

Effects of Maternal Nutrition on Embryonic Development and Growth in Wistar Rat Pups

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ABSTRACT

Maternal nutrition is a major determinant of fetal growth, embryonic development, and long-term offspring health. Although several studies had investigated the developmental consequences of maternal nutritional imbalance, the specific effects of diets predominantly composed of individual macronutrients on embryonic growth remain insufficiently understood, hence this study. Twenty female Wistar rats and eight fertile male Wistar rats were used. The females were assigned into four groups (n = 5): a control group fed with standard balanced diet, (a high-protein diet group, a high-carbohydrate diet group, and a high-fat diet group), followed by administration of the experimental diet for 28 days, then mating continued throughout gestation. Pregnancy was confirmed by vaginal smear examination, and maternal body weight was monitored throughout pregnancy. Following delivery, neonatal growth parameters including birth weight, crown-rump length (CRL), and PCV were measured. The CRL of Wistar Rat Pups showed a statistically significant reduction in fetal crown length in all experimental diet groups compared to the control (p = 0.0056). Similar with the (PCV %) (p= 0.0001), and fetal weight (p= 0.0001). However, maternal weight was significantly higher in the experimental groups compared to the control group (p= 0.0022). These findings underscore the importance of adequate maternal nutrition during pregnancy and provide experimental evidence supporting nutritional interventions aimed at improving fetal growth and developmental outcomes. Therefore, it is recommended that women of reproductive age and pregnant mothers maintain a balanced and nutritionally adequate diet before and throughout pregnancy to promote optimal embryonic growth, fetal development, and favourable birth outcomes.

Received: 22 Aug. 2026

Accepted: 28 Aug. 2026

Published: 03 Sep. 2026

Keywords:

Maternal nutrition, Embryonic development, Packed cell volume, Fetal growth, Crown-rump length, Birth weight

INTRODUCTION

Maternal nutrition is one of the most important determinants of successful pregnancy outcomes and healthy fetal development (Dakare *et al.*, 2025). It encompasses the intake of adequate energy, macronutrients, and micronutrients required before conception, throughout pregnancy, and during lactation to support maternal health, placental function, fetal growth, and postnatal development. Nutritional status during pregnancy influences fetal organogenesis, brain maturation, immune competence, and long-term metabolic programming (Abu-Saad & Fraser, 2010; Koletzko *et al.*, 2019). The concept of the Developmental Origins of Health and Disease (DOHaD) proposes that environmental exposures during critical periods of prenatal development, particularly maternal nutritional status, permanently influence fetal physiology and susceptibility to diseases later in life (Barker, 2004). Adequate maternal nutrition promotes optimal placental growth, nutrient transfer, and fetal development, whereas nutritional deficiencies or excesses may induce structural, physiological, and epigenetic alterations that persist into adulthood (Godfrey *et al.*, 2017).

Current nutritional guidelines recommend that pregnant women consume a balanced diet containing sufficient amounts of proteins, carbohydrates, fats, vitamins, and

minerals to meet the increased metabolic demands of pregnancy (Koletzko *et al.*, 2019). Appropriate maternal weight gain and nutritional status before and during pregnancy have consistently been associated with improved birth outcomes, including normal birth weight, healthy fetal growth, and reduced neonatal morbidity (IM, 2009).

Despite improvements in maternal healthcare, maternal malnutrition remains a significant public health problem, particularly in low- and middle-income countries. Malnutrition includes undernutrition, micronutrient deficiencies, overweight, obesity, and imbalances in nutrient intake (WHO, 2021). Maternal undernutrition has been associated with intrauterine growth restriction (IUGR), low birth weight, preterm birth, impaired neurodevelopment, and increased neonatal mortality (Black *et al.*, 2013). Deficiencies in essential micronutrients such as folate, iron, iodine, zinc, and vitamin A have also been linked to congenital anomalies, neural tube defects, impaired cognitive development, and compromised immune function (Abu-Saad & Fraser, 2010). Experimental studies using animal models have demonstrated that fetal development is particularly vulnerable to maternal nutrient restriction during early gestation, especially during implantation and rapid placental development. Nutrient deprivation during these critical windows adversely affects placental vascularization, fetal organ development, and gene

