

# Alterations in the Survivability and Gross Morphology of Chick Embryos Following Exposure to Glucose

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## ABSTRACT

### Objective:

To evaluate the effects of administration of glucose on survivability and gross developmental malformations of the chick embryos in comparison with age matched controls.

### Methodology:

This was an experimental study carried out at Anatomy Department, Regional Centre, College of Physicians and Surgeons Pakistan, Islamabad. Glucose (5% weight/volume solution) was injected in the albumin of fertilized eggs before incubation in experimental group B (n=60), while normal saline was given in control group A (n=60). Both groups (A and B) were compared for survivability and gross developmental malformations. Eggs in the subgroups A1 (n=30) and B1 (n=30) were opened on day 10 of incubation while subgroups A2 (n=30) and B2 (n=30) were opened at hatching or day 22.

### Results:

In the glucose exposed group B1, 22 (73.3%) chicks were alive while 8 (26.6%) were dead. Upon hatching, 23 (76.6%) of the chicks belonging to the experimental group B2 survived, while 23.3% died. In control group 29 (96.6%) embryos survived in A1 while 1 (3.3%) was dead. All embryos from subgroup A2 survived. The difference of survivability between the experimental and the control groups was significant at the embryonic stage ( $p=0.005$ ) as well as at the hatching stage ( $p=0.013$ ). The experimental animals showed a number of gross deformities with failure of closure of neural tube and anterior abdominal wall. There was herniation of brain, thoracic and abdominal viscera. Some experimental animals demonstrated absent lower limbs and others were unable to stand and/or walk properly. None of these anomalies were shown by embryo of the control group.

### Conclusion:

Glucose exposed embryos demonstrated decreased survivability and gross developmental malformations.

### Key words:

Glucose, chick embryos, malformations, diabetes

## INTRODUCTION

Diabetes is a common yet manageable disease. The first WHO Global Report on Diabetes reveals that the number of adults suffering from diabetes has augmented from 108 million in 1980 to 422 million in 2014.<sup>1</sup> The occurrence of this metabolic disorder has been rising more rapidly in middle- and low-income countries. The number of people suffering from diabetes is likely to rise from 382 million in

2013 to 592 million in 2035. The study also shows that in Pakistan this number will rise from 6,713,000 in 2013 to 12,798,000 in 2035.<sup>2</sup>

A common effect of uncontrolled diabetes is hyperglycemia, which is raised blood glucose levels. The maternal-diabetes induced embryopathies are caused by exposure of the embryo to maternal energy fuels which are excesses of glucose and ketones.<sup>3,4</sup> Because good control

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of blood glucose level is linked with a lower incidence of pregnancy loss and congenital malformations, glucose is generally said to be main teratogen. This is proved by glucose administration to experimental animals.<sup>5,6</sup>

Chick embryo and its egg is a worthy comparative model for research purposes because of rich background of information, short breeding time and ease of maintenance. The genetic analysis of chicken has been carried out and substantial similarities were found between the human and chicken genomes.<sup>7</sup>

The aim of this study is to apprehend the adverse outcome of human pregnancy complicated by hyperglycemia as a result of maternal diabetes by using chick embryos. The study was intended to look into the effect of administered glucose on survivability and gross developmental malformations in chick embryos keeping in mind that the increasing incidence of diabetes especially in women of childbearing age.<sup>8</sup> Significantly, this model isolates the effects of hyperglycemia only, sidestepping the multiple effects of maternal diabetes.

## METHODOLOGY

The study design of this project was experimental. The study was carried out at Anatomy Department of College of Physicians and Surgeons Pakistan, Islamabad. The number of eggs used was 120 of *Gallus gallus domesticus*. We collected these fertilized eggs from Poultry Research Institute Punjab, Rawalpindi. The exclusion criteria was the eggs which were cracked and kept in the fridge. Simple random tables were used to divide the eggs into an experimental group B (n=60) and its control group, A (n=60). Eggs in the subgroups A1 (n=30) and B1 (n=30) were opened on day 10 of incubation while subgroups A2 (n=30) and B2 (n=30) were opened at hatching or day 22 (which ever was earlier).

10 mg of 5% weight/volume solution of glucose was injected into egg albumin belonging to the experimental group while the control group was injected with same volume of normal saline. The dose of glucose was selected after giving preliminary doses in pilot project and the dose was such that the animals remained alive and showed malformations. An incubator (manufactured by Memmert Electric Company Germany) was used to place the eggs. The temperature of incubator was conserved at 38 °C and relative humidity between 60-70%.

