International Journal of Innovative Research and Review ISSN: 2347 – 4424 (Online)
An Online International Journal Available at http://www.cibtech.org/jirr.htm
2016 Vol. 4 (3) July-September, pp.7-13/ Bokariya et al.
Review Article

EFFECT OF INSULIN ON DEVELOPING CHICK EMBRYOS: A SYSTEMATIC REVIEW

***Pradeep Bokariya, Ruchi Kothari, Doshi MA, MR Shende and Mrinmayee Debbarma**
Department of Anatomy, Mahatma Gandhi Institute of Medical Sciences, Sevagram, Wardha - 442102 (Maharashtra)
**Author for Correspondence: pradeepbokariya@mgims.ac.in*

ABSTRACT
Insulin is a peptide hormone, produced by the beta cells in the pancreas and is central to regulating carbohydrate and fat metabolism in the body. It is one of the best-known teratogenic agents which has been studied profoundly in the chick embryo. Extensive studies have been undertaken from time to time to study effect of drugs on developing chick embryo. In this review, we present a comprehensive account of the research that is conducted so far in this regard and which deal with effect of insulin on chick embryo with a desire as well as concern for further exploration of this aspect.

Keywords: *Insulin, Chick Embryo, Teratogen*

INTRODUCTION

The use of insulin is quite rampant in pregnant women. In ovo experiments with the chick have been the most popular means of investigating the teratogenic action of insulin. Easy availability of fertilized eggs has added advantage for developing chick embryo to be used as a very important animal model for experimental studies. Many studies are being conducted from time to time to study effect of drugs on developing chick embryo. Insulin is a peptide hormone, produced by beta cells in the pancreas, and is central to regulating carbohydrate and fat metabolism in the body. It causes cells in the skeletal muscles, and fat tissue to absorb glucose from the blood. Insulin is a very old protein that may have originated more than one billion years ago (Alzira and López, 2004). The molecular origins of insulin go at least as far back as the simplest unicellular eukaryotes (Le *et al.,* 1985). Insulin (De *et al.,* 1982) and receptors for insulin (Bassas *et al.,* 1987; Girbau *et al.,* 1992) have been shown to be present in developing chick embryo too. There are few studies which have reported effects of insulin on other animal models.

Effects of Insulin on Chick Embryo

Early Studies

One of the best-known teratogenic agents is insulin, which has been used extensively in the chick embryo. When implanted into the yolk sac at various stages of development it readily induces abnormalities closely resembling certain mutant conditions. Landauer (1945) produced rumplessness in chicks by injecting insulin into the yolk sac at 24 hours of incubation. Later Landauer (1947) found abnormalities of the beak, extremities, and eyes after giving it at 5-6 days of incubation. Duraiswami (1950) also found abnormalities of the beak, eyes, and limbs with insulin treatment of the chick embryo.

Insulin, in varying concentrations, was applied to the chick embryo by Barron and McKenzie (1962) explanted after 24 hours incubation at stages 3-8 and cultured for 22-24 hours *in vitro.* It was demonstrated that, in whatever manner insulin is applied to the embryo, its effects are most frequently manifest in the brain and neural tube. Somites are inhibited to a much lesser extent and heart, except with the higher doses, is unaffected. Application of either sodium pyruvate or nicotinamide with the insulin has very little protective action and larger doses of both these substances potentiate the inhibitory action of insulin. Diphosphopyridine nucleotide in the oxidized form given with the insulin provides complete protection, but the reduced form of DPN has no such effect. They suggested that the heart, in contrast to the brain, can if necessary survive for a period of time under anaerobic conditions but, where possible, it makes full use of aerobic metabolic pathways. The complete protection afforded by DPN but not by

DPNH implied that the site of action of insulin lies in the re-oxidation of the pyridine nucleotides, i.e. in a hydrogen transport system.