expression, resulting in permanent alterations in growth and metabolism (Wu *et al.*, 2006; Igbokwe *et al.*, 2021). Similarly, maternal undernutrition during pregnancy has been shown to reduce placental efficiency and fetal growth in several domestic animal species, including cattle, sheep, and pigs (Bell & Ehrhardt, 2002). Conversely, maternal overnutrition and obesity are increasingly recognized as important contributors to adverse pregnancy outcomes. Excess maternal nutrient intake before or during pregnancy has been associated with insulin resistance, gestational diabetes mellitus, hypertensive disorders, excessive fetal adiposity, and increased risks of neonatal morbidity and mortality (Rasmussen & Yaktine, 2009; McKnight *et al.*, 2011). Animal studies further indicate that excessive maternal nutrition may impair placental function, alter fetal growth trajectories, and predispose offspring to obesity, insulin resistance, and cardiovascular disease in later life (Wu *et al.*, 2006).

Increasing evidence suggests that maternal nutrition influences fetal development through epigenetic mechanisms that regulate gene expression without altering DNA sequences. Nutritional factors during pregnancy can modify DNA methylation, histone modification, and non-coding RNA expression, thereby influencing fetal programming and increasing susceptibility to chronic diseases such as obesity, hypertension, cardiovascular disease, and type 2 diabetes during adulthood (Godfrey *et al.*, 2017).

Rodent models, particularly Wistar rats, have become indispensable in investigating the biological mechanisms underlying maternal nutrition and fetal development because of their short gestational period, well-characterized reproductive physiology, and physiological similarities to human pregnancy. These models provide valuable insights into how maternal dietary manipulation affects embryonic growth, organ development, birth outcomes, and postnatal health. Although numerous studies have investigated maternal nutrition in humans, relatively fewer controlled experimental studies have evaluated its direct effects on embryonic growth and development using laboratory animal models under standardized conditions. Thus, understanding the effects of maternal nutrition during pregnancy remains essential for developing effective nutritional interventions aimed at improving pregnancy outcomes and offspring health. This study therefore investigated the effects of maternal nutrition on embryonic development and growth in Wistar rat pups. Based on the crucial role of maternal diet in fetal programming, it is hypothesized that pregnant Wistar rats fed a nutrient-deficient diet will show altered placental transport mechanisms, which will subsequently result in significantly reduced birth weights, CRL, blood volume, impaired skeletal ossification, and delayed organogenesis in the offspring compared to pups born to mothers receiving a standard, nutritionally balanced diet.

MATERIALS AND METHODS

Study Design

This study employed a randomized controlled experimental design to investigate the effects of maternal nutrition on embryonic development and growth in Wistar rat pups. Adult female Wistar rats were randomly allocated into four experimental groups according to the type of diet administered prior to conception and throughout gestation. Pregnancy outcomes and neonatal growth parameters were subsequently evaluated and compared among the groups.

Study Location

The experiment was conducted at the Animal House, Department of Anatomy, Faculty of Basic Medical Sciences, Nile University of Nigeria, Abuja, Nigeria. All laboratory procedures, animal breeding, and data collection were performed under standard laboratory conditions between January and March 2025.

Ethical Approval

Ethical approval for this study was obtained from the Research Ethics Committee of Nile University of Nigeria prior to the commencement of the experiment. All experimental procedures complied with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NRC, 2011) and were conducted in accordance with the ARRIVE 2.0 guidelines for reporting animal research (Percie *et al.*, 2020). Every effort was made to minimize animal suffering and reduce the number of animals used throughout the study.

Experimental Animals

Twenty (20) healthy adult female Wistar rats aged 8–10 weeks and weighing between 180 and 205 g were used for this study. Eight (8) proven fertile adult male Wistar rats of similar age were used solely for mating purposes. The animals were obtained from the National Institute for Pharmaceutical Research and Development (NIPRD), Idu Industrial Area, Abuja, Nigeria. Only clinically healthy animals with no obvious congenital abnormalities or signs of disease were included in the study.

