group.

### 5.3 Proposed Improvements

Figures 4 and 5 collectively demonstrate a consistent difference in suture gap measurements between the control (K) and treatment (P) groups based on Micro-CT analysis. Figure 4, which presents the boxplot distribution, shows that the control group generally has higher median values and wider interquartile ranges, indicating greater variability in suture gap dimensions. Several control samples display broader data dispersion, and isolated outliers are also observed. In contrast, the treatment group exhibits lower median values and a more compact distribution pattern, suggesting narrower suture gaps with relatively reduced variability. Although minor dispersion and occasional outliers are present in the treatment group, the overall central tendency remains lower than that of the control group. Figure 5 further supports these findings by illustrating the mean comparison in a bar chart format. The average suture gap in the control group is 1.329 mm, whereas the treatment group shows a lower mean value of 1.037 mm. This clear reduction in the treatment group confirms the trend observed in the boxplot analysis. Overall, both graphical representations consistently indicate that the treatment condition is associated with decreased suture gap measurements compared to the control group, reinforcing the quantitative results of the Micro-CT evaluation.

### 5.4 Validation

Suture gap measurements using two-dimensional (2D) Micro-CT are systematically performed by scanning craniofacial specimens until high-resolution 2D image slices are obtained that are perpendicular to the suture direction

and anatomically consistent between specimens. The images are then segmented using a uniform grayscale threshold to separate mineralized bone tissue from the suture space. Next, a Region of Interest (ROI) is selectively defined in the suture area by excluding the surrounding cortical bone so that the analysis represents the true suture gap. Suture width is measured linearly as the shortest distance between two opposing bone surfaces on the selected slice, with measurements taken at multiple points or several consecutive slices to increase reliability, then averaged and reported in micrometers. This 2D Micro-CT approach allows for a simple, standardized, and reproducible evaluation of suture morphology to compare changes in suture widening or narrowing, although its limitation lies in its inability to capture variations in suture microarchitecture in three dimensions. In this study, as shown in Figure 5, the average height of the control group was 1,329 mm and the treatment group 1,037 mm.

Vitamin D deficiency during pregnancy or early life is associated with impaired mineralization and craniofacial bone formation, which can affect suture closure and cranial bone strength. Clinical studies and case reports indicate that maternal vitamin D deficiency is often associated with skeletal manifestations in newborns, such as craniotabes (softening and thinning of the skull bones), which is an early sign of impaired bone mineralization associated with rickets due to vitamin D deficiency. This condition reflects a delay or abnormality in cranial bone maturation, resulting in softer and less mineralized sutures than normal, due to impaired osteoid mineralization due to low maternal vitamin D, resulting in suboptimal fetal bone calcification (Weernink et al., 2016).

On the other hand, suture closure is biologically and clinically linked to stunting, primarily through mechanisms of impaired bone growth, nutrition, and endocrine regulation during early growth. Physiologically, cranial sutures function as intramembranous bone growth centers that allow skull expansion as the brain and body grow. Stunting, which is generally caused by chronic malnutrition (including deficiencies of protein, energy, vitamin D, calcium, and other micronutrients), disrupts osteogenesis and bone remodeling. Essential nutrient deficiencies can lead to slowed osteoblast proliferation, impaired mineralization, and altered osteocyte signaling, resulting in suboptimal bone maturation. Consequently, suture closure can be delayed or exhibit an abnormal pattern, reflecting overall skeletal growth retardation. Furthermore, stunting is often associated with hormonal dysfunction, such as disruption of the GH and IGF-1 axes, hypocalcemia, and vitamin D deficiency, all of which play a crucial role in craniofacial bone growth. This disorder can reduce the rate of suture bone growth and alter suture microarchitecture, preventing suture closure from occurring according to chronological age. Therefore, suture condition, whether delayed or abnormal, can be viewed as a skeletal indicator of stunted growth, consistent with the characteristics of stunting.

Based on the preliminary findings of Micro-CT, it can be concluded that the cranial suture bones of fetuses originating from mothers with vitamin D deficiency experience a decrease in the overall quality of osteogenesis. This condition is characterized by a decrease in relative bone volume, impaired suture closure dynamics, a reduction in bone-forming and maintenance cells, and a predominance of immature, less mineralized bone tissue. Biologically, these findings confirm the central role of maternal vitamin D in the regulation of mineralization, bone cell differentiation, and the balance of craniofacial bone remodeling during fetal development (Holick, 2007; Kovacs, 2013). This study's contribution revealed structural changes linked to maternal vitamin D deficiency, with the treatment group's mean cranial measurement being lower (1.037 mm) than the control group's (1.329 mm). Maternal vitamin D deficiency is biologically linked to lower craniofacial bone quality, delayed osteogenesis, and poor mineralization during fetal development.

## 6. Conclusion

In conclusion, two-dimensional (2D) Micro-CT analysis provides a standardized and reproducible method for evaluating cranial suture morphology through systematic slice acquisition, uniform threshold segmentation, and precise linear measurement of suture gaps. By defining a specific region of interest and averaging measurements across multiple slices, this approach ensures reliable quantification of suture width, expressed in micrometers. The findings of this study demonstrated a lower mean cranial measurement in the treatment group (1.037 mm) compared with the control group (1.329 mm), indicating structural alterations associated with maternal vitamin D deficiency. Although 2D Micro-CT does not fully capture the three-dimensional complexity of suture microarchitecture, it remains an effective tool for detecting comparative differences in suture widening or narrowing. Biologically, maternal vitamin D deficiency is strongly associated with impaired mineralization, delayed osteogenesis, and reduced craniofacial bone quality during fetal development. Insufficient vitamin D disrupts osteoblast function, osteoid mineralization, and bone remodeling balance, resulting in softer, less mineralized sutures and compromised suture closure dynamics. These alterations are consistent with skeletal manifestations such as craniotabes and reflect suboptimal fetal bone calcification. Collectively, the Micro-CT findings support the conclusion that maternal vitamin

D plays a critical regulatory role in fetal craniofacial bone formation, and its deficiency contributes to decreased osteogenic quality, delayed suture maturation, and overall skeletal growth impairment.

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