International Journal of Zoology Studies
www.zoologyjournals.com
ISSN: 2455-7269
Received: 18-07-2026, Accepted: 17-08-2026, Published: 01-09-2026
Volume 11, Issue 3, 2026, Page No. 72-76

Metformin-induced developmental toxicity in chick embryos: Relevance to gestational diabetes mellitus

Sreenanda G, Suresh K K*, Nayana S, Sandra Sajeevan
Department of Zoology, NSS Hindu College, Changanacherry, Kerala, India
Corresponding Author: Suresh K K

Abstract
Metformin, a member of the biguanide class, is an anti-diabetic medication used to manage Type 2 diabetes, Gestational diabetes mellitus and Polycystic ovary syndrome. Prenatal exposure to increased Metformin levels can have lasting effects on mothers with diabetes, as chronic and persistent high blood glucose (hyperglycemia) is prevalent in diabetes mellitus. Although metformin can cross biological barriers and influence cellular metabolic pathways, its effects on early embryonic development require further investigation. The present study evaluated the impact of different doses of metformin on embryonic growth and development using chick embryo (*Gallus gallus domesticus*). Chick embryos serve as a classic model for studying different stages of development and can be utilized in regenerative therapy. The fertilized eggs were categorized into two primary groups; group A served as the control group (normal saline), while group B represented the experimental group (metformin). The experimental group was further divided into three subgroups, each receiving different concentrations of metformin injections. Eggs from both group A and group B were injected at the 24-hour mark of incubation and examined on day 5 and 10 of incubation. Administering Metformin led to initial growth delays in chick embryos, along with alterations in their morphology. Metformin exposure was associated with alterations in embryonic morphology and extraembryonic vascular development compared with the control group. Treated embryos exhibited reduced peripheral vascular branching, vessel thinning, disruption of vascular organization, and reduced vascular density, with more pronounced alterations. These findings demonstrate that exposure to metformin during early chick embryonic development is associated with developmental and vascular alterations. The chick embryo model may therefore be useful for investigating the dose-dependent effects and underlying mechanisms of metformin-associated developmental toxicity. Further, molecular and mechanistic studies are required to clarify the pathways responsible for these developmental alterations.

Keywords: Metformin, chick embryo, *Gallus gallus domesticus*, embryonic development, developmental toxicity, vascular development

Introduction

Gestational diabetes mellitus refers to varying degrees of glucose intolerance that either begin or are discovered during pregnancy [1]. In recent years, especially in developing countries, this condition has been increasing rapidly globally. It affects approximately 14% of pregnancies every year and has effects on both the mother and the baby [2]. This condition is considered chronic and can lead to significant problems with insulin function and glucose regulation. Pregnancy is marked by high metabolic activity, making maintaining stable glucose levels essential [3]. Pregnancy with diabetes is associated with an increased risk of miscarriage at both early and late stages, congenital abnormalities, excessive fetal growth, and a higher chance of shoulder dystocia and birth-related injuries [4, 5].

The main strategy for managing GDM is early detection and treatment with several anti-hyperglycemic medications such insulin, metformin, and glyburides [6]. Insulin is safe and appropriate because it doesn't pass through the placenta [7]. Metformin, which is primarily used to treat type 2 diabetes, is now more commonly prescribed for polycystic ovary syndrome (PCOS) due to the shared link with insulin resistance [8]. It is also used for women diagnosed with Gestational diabetes and is currently categorized as a class B medication for use during pregnancy [8]. However, Metformin, is utilized in place of insulin treatment due to a number of obstacles, including cost, needle phobia, and availability [8, 9]. Since metformin can cross the placental barrier, it may affect fetal physiology and embryonic development, raising concerns about its safety during early development or conception [10]. The placenta plays a crucial role in transporting medications from the mother to the fetus, and metformin may directly influence fetal physiology and development. Metformin has been detected in umbilical cord blood at levels similar to those found in maternal blood [11]. High doses of metformin may lead to harmful or embryotoxic effects during chick embryo development. Metformin influences early embryonic growth and raises concerns about its use during conception. Administering metformin to one-day-old chicks affects lipid metabolism in the yolk sac of the embryo, leading to lipid depletion [12]. The activation of metformin inhibits complex I of the mitochondrial respiratory chain, reducing mitochondrial function and cellular respiration, which results in anaerobic respiration and an increased production of lactate [13, 14, 15]. Due to the significant risk of lactic acidosis and cardiovascular disorders associated with their usage, the American Food and Drug Administration (FDA) has removed phenformin and buformin biguanides, which are comparable to metformin [16]. Metformin causes gastrointestinal problems and lowers vitamin B12 [16]. When compared to treatment with a placebo in pregnant women who were obese but not diabetic, the Fetal Medicine Foundation trial (2010–2015) found that metformin can