Animal Housing

After procurement, the animals were acclimatized for two weeks before commencement of the experiment. They were housed in well-ventilated polypropylene cages fitted with stainless steel wire covers and clean wood-shaving bedding. The experimental animals were acclimatized to a 12-hour light and dark cycle with an ambient temperature of $25 \pm 2^\circ\text{C}$ and 45–55% relative humidity. They were given regular pellets to eat and unlimited water. Procurement of feed, the Chikun feed is produced by Ola Chikun and Ultima Poultry Feed. The cages were cleaned regularly, and bedding materials were replaced every 48 hours to maintain hygienic conditions. Animals had unrestricted access to clean drinking water throughout the study.

Experimental Diets

Four dietary regimens were prepared for the study as detailed below:

Group A (Control)

Animals received commercially available standard laboratory rat pellets formulated to provide balanced amounts of carbohydrates, proteins, fats, vitamins, minerals, and fibre.

Group B (High-Protein Diet)

Animals received an experimental diet predominantly composed of protein (approximately 95% protein with minimal carbohydrate and fat content).

Group C (High-Carbohydrate Diet)

Animals received an experimental diet predominantly composed of carbohydrates (approximately 95% carbohydrate with minimal protein and fat).

Group D (High-Fat Diet)

Animals received an experimental diet predominantly composed of fat (approximately 95% fat with minimal protein and carbohydrate).

Experimental diets were prepared under hygienic laboratory conditions and supplied fresh daily. Water was provided *ad libitum*.

Experimental Grouping

The twenty female Wistar rats were randomly assigned into four groups consisting of five animals each as detailed in Table 1.

Table 1: Grouping Of Experimental Animals

Group	Diet	Number of Animal
Group A	Standard balanced diet (Control)	5
Group B	High-protein diet	5
Group C	High-carbohydrate diet	5
Group D	High-fat diet	5

Randomization was performed to reduce allocation bias and ensure equal representation among treatment groups.

Dietary Intervention

The assigned diets were administered orally by voluntary feeding for 28 consecutive days prior to mating to establish the desired maternal nutritional status. Following confirmation of pregnancy, each animal continued to receive its assigned diet throughout the entire gestational period until delivery. Daily food intake and water consumption were monitored, while maternal body weight was recorded weekly using a calibrated digital weighing balance.

Mating Procedure

The mating procedure was monitored by adopting the procedure described by Halaweh *et al.* (2025). Following the dietary intervention period, mating was carried out using a female-to-male ratio of 2:1. The reproductive cycle of each female was monitored by assessing vaginal opening and vaginal cytology to identify the estrus phase, during which fertility is maximal. Females identified in estrus were paired overnight with proven fertile males.

Successful copulation was confirmed the following morning by presence of spermatozoa in vaginal smear microscopy, and presence of a copulatory (vaginal) plug. The day on which spermatozoa were detected was designated Gestational Day 0 (GD0). Pregnant females were thereafter housed individually until parturition.

Pregnancy Monitoring

Throughout gestation (Pangestuti *et al.*, 2025), maternal body weight was measured at regular intervals using a calibrated electronic balance (Charakida *et al.*, 2025). Animals were observed daily for behavioural changes, food intake, water intake, and general health status. The average gestational period was approximately 21–23 days.

Assessment of Neonatal Growth Parameters

Immediately after delivery, all live pups were carefully examined. The following growth parameters were recorded:

Birth Weight

Individual pup birth weights were measured within 24 hours after birth using a precision electronic balance and recorded to the nearest 0.01 g as described by Basnet *et al.* (2025).

Crown-Rump Length (CRL)

Crown-rump length (CRL) was measured, as described by Jakubowski *et al.* (2020), from the tip of the nose to the base of the tail using a calibrated digital Vernier caliper. Measurements were recorded in millimetres (mm).

Packed Cell Volume (PCV)

PCV was measured by adopting the protocol earlier described by Chidozie *et al.* (2020). Blood samples were collected from neonatal pups using standard micro-haematocrit techniques. The blood collection tube gently inverted 5 to 7 times to spread the cells evenly. The end of a capillary tube was allowed to touch the blood, so that by capillary action, the cells filled the tube to about two-thirds to three-fourths full. Push the dry (empty) end of the tube into the sealing clay vertically to create a 4-to-6 mm clay plug. Thereafter, place the tube in the micro-hematocrit centrifuge with the sealed clay end facing outward against the outer rim by balancing it with another tube of identical weight directly opposite. The lid securely closed and centrifuged at 10,000 to 12,000 RPM for 5 minutes. The bottom of the red blood cell column (above the clay) aligned at the 0% line on a microhematocrit reader. The top of the plasma layer was moved to the 100% line, after which the percentage value where the packed red cells meet the buffy coat was read as the PCV value and recorded and expressed as percentage.