The number of alive and dead embryos were recorded and analyzed by Chi square test for measuring any significant differences between control and experimental groups. P

value < 0.05 was considered significant. The gross developmental anomalies were also recorded in both groups.

## RESULTS

Hyperglycemia decreases the survivability of chick embryos and resulted in multiple gross developmental anomalies. Out of total of 30 embryos belonging to the glucose exposed embryonic group B1, 73.3% (n=22) were dissected alive while 26.6% (n= 8) were dead. From the chicks belonging to the age matched control group A1 96.6% (n=29) were dissected alive. This difference of survivability at the embryonic stage between the groups A1 and B1 was statistically significant (p=0.005).

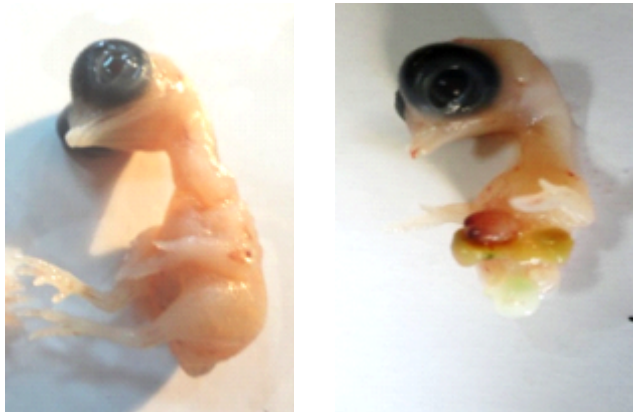
Regarding the fully hatched group, 76.6% (n=23) of the chicks from the experimental group B2 were hatched alive and 23.3% (n=7) did not survive. From the age matched control group A2, 100% chicks survived. The difference of survivability between the fully hatched experimental and the control groups B2 and A2 respectively was significant (p=0.002) (Table-1). Beside survivability, 6.5% animals from experimental group demonstrated failure of closure of neural tube and anterior body wall with herniation of brain and thoracoabdominal contents (Fig.3). Some experimental animals (4.2%) exhibited absent lower limbs with herniation of abdominal contents (Fig.1). About 5% hatched chicks were unable to stand and walk properly with flexed lower limbs (Fig-2, Table-2). No animal from control group showed any of these gross abnormalities.

Subgroup	n	Number of alive embryos/chicks n (%)	Number of dead embryos/chicks n (%)	P value
B1	30	22 (73.33)	08 (26.67)	0.005*
A1	30	29 (96.66)	01 (0.33)	
B2	30	23 (76.66)	07 (23.33)	0.002*
A2	30	30 (100)	00	

Table1; Comparison of total number of dead and alive chicks in control and experimental groups (chi-square test)

S. No	Gross Developmental Defects	Percentage
1	Failure of closure of neural tube and anterior abdominal wall	6.5%
2	Absent lower limbs	4.2%
3	Inability to stand	5%

Table 2; Frequency of gross developmental defects in experimental group



A1

B1

Figure 1: Photograph of day 10 chick embryo belonging to experimental group (B1) with herniation of abdominal contents and absent lower limbs. Compare with age matched control (A1).



Figure 2: Photograph of chick embryo belonging to experimental group (B2) with inability to stand (arrow) compared with age matched control (A2)



Figure 3: Photograph of newly hatched glucose exposed chick showing exencephaly with anterior body wall defect (B2).

## DISCUSSION

The data we represent here shows that administration of glucose adversely affects the survival of chick embryos. Moreover, glucose induced teratogenicity is manifested by deranged development of neural tube, heart, limbs and abdominal wall.

Diabetes during pregnancy has a high risk for infants to be born with congenital anomalies leading to complications in childhood and later in adult life.<sup>9,10</sup> In our study, glucose decreased the survivability and altered the morphology of developing embryos.

The survival of a species is extremely important for maintenance of the species robustness. Survivability depends on various factors. Regarding decreased survivability, our observations are in agreement with the findings of the research done by Savita Datar and colleagues<sup>11</sup> which showed that glucose exposure to chick embryos in a shell less culture system had augmented mortality. Another research demonstrated that excess glucose resulted in less expanded blastocyst with decreased number of inner cell mass in mouse embryos.<sup>12</sup> Consistent with findings of our study, a mouse model with hyperglycemia demonstrated that oocytes are smaller with delayed meiotic maturation when compared to oocytes

## CONCLUSION

Exposure to high glucose concentrations during development of chick embryos may result in embryo resorption. Disorders in embryonic glucose metabolism are found to be the potential reasons for embryopathies during development of bovine blastocysts.<sup>15,16</sup>