reduce maternal weight gain [17]. One of the biggest disadvantages of metformin is the short- and long-term adverse effects on the children following exposure to the drug during intrauterine growth. The teratogenic effects of metformin hydrochloride include delayed development of brain vesicles, eyes, heart loops, branchial arches, somites and limb buds, as well as delayed closure of anterior and posterior neuropores, which resulted in anomalies in the brain [18]. The risk of early-stage chick embryonic development abnormalities, retardation, and mortality increased with increasing metformin concentrations [18]. Metformin increases the prevalence of central adiposity, obesity and post-natal growth [16]. This study aims in identifying the impacts of in-utero exposure of Metformin and how it influences early embryonic development.

Experimental models play a crucial role in elucidating the pathogenesis and molecular mechanisms involved in the developmental alterations caused by maternal hyperglycemia [19]. In this study, we utilize chick embryos as the animal model to investigate the impact of metformin on patients with gestational diabetes mellitus (GDM). Chick embryos are chosen due to their significant resemblance to mammalian embryos [19, 20]. This model offers greater accessibility, enabling the handling of embryos from very early developmental stages and facilitating a thorough examination over several days of development [21]. The extraembryonic membranes of chicken embryos are commonly utilized as a screening method for drugs [22] and teratogenic substances [23]. This study aims in identifying the impacts of in-utero exposure of Metformin and how it influences early embryonic development.

Methodology

Experimental Design and Incubation Conditions

Fertilized chicken eggs (*Gallus gallus domesticus*) were collected, and an experimental study was conducted on two main groups consisting of 40 eggs. Group A served as the control group, with 10 eggs receiving normal saline injections. Group B, the experimental group, consisting of 30 eggs injected with metformin. This group was further divided into three subgroups- B1, B2 and B3 with each group containing 10 eggs. The control group received saline injections (0.3 ml) into the egg albumen. Similarly, Group B received 0.3 ml of metformin solutions at concentrations of 3 mg/ml, 6 mg/ml and 9 mg/ml corresponding to 0.9 mg/egg, 1.8 mg/egg and 2.7 mg/egg respectively. All injections were administered into the egg albumen at 24 hr of incubation. The eggs were placed inside the incubator, with the starting day marked as 0. Each day, the eggs were rotated by 180º manually throughout the incubation period. Eggs were maintained at standardized conditions at 38 ± 0.5°C with relative humidity 60 -70%, and adequate fresh air circulation.

Embryo Evaluation

Embryos were evaluated on the 5th and 10th day of incubation corresponding to Hamburger-Hamilton stages of development (HH26 and HH38). At each point of time, eggs were opened and the embryos were examined for viability and developmental abnormalities. Embryo viability was assessed by the presence of a visible heart beat at the time of examination. The mean weight of the embryos was measured using analytical weighing balance. The morphogenetic movements of the embryos were examined for gross morphological abnormalities and deviations from normal embryonic development. Using skeletonized images using ImageJ software, the embryo area, vessel length, branch points, and vessel density were quantified and compared with those of the control embryos.

Statistical Analysis

All data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism Software. Differences among the control and metformin-treated groups were assessed using one-way analysis of variance (ANOVA). A value of $p < 0.05$ was considered statistically significant.

Results and Discussion

An *"in-ovo"* model was created using chick embryos to mimic the exposure of foetus to Metformin in mothers with diabetes. Injection of Metformin enabled direct exposure of the developing chick embryos to the drug within the *in-ovo* environment. The mean body weight of the embryos on day 10 was measured as an indicator of embryonic growth and were compared with those of the control groups. The administration of Metformin resulted in significant reduction in mean body weight with the highest growth suppression observed at 9 mg/ml dosage (Fig.1). Studies suggest that Metformin, the commonly prescribed anti-diabetic drug has been associated with significant weight loss [24].