Statistical Analysis

Data obtained from the study were entered into Microsoft Excel and analyzed using IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean $\pm$ standard deviation (SD). Comparisons among the four experimental groups were performed using one-way analysis of variance (ANOVA). Tukey's post hoc multiple comparison test was employed to determine pairwise differences between groups. Statistical significance was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

As shown in Figure 1, there was a statistically significant ($p < 0.05$) reduction in fetal crown length in experimental group that receive protein, carbohydrate and fats diet compared to the control ($p = 0.0056$). This suggests that maternal macronutrient imbalances may slightly impair embryonic growth.

Figure 2 showed that maternal diets enriched with macronutrient (protein, carbohydrate, or fat) significantly ($p < 0.05$) reduced PCV of wistar rat pups when compared to the control group ($p = 0.0001$). Lower PCV suggests possible mild anemia or hemodilution, potentially linked to nutrient imbalance or poor iron absorption/utilization in experimental diets.

Figure 3 revealed that there was a significant ($p < 0.05$) reduction in fetal weight in all experimental diet groups compared to the control ($p = 0.0001$). This suggests that deviations from a balanced maternal diet whether high in

protein, carbohydrates, or fats, may negatively influence fetal growth and development. The uniform trend across all experimental groups points to the importance of nutritional balance rather than macronutrient enrichment alone in supporting optimal fetal outcomes.

Figure 4 showed that maternal weight was significantly ($p < 0.05$) higher in the protein, carbohydrate, and fat groups

compared to the control group ($p = 0.0022$). This indicates that diets enriched with individual macronutrients led to greater overall weight gain in pregnant rats. The findings suggest that macronutrient composition influences maternal body mass during gestation, potentially through increased energy storage or altered metabolic regulation.

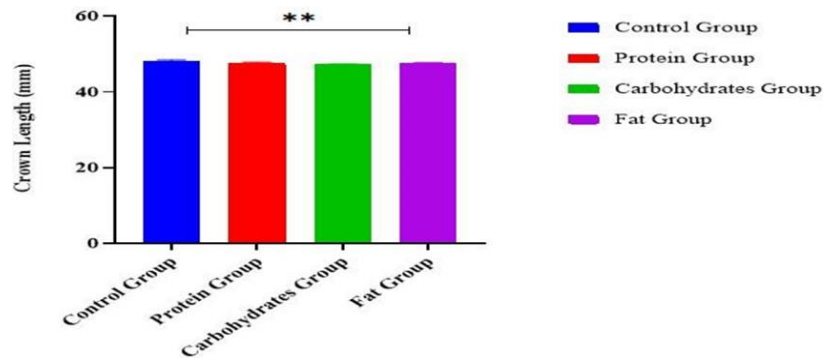


Figure 1: Effect of Macronutrient-Enriched Maternal Diets on Crown rump Length of Wistar Rat Pups

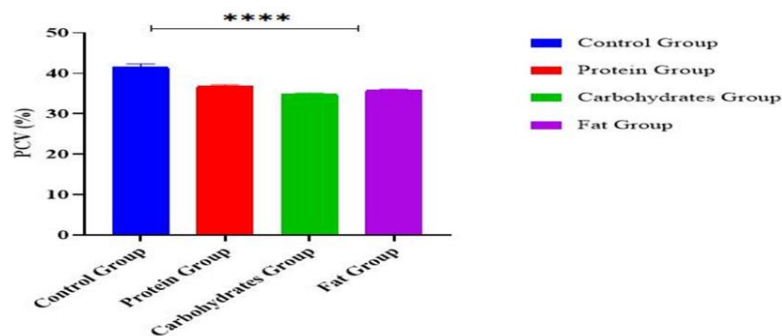


Figure 2: Effect of Different Maternal Macronutrient Diets on Packed Cell Volume (PCV%) in wistar rat pups