Furthermore, many genes had been identified which regulate the developmental process, and the mechanisms by which morphogenesis is regulated by these genes has been studied. Maternal hyperglycemia is found to hinder the expression of the genes that are responsible for regulation of embryogenesis. The role of Insulin-like growth factor (IGF) -I and -II has been explained to act as proliferation and differentiation factors in cultured fetal cells.<sup>17</sup> Exposure of rabbit blastocyst to glucose resulted in down regulation of expression of IGF-I receptor and their metabolic target genes. The embryonic disc showed decreased number of apoptotic cells with delay in onset and progression of gastrulation.<sup>18</sup> In the current study, we expected that glucose exposure has resulted in altered concentrations of these regulatory factors that are essential for normal growth leading to decreased survivability.

In our study, glucose exposed embryos had closure defects in thoraco-abdominal wall. The heart was located outside the chest wall not even covered with skin. The abdominal viscera were also exposed including small intestine, pancreas and liver. The ventral body wall formation is a complex developmental process. A number of studies have been conducted on different embryonic models to understand this process. The lateral plate and paraxial mesoderm both have essential role in the formation of ventral body wall as was discussed by Sadler and Feldkamp.<sup>19</sup>

The etiology of ventral body wall defects is not exactly known, but it may be linked to abnormalities in the lateral body wall folds which are important for closing the thoracic, abdominal, and pelvic portions of the body wall. The fusion process between the folds consists of adhesion between the cells, their migration and reorganization. Any of these processes if disrupted can lead to malformations. This complex process also involves many genes. For example, Pitx2 (Pituitary homeobox 2) is involved in mediating cell proliferation, the absence of this gene causes body wall closure defects.<sup>20</sup> In a research work, culture of mouse embryo in high glucose medium resulted in loss of Pitx2 expression.<sup>21</sup>

In our study, hyperglycemia may have worked in a similar mechanism resulting in ventral body wall closure defect. An additional molecular factor that is involved in regulation of certain physiological functions like adhesion, contraction, proliferation, apoptosis and migration is Rho associated protein kinase (ROCK). This is an important regulator of actin cytoskeleton assembly and cell contractility, the process that is involved in body wall closure.<sup>22,23</sup> In a research

work, chick embryos demonstrated failure of ventral body wall closure when treated with ROCK inhibitor.<sup>24</sup> Inhibition of this regulatory factor alters the cytoskeleton arrangement during early chick embryogenesis which results in closure defects at later stages of development. A marked down regulation of genes of key components of cytoskeleton including ROCK genes had been observed by exposing the embryos to hyperglycemia.<sup>6</sup> The developing experimental chick embryos in our study might face changes in this regulatory pathway and failed to achieve the developmental milestone that is closure of body wall.

One rare gross anomaly seen in our study is anterior body wall defect accompanied by absent lower limb (Figure 1). Literature reviewed demonstrated it as 'limb body wall complex' which is an unusual fetal malformation observed in human beings. The diagnosis of this condition has been based on the presence of exencephaly, thoracoschisis and abdominoschisis along with limb defects. The precise underlying mechanism of this condition is still unclear. The most accepted theory is early embryonic dysplasia put forward by Hartwig et al. in 1989<sup>25</sup> that abnormal folding of the embryo might be the reason. This resulted in faulty closure of embryonic abdominal wall. Some authors propose that disruption of blood supply in the affected area may be the reason.<sup>26</sup> The studies have provided evidence that hyperglycemia-induced vascular complications are associated with extensive tissue and organ damage<sup>27</sup> and this might be a factor that affected the development of glucose exposed embryos in our study.

Another gross developmental defect seen in our study was failure of closure of neural tube in its cephalic part. As a result, the vault of the skull did not form, leaving the brain exposed. Neurulation is a complex morphogenetic process that involves the synchronization of many cellular and molecular events, and is regulated by numerous genetic factors.<sup>28</sup>

An important step in process of neurulation is the migration of cranial neural crest cells from the edges of neural tube. This suggest a possible link between timely neural crest cell migration and neural tube closure. Literature reviewed had shown that elevated glucose inhibit the migration of NCC. It is proposed that apoptosis is involved in the inhibition of cranial neural crest cells that is induced by exposure of chick embryos to high glucose levels.<sup>29</sup> This may possibly explain the underlying mechanism for exencephaly in our work.



## CONCLUSION

Exposure of developing chick embryos to elevated glucose decreased the survivability and resulted in multiple gross developmental anomalies. Our findings thus reinforce the need for strict avoidance of hyperglycemia during organogenesis in women with diabetes.

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