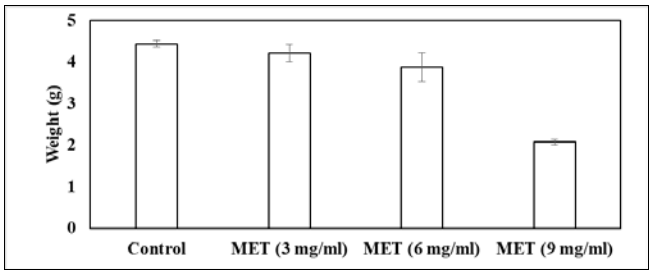

Fig 1: Effect of different concentrations of metformin on chick embryo body weight. A reduction in mean embryo body weight was observed following metformin treatment, with the greatest reduction observed in the MET (9 mg/ml) group. Data are presented as mean ± SD.

Representative images obtained on day 5 of incubation demonstrated differences in embryonic morphology and extra embryonic vascular organization between the control and Metformin treated groups. The control embryos exhibited normal morphology and a well-developed vascular network characterized by extensive branching and a dense peripheral vascular system. In contrast, embryos administered with Metformin showed alterations in vascular architecture including reduced peripheral branching, vessel thinning, discontinuities in vascular network and a reduction in overall network density. The most pronounced alterations were observed in the MET (9 mg/ml) which showed a sparse peripheral vascular network, fewer branches and marked disruption of the normal development (Fig. 2).

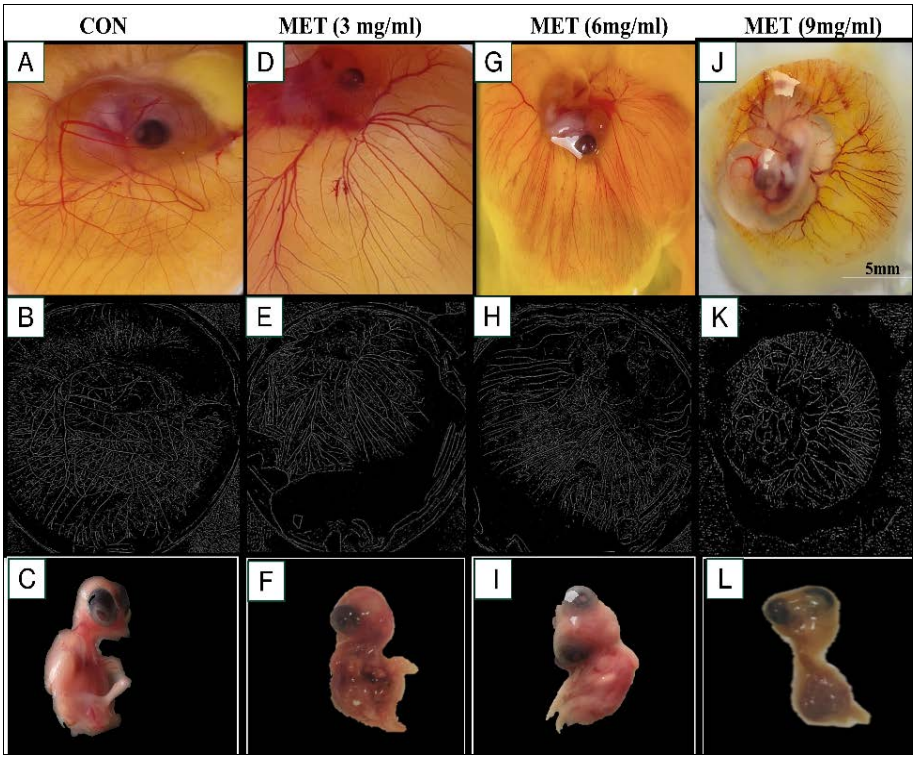

Fig 2: Effects of metformin exposure on embryonic growth and extraembryonic vascular development in chick embryos. Representative images of control and metformin-treated embryos on days 5 and 10 of incubation. (A–C) Control: (A) normal embryonic morphology with a well-developed, extensively branched extraembryonic vascular network (B) corresponding ImageJ-processed vascular image and (C) representative day 10 embryo. (D–F) Metformin (3 mg): reduced peripheral branching and vessel thinning on day 5, corresponding processed vascular image, and representative day 10 embryo. (G–I) Metformin (6 mg): altered vascular organization and embryonic morphology, corresponding processed vascular image, and representative day 10 embryo. (J–L) Metformin (9 mg): marked reduction in vascular branching and sparse peripheral vascular network, corresponding processed vascular image, and representative day 10 embryo.