The preparation of cell suspensions by treatment of chick embryo hearts with collagenase at various stages of development was described by Guidotti *et al.,* (1969). Measurements of oxygen consumption, incorporation of labeled leucine into protein and accumulation of labeled α-amino isobutyric acid against a concentration gradient indicated a long-lasting viability of the isolated heart cells in vitro; a satisfactory preservation of sub-cellular structures, including plasma meiibrane, was assessed by electron microscopic examination. The rate of α-amino isobutyric acid accumulation by cardiac cells isolated from hearts at different stages of embryological development decreased with aging; insulin stimulated the intracellular accumulation of this amino acid analogue.

Insulin increased the uptake by isolated heart cells of several 14C-labelled naturally occurring amino acids; however, the fraction of amino acid taken up by the cells that was recovered free intracellularly, and therefore the concentration ratio (between intracellular water and medium), was enhanced by the hormone only with glycine, proline, serine, threonine, histidine and inethionine. When isolated heart cells were incubated in the presence of a mixture of labeled amino acids, the addition of insulin increased the disappearance of radioactivity from the medium. The general pattern of amino acid transport (in the absence and in the presence of insulin) in isolated cardiac cells was similar to that found in intact hearts.

The effect of treatment of chick embryos during the first day of incubation with a number of sugars was described in a previous study by Hughes *et al*., (1974). To some embryos solid sugars were applied in opened eggs; for others the substances were injected in solution. By both methods, all sugars tested were found to be teratogenic, but no apparent general differences between mono-, di-, and trisaccharides were found.

Nor were there any correlations between those which can be metabolised at these stages and their teratogenicity. The range of defects produced is similar to those found when embryos of this age are treated with other substances. In embryos treated with [14C] sucrose, some of the label is retained within the tissues in a bound, insoluble form.

To study the survival rates, embryo weights, blood sugars, liver and tibiotarsus glycogen histochemistry, and pancreatic alpha and beta tissue histogenesis glucagon concentrations ranging from 116 to 3000/tg/01 ml diluent were injected by Anderson and Gibson (1981) into the yolk of chick embryos on incubation days 8, 10, and 12.

Studies were undertaken by them during the 9- to 16-day incubation period. Glucagon dosages of 37-5 and 1500 /*g/01 ml diluent gave the best survival rates. Glucagon caused an increase in embryo weight, increased liver glycogen storage, a chondrocyte glycogen storage pattern which correlated with blood sugar levels, an increase in pancreatic beta tissue and a decrease in pancreatic alpha tissue. Studies of blood sugars following glucagon treatment showed that most concentrations caused an initial (first 16h) hyperglycemia.

Following this, two general patterns were exhibited: (1) the lower glucagon concentrations caused hypoglycemia after about 24 h, and (2) the higher concentrations caused a more prolonged hyperglycemia when administered on incubation day 10 but caused hypoglycaemia when administered on days 8 and 12. Interpretation of these results is based on the contribution of three factors to the expression and duration of the glucagon effect: (1) concentration of glucagon administered, (2) insulin secretion, and (3) levels of glycogen storage at the incubation stage of administration.

The teratogenic effect of insulin in early vertebrate embryos is controversial and the mechanisms involved are unknown. The effects of pharmacological doses of insulin in chick embryos during the period of differentiation were studied by dePablo *et al.,* (1985). They compared the effects of insulin with two proinsulins, desoctapeptide-insulin and multiplication-stimulating activity, peptides that have little insulin-like metabolic activity while they have significant growth effects. Chick embryos at 46 h of development were injected with the different peptides. At 96 h the mortality and abnormal growth elicited by the peptides were dose-dependent. Considering the indices of lethality (LD50) and affected embryos (ED50) as 100% for insulin, proinsulin was 59-66% as potent as insulin, desoctapeptide-insulin 2-6% and

multiplication-stimulating activity 176-204%. In the surviving embryos, insulin (5 micrograms, decreased DNA, RNA and protein content by 49%, 40% and 48% respectively compared with controls.

The effects of insulin were not corrected by simultaneous glucose injections. These data suggested that insulin, at pharmacological doses, interferes with embryo development through a non-metabolic pathway, probably via a growth-type receptor.