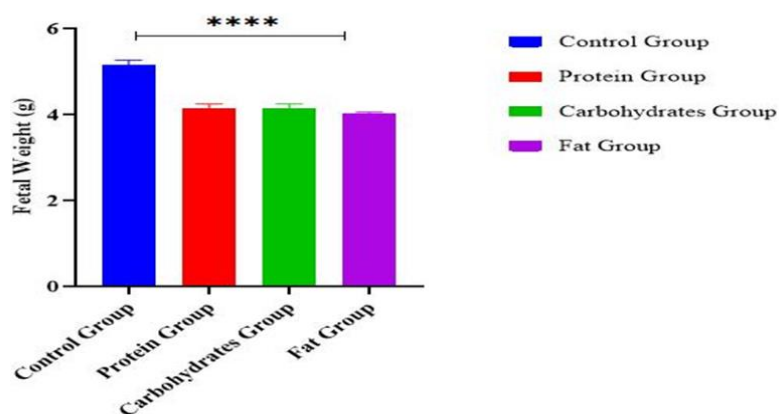


Figure 3: Impact of Maternal Macronutrient-Based Diets on Fetal Weight of Wistar Rat pups

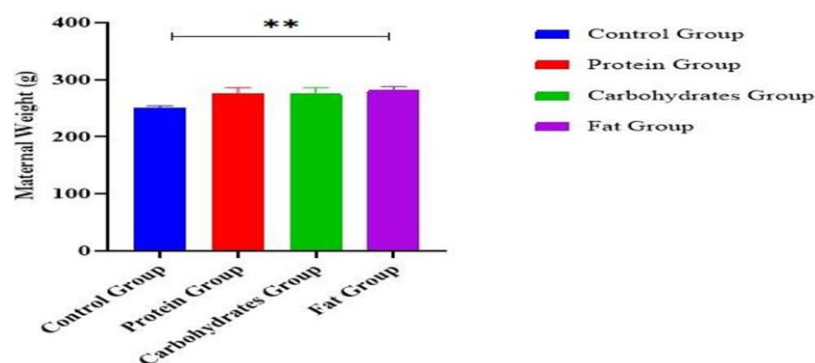


Figure 4: Impact of Macronutrient-Based Diets on Maternal Weight in Experimental Groups

Discussion

Maternal nutrition during the preconception and gestational periods significantly influences embryonic growth and neonatal development in Wistar rats. Diets that are nutritionally imbalanced adversely affect fetal growth, whereas balanced maternal nutrition promotes optimal neonatal development (Meraiyebu *et al.*, 2022).

Findings from the present study revealed that there was a significant reduction in fetal crown rump length across all experimental diet groups compared to the control. This observation suggests that maternal diets lacking in nutritional balance despite being enriched with a single macronutrient, may impair embryonic growth. Crown rump length is a reliable early indicator of fetal development and intrauterine health. The shortening of this parameter across all test groups points to a possible disruption in cellular proliferation or differentiation processes, which are highly sensitive to micronutrient availability and metabolic homeostasis (Fu *et al.*, 2022; Lawan *et al.*, 2026). Embryonic growth depends not only on macronutrient quantity but also on the appropriate proportion and synergy among macro- and micronutrients. Excessive protein, carbohydrate, or fat in isolation may create imbalances in amino acid utilization, glucose metabolism, or lipid signaling, all of which play pivotal roles in embryogenesis (Eze *et al.*, 2018; Dasgin *et al.*, 2024).

Furthermore, significant reduction in the packed cell volume (PCV) of Wistar rat pups across experimental groups compared to the control group was observed. PCV is a crucial hematological indicator of oxygen-carrying capacity and red blood cell mass. A reduction in PCV may indicate mild anemia or hemodilution, potentially arising from disrupted iron absorption, reduced erythropoiesis, or metabolic inefficiencies associated with unbalanced diets (Bayat *et al.*, 2025). These findings align with prior research by de Oliveira-Silva *et al.* (2023), which noted that maternal protein restriction can disrupt offspring development, including hematological parameters.