Table 1: Quantitative assessment of embryo area, vessel length, and vessel density in control and metformin-treated chick embryos.

Group	Embryo area (Px^2)	Vessel length (Px)	Vessel density
Control	120000	86127	.7177
Met (3 mg)	115000	72112	.6270
Met (6 mg)	110000	68500	.6227
Met (9 mg)	108000	65896	.6102

The table shows Embryo area and vessel length were measured using ImageJ and expressed in pixels squared (Px^2) and pixels (Px), respectively. Vessel density was calculated as the ratio of vessel length to embryo area

Quantitative image analysis supported the morphological observations (Table 1). Embryo area decreased in the metformin group compared to the control and the vessel length was also lowered in MET groups. All the metformin treated groups exhibited lower vessel density than control with the lowest vessel density observed in MET (9 mg/ml) on day 5. The decrease in the vessel density leads to complications in respiratory exchange thereby resulting in the abnormal growth of the embryo. The metformin treated groups displayed developmental defects compared with the normal morphology observed in control groups on day 10 confirming Metformin restricts normal development and is associated with increased risk of abnormalities thereby disturbing normal homeostasis (Fig. 2). The control embryo exhibited normal gross morphology and appropriate developmental progression. The 3 mg metformin-treated embryo showed mild growth retardation and altered body morphology compared with the control. In the 6 mg group more pronounced developmental delay and morphological abnormalities were evident, including abnormal body curvature and apparent incomplete ventral body wall closure with protrusion of abdominal contents. The 9 mg metformin-treated embryo exhibited severe growth retardation, marked reduction in overall body size, and pronounced developmental dysmorphology, indicating severe impairment of embryonic development at the highest dose. Administration of Metformin in high doses results in embryotoxicity and acts as a teratogen.

Conclusion

Metformin is a drug used to lower blood sugar levels, and its potential to harm embryos or cause birth defects has not been thoroughly studied. This study aimed to examine the teratogenic effects of metformin on chick embryo development by comparing results from the experimental group with those from the control group. Metformin delayed embryo development and caused an increase in abnormalities. This research aims to enhance the understanding of how metformin affects the fetus when taken during pregnancy. Our study revealed that administration of metformin has negative impacts on chick embryo in the early stages of development. Administration of Metformin resulted in growth retardation, changes in morphogenetic movements, decreased blood vessel density as well as other morphological defects when compared with the control. In this study, the results from statistical analysis, combined with findings from prior research, indicate that metformin led to visible changes in the structure of

developing chick embryos when compared to a control group. While this study provides initial insights, further investigation is necessary to explore the molecular mechanisms, such as the specific pathways and genes involved in metformin metabolism, and how these might be affected when the drug is administered in higher doses.

Competing interests

The authors declare no competing or financial interests.

Acknowledgements

The authors would like to take this opportunity to thank the management of NSS Hindu College for providing the necessary facilities and encouragement to carry out this work.