Biological effects of insulin-like growth factors (IGF) I and II on primary cultures of chick embryo liver cells have been investigated by Widmer *et al.*, (1985) and compared 1) with the biological effect of insulin and 2) with competitive binding of the three hormones to their respective binding sites. IGF I and II stimulate the incorporation of D[U-14C]-glucose into liver cell glycogen in a time- and dose-dependent manner, but with a 5-10-fold lower potency than insulin. Both IGFs also lead to enhanced incorporation of 5-[3H] uridine and L[U-14C]valine into trichloroacetic acid (TCA) insoluble material and to activation of ornithine decarboxylase activity. Their potency in stimulating RNA synthesis and ornithine decarboxylase activity is comparable to that of insulin. Protein synthesis is maximally stimulated at 3 nM by all three hormones. In the competitive binding studies, IGF I and II are 10-fold less potent than insulin in competing for [125I] insulin binding, but 100-fold more potent than insulin in competing for [125I] IGF I or II binding. These studies show that IGF I and II stimulate the same metabolic indices as insulin in the chick embryo liver. By comparing these biological effects with competitive binding data it appears that IGFs act on glucose metabolism in the chick embryo liver via the insulin receptor, whereas stimulation of growth indices by IGFs and insulin appears to be mediated by their own specific receptors.

Hepatocytes were isolated from 17-day-old chick embryos by Onoagbe (1994) to study the effect of insulin along with steroid hormones. In the isolated hepatocytes, tyrosine aminotransferase (TAT) activity was not altered by insulin or steroid hormones, when combined or given alone. However, TAT was stimulated by glucocorticoids in mixed hepatocyte and fibroblast cocultures; hormonal effects were not observed in pure hepatocyte cultures. Administration of hydrocortisone, dexamethasone, or triamcinolone (singly) to chick embryos *in ovo* resulted in an increase in hepatic TAT activity; insulin injection was without effect on the enzyme. The stimulation of TAT activity evoked by glucocorticosteroids *in ovo* was abolished by injection of cycloheximide or cordycepin. These observations contrast with reported glucocorticosteroid actions on TAT activity in fetal rat liver. It would appear that the differential regulatory effects on hepatic TAT by glucocorticosteroids are imposed by the distinct nutrient environments of chick embryos and fetal rats.

It was postulated by Dealy and Kosher (1995) that the apical ectodermal ridge (AER) promotes the proliferation and directed outgrowth of the subridge mesodermal cells of the developing limb bud, while suppressing their differentiation. Insulin-like growth factor-I (IGF-I) and its receptor are expressed by the subridge mesodermal cells of the chick limb bud growing out in response to the AER, and specific insulin receptors are present in the limb bud during its outgrowth. To study the possible roles of IGF-I and insulin in limb outgrowth, the authors examined their effects on the morphogenesis of posterior and anterior portions of the distal tip of stage 25 embryonic chick wing buds subjected to organ culture in serum-free medium in the presence or absence of the AER and limb ectoderm. The distal mesoderm of control posterior explants lacking an AER or all limb ectoderm ceases expressing IGF-I mRNA, exhibits little or no proliferation, fails to undergo outgrowth, and rapidly differentiates. Exogenous IGF-I and insulin promote the outgrowth and proliferation and suppress the differentiation of distal mesodermal cells in posterior explants lacking an AER or limb ectoderm, thus mimicking at least to some extent the outgrowth promoting and antidifferentiative effects normally elicited on the subridge mesoderm by the AER. Furthermore, IGF-I and insulin-treated posterior explants exhibit high IGF-I mRNA expression, indicating that IGF-I and insulin maintain the expression of endogenous IGF-I by the subridge mesoderm.

They found IGF-I and insulin can affect the morphology and activity of the AER. When the posterior portion of the wing bud tip is cultured with the AER intact in control medium, on day *4-5* the AER flattens, ceases expressing high amounts of the AER-characteristic homeobox-containing gene *MsxZ*, and concomitantly an elongated cartilaginous element differentiates in the subridge mesoderm. In contrast, in the presence of exogenous IGF-I or insulin the AER of such explants does not flatten, continues

expressing high amounts of *MsxZ,* and the subridge mesoderm remains undifferentiated and proliferative. Thus, exogenous IGF-I and insulin maintain the thickness of the AER and sustain its expression of *Msx2,* while sustaining the anti-differentiative effect normally elicited on the subridge mesoderm by a thickened functional AER.