Similarly, Carvalho *et al.* (2021) reported vascular and hematologic changes in rat pups from mothers subjected to dietary manipulation. The PCV decrease may also result from impaired gastrointestinal absorption of iron due to excessive dietary fat or carbohydrate interfering with iron bioavailability or hepcidin-mediated regulation (Blais *et al.*, 2022). These outcomes suggest the need for dietary frameworks that ensure optimal micronutrient assimilation alongside energy provision.

More so, fetal weights were significantly reduced in all macronutrient-enriched groups compared to the control. Fetal weight is a direct marker of intrauterine growth, and its reduction indicates that isolated macronutrient enhancement may not support comprehensive fetal development. This

uniform trend across experimental groups emphasizes that the issue lies not with the macronutrients themselves but with the lack of dietary balance. Macronutrients serve as substrates for tissue synthesis and energy production, but imbalances can lead to metabolic disturbances, inflammatory responses, or oxidative stress factors that are deleterious to fetal growth (Zouridis *et al.*, 2021). Shankar *et al.* (2019) have also observed that maternal overnutrition or undernutrition can program offspring metabolism in ways that limit growth potential. The observed fetal weight reduction underscores that diets must be optimized not just for caloric content but for appropriate macro- and micronutrient distribution to support placental function and nutrient transfer.

Interestingly, findings revealed that maternal weight was significantly higher in the protein, carbohydrate, and fat groups relative to the control group. This finding suggests that individual macronutrient enrichment leads to greater energy storage, possibly in the form of adipose tissue, or reflects altered metabolic regulation during gestation. Maternal weight gain in pregnancy is influenced by factors such as hormonal changes, basal metabolic rate, and nutrient partitioning (Syggelos *et al.*, 2021). While some weight gain is expected and essential, excessive or poorly regulated weight increases can impair placental efficiency and contribute to gestational metabolic disorders. Furthermore, maternal overnutrition particularly from specific macronutrient sources can predispose offspring to metabolic diseases later in life, a phenomenon supported by the concept of developmental origins of health and disease (DOHaD) (Shankar *et al.*, 2019; Fu *et al.*, 2022).

Taken together, the findings suggest that while individual macronutrient supplementation increases maternal weight, it does not translate into improved fetal development. On the contrary, fetal crown rump length and weight are significantly reduced, and PCV levels in pups are compromised. This paradox indicates that the maternal-fetal nutrient transfer may be inefficient under such imbalanced conditions, possibly due to metabolic bottlenecks or impaired placental signaling. Nutrient oversupply in one category may trigger homeostatic countermeasures that affect absorption and utilization of other essential nutrients (Dasgin *et al.*, 2024).

Additionally, the findings raise questions about the role of gut microbiota and digestive enzyme expression under macronutrient-skewed diets. Previous studies have shown that excessive fat or protein alters gut microbiota composition, which in turn affects nutrient absorption and immune modulation (Blais *et al.*, 2022). These microbiota-mediated changes might impact both maternal health and fetal development in ways not directly measurable through weight or PCV alone.

The implications of this research extend beyond animal studies. In human populations, maternal dietary patterns similarly affect pregnancy outcomes. Both undernutrition and overnutrition have been associated with adverse birth outcomes such as low birth weight, preterm birth, and developmental delays.

CONCLUSION

This study demonstrates that maternal diets enriched with individual macronutrients such as protein, carbohydrate, or fat can negatively impact critical developmental and physiological outcomes in Wistar rat pups, including significant reductions in fetal crown length, fetal weight, and packed cell volume (PCV), alongside increased maternal weight. These results suggest that unbalanced macronutrient intake during gestation may impair fetal growth and hematological function, possibly due to disrupted nutrient absorption or metabolic regulation. The findings from this study reinforce the need for public health strategies that emphasize balanced maternal nutrition over targeted macronutrient supplementation. Prenatal nutrition programs should integrate culturally relevant, balanced meal planning that includes adequate protein, complex carbohydrates, healthy fats, and essential micronutrients like iron, folate, and calcium. Based on the findings of this study, it is recommended that women of reproductive age and pregnant mothers maintain a balanced and nutritionally adequate diet before and throughout pregnancy to promote optimal embryonic growth, fetal development, and favourable birth outcomes, while further well-controlled experimental and clinical studies are encouraged to elucidate the underlying mechanisms of maternal nutritional programming.

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