References

1. Plows JF, Stanley JL, Baker PN, Reynolds CM, Vickers MH. The Pathophysiology of Gestational Diabetes Mellitus. International Journal of Molecular Sciences,2018:19(11):3342.
2. International Diabetes Federation. IDF Diabetes Atlas. 8th edition. IDF; Brussels, Belgium: 2017.
3. Anovskaya TN, Nekhayeva IA, Patrikeeva SL, Hankins GDV, Ahmed MS. Transfer of metformin across the dually perfused human placental lobule. American Journal of Obstetrics and Gynecology,2006:195(4):1081-1085.
4. International Association of Diabetes and Pregnancy Study Groups Consensus Panel, Metzger BE, Gabbe SG, Persson B, Buchanan TA, et al. international association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care,2010:33:676-682.
5. Esakoff TF, Cheng YW, Sparks TN, Caughey AB. The association between birthweight 4000 g or greater and perinatal outcomes in patients with and without gestational diabetes mellitus. American Journal of Obstetrics and Gynecology,2009:200:672.e1-672.e4.
6. Yu DQ, Xu GX, Teng XY, Xu JW, Tang LF, Feng C, et al. Glycemic control and neonatal outcomes in women with gestational diabetes mellitus treated using glyburide, metformin, or insulin: A pairwise and network meta-analysis. BMC Endocrine Disorders,2021:21:199. DOI: 10.1186/s12902-021-00865-9.
7. Lv S, Wang J, Xu Y. Safety of insulin analogs during pregnancy: A meta-analysis. Archives of Gynecology and Obstetrics,2015:292(4):749-756. DOI: 10.1007/s00404-015-3692-3.
8. Rai L, Meenakshi D, Kamath A. Metformin—a convenient alternative to insulin for Indian women with diabetes in pregnancy. Indian Journal of Medical Sciences,2009:63(11):491-497. DOI: 10.4103/0019-5359.58878.
9. Wu R, Zhang Q, Li Z. A meta-analysis of metformin and insulin on maternal outcome and neonatal outcome in patients with gestational diabetes mellitus. The Journal of Maternal-Fetal & Neonatal Medicine,2024:37(1):2295809. DOI: 10.1080/14767058.2023.2295809.
10. Schwartz RA, Rosenn B, Aleksa K, Koren G. Glyburide transport across the human placenta. Obstetrics & Gynecology,2015:125(3):583-588. DOI: 10.1097/AOG.0000000000000672.
11. Eyal S, Easterling TR, Carr D, Umans JG, Miodovnik M, Hankins GD, et al. Pharmacokinetics of metformin during pregnancy. Drug Metabolism and Disposition,2010:38(5):833-840. DOI: 10.1124/dmd.109.031245.
12. Nayak G, Salian SR, Agarwal P, Suresh Poojary P, Rao A, Kumari S, et al. Antidiabetic drug metformin affects the developmental competence of cleavage-stage embryos. Journal of Assisted Reproduction and Genetics,2020:37(5):1227-1238. DOI: 10.1007/s10815-020-01709-6.
13. Han X, Tai H, Wang X, Wang Z, Zhou J, Wei X, et al. AMPK activation protects cells from oxidative stress-induced senescence via autophagic flux restoration and intracellular NAD (+) elevation. Aging Cell,2016:15:416-427. DOI: 10.1111/acel.12446.
14. Hernandez-Sanchez C, Rubio E, Serna J, de la Rosa EJ, de Pablo F. Unprocessed proinsulin promotes cell survival during neurulation in the chick embryo. Diabetes,2002:51:770-777. DOI: 10.2337/diabetes.51.3.770.
15. Luft D, Schmülling RM, Eggstein M. Lactic acidosis in biguanide-treated diabetics: A review of 330 cases. Diabetologia,1978:14:75-87.
16. McIntyre HD, Catalano P, Zhang C, Desoye G, Mathiesen ER, Damm P. Gestational diabetes mellitus. Nature Reviews Disease Primers,2019:5(1):47. DOI: 10.1038/s41572-019-0098-8.
17. Syngelaki A, Nicolaides KH, Balani J, Hyer S, Akolekar R, Kotecha R, et al. Metformin versus placebo in obese pregnant women without diabetes mellitus. New England Journal of Medicine,2016:374(5):434-443. DOI:10.1056/NEJMoa1509819.
18. Siripattanaphol K, Rungruang T, Plakornkul V, Viravud Y, Roongruangchai J. Teratogenic effects of metformin hydrochloride on developing chick embryo, The 15th RSU National Graduate Research Conference, 2020, 2706-2712.
19. Smith CP. Animal models in biomedical research: Poultry. National Agricultural Library, Beltsville, Maryland, 1992, 1-80.
20. Machado-Costa D, Miranda G, Correia-Pinto J, Moura RS. Exploring hyperglycemia's impact on embryonic development: Insights from the chicken embryo model. Tissue and Cell, 2025, 102925.
21. Brown WRA, Hubbard SJ, Tickle C, Wilson SA. The chicken as a model for large-scale analysis of vertebrate gene function. Nature Reviews Genetics,2003:4(2):87-98. DOI: 10.1038/nrg998.
22. Vargas A, Zeisser-Labouèbe M, Lange N, Gurny R, Delie F. The chick embryo and its chorioallantoic membrane (CAM) for the in vivo evaluation of drug delivery systems. Advanced Drug Delivery

Reviews,2007:59(11):1162-1176. DOI: 10.1016/j.addr.2007.04.019.
23. Kotwani A. Use of chick embryo in screening for teratogenicity. Indian Journal of Physiology and Pharmacology,1998:42(2):189-204.
24. Seifarth C, Schehler B, Schneider HJ. Effectiveness of metformin on weight loss in non-diabetic individuals with obesity. Experimental and Clinical Endocrinology & Diabetes,2013:121(1):27-31.