Notably, they also found that exogenous IGF-I and insulin induce the formation of a thickened ridge-like structure that expresses high amounts of *Msx2* from the normally thin distal anterior ectoderm of the limb bud, while promoting dramatic outgrowth and proliferation of the anterior mesoderm, which normally undergoes little outgrowth or proliferation.

These studies provide support for the hypothesis that endogenous IGF-I and insulin may be involved in promoting the outgrowth and suppressing the differentiation of limb mesoderm in response to the AER, and also in regulating and/or maintaining at least some aspects of AER activity.

IGF-I, insulin, FGF-2 and FGF-4 have been implicated in the reciprocal interactions between the apical ectodermal ridge (AER) and underlying mesoderm required for outgrowth and patterning of the developing limb. To study the roles of these growth factors in limb outgrowth, same workers (Dealy and Kosher, 1996) examined their effects on the in vitro morphogenesis of limb buds of the amelic mutant chick embryos *wingless* (*wl*) and *limbless* (*ll*). Limb buds of *wl* and *ll* mutant embryos form at the proper time in development, but fail to undergo further outgrowth and subsequently degenerate.

Wl and *ll* limb buds lack thickened AERs capable of promoting limb outgrowth, and their thin apical ectoderms fail to express the homeobox-containing gene *Msx-2*, which is highly expressed by normal AERs and has been implicated in regulating AER activity. Here we report that exogenous IGF-I and insulin, and, to a lesser extent, FGF-2 and FGF-4 induce the proliferation and directed outgrowth of explanted *wl* and *ll* mutant limb buds, which in vitro, like in vivo, normally fail to undergo outgrowth and degenerate. IGF-I and insulin, but not FGFs, also cause the thin apical ectoderms of *wl* and *ll* limb buds to thicken and form structures that grossly resemble normal AERs and, moreover, induce high level expression of *Msx-2* in these thickened AER-like structures. Neither IGF-I, insulin nor FGFs induce expression of the homeobox-containing gene *Msx-1* in the subapical mesoderm of *wl* or *ll* limb buds, although FGFs, but not IGF-I or insulin, maintain *Msx-1* expression in normal (non-mutant) limb bud explants lacking an AER.

Research in the Twentieth Century

The cardiovascular effects of volatile anesthetics in prenatal hearts were not well investigated till Wojtczak (2000) conducted a study to determine whether the embryonic cardiovascular system is sensitive to an exposure to clinically relevant, equipotent concentrations of halothane and isoflurane. Stage 24 (4-day-old) chick embryos were exposed to 0.09 and 0.16 mM of halothane and 0.17 and 0.29mMof isoflurane.

Dorsal aortic blood velocity was measured with a pulsed- Doppler velocity meter. Halothane, but not isoflurane, caused a significant decrease in cardiac stroke volume and maximum acceleration of blood (dV/dtmax), an index of cardiac performance. This effect was reversible, and during washout, stroke volume and dV/dtmax increased above control levels. Embryonic heart rate was not affected by either drug. Chick and human embryos are similar during early stages of development; therefore, chick embryo may be a useful model to study the cardiovascular effects of anesthetics.

In the study performed by Lamošová and Zeman (2005), they used the primary cultures of chick embryonic muscle and liver cells as a model for potential mutual combination effects of leptin and insulin, respectively. The influence of both hormones on the proliferation and protein synthesis was dose-dependent and related to the age of embryos from which the cells were isolated. Leptin (10 and 100 ng/well) increased the proliferation (estimated by DNA content and incorporation of labeled thymidine into DNA) and protein synthesis (determined by incorporation of labeled leucine into proteins) of muscle cells. The effect of leptin and insulin in muscle cells was similar. In younger embryo (11-day-old) the lower dose of leptin was more effective than the higher one compared to the insulin effect. Mutual effects of leptin and insulin were neither additive nor synergistic and were equivalent to the effects of individual

hormones. In hepatocytes the influence of leptin was dependent on the age at which the cells were isolated (11- and 19-day-old embryos). The presence of insulin neither potentiated nor inhibited the effect of leptin.

Hypoglycaemia was induced in primigravida Wistar albino rats during early pregnancy and its effect was studied by Singh *et al.,* (2002) on the newborn. On d 9.5 of gestation, 1.6 mU of Actrapid human insulin per gram body weight was infused intraperitoneally to two groups of animals. One group was allowed to become hypoglycaemic for one hour and subsequently restored to euglycaemic level while the other group was maintained at euglycaemic levels throughout the experiment and afterwards by intraperitoneal infusion of exogenous dextrose.

The same experiment was repeated on d 10.5 with another set of animals. All the animals were allowed to deliver normally. Significant decrease in the mean body weight and crown rump length was observed among the neonates from hypoglycaemic mothers. Gross and histological examination showed that 4.61% neonate of hypoglycaemic animals induced on d 9.5 and 7.93% on d 10.5 had a patent foramen ovale. In one neonate of d 10.5, there was microphthalmia, aphakia and failure to develop neural layer of retina, and the optic nerve anlage was occupied by glial cells covered by connective tissue. This study revealed that brief maternal hypoglycaemia during early pregnancy induced teratogenic effects on various unreported systems too, in the newborn albino rats and its implications in the human beings need to be taken into account.

A study was conducted by Patwardhan *et al.,* (2004) pertaining to effect of insulin on developing chick embryo. In this study they showed that insulin accelerates early morphogenesis in gastrulating chick embryo explants cultured in vitro. Comparison between length of body axis of control and treated embryos clearly brings out the significant acceleration of development by excess insulin (0.175 to 17.5 nM).

In embryos treated with 87.5 and 175 nM insulin, a high occurrence of abnormalities is observed.

To evaluate the effects of insulin-like growth factor-1 (IGF-1) on the histological development of the thoracic part of the m. longus colli dorsalis (m. spinalis thoracis) in Japanese quail embryos, a study was performed by Deprem and Gülmez (2007). A single in ovo dose of recombinant human IGF-1 (rhIGF-1) (100 ng embryo–1) was administered through the blunt end of eggs via a single hole made with a dental drill bit, without penetrating the chorioallantoic membrane. For histological evaluation, the embryos were collected daily from days 7 to 16 of embryonic development (E). In ovo administration of rhIGF-1 increased the diameter of muscle fibers on E7, 9, 10, 11, and 13. Additionally, in ovo rhIGF-1 also increased the number of muscle fibers ($P < 0.001$). It was concluded that rhIGF-1 accelerated skeletal muscle development in the quail embryos.

The direct acute effects of food mycotoxin deoxynivalenol (vomitoxin, DON) produced by *Fusarium graminearum* and *F. culmorum* on the chick immune-related embryo tissues such as embryonic liver and spleen were investigated by Moon *et al.,* (2007). Direct DON administration into the embryonic eggs caused toxin accumulation in liver in a time-dependent manner. Electron microscopic observation showed a notable accumulation of fat droplet in the liver tissue and the re-exposed hatched chicken showed more distinguishing enlarged fat globules, so-called fatty cysts like human steatosis. Regarding effects of deoxynivalenol on the chick embryonic spleen, fatty change was also observed in splenocytes. Functionally, mitogen-stimulated cellular and humoral lympho-proliferations were suppressed in the DON-treated embryo. Conclusively, acute direct exposure to deoxynivalenol in the chick embryo caused toxic histological alterations in the liver and spleen and suppressed *in vitro* lymphoblastogenesis**.**

As there was a dearth of published data as far as lethal doses of insulin is concerned, a recent study was conducted by Bokariya and Umarji (2012) to estimate the same and median lethal dose obtained was 3 IU. The Lowest Observable Adverse Effect Level (LOAEL) came out to be 2 IU.

This study also enlightens regarding the abuse and misuse of medication on poultry farms without consultation with qualified veterinarians and the data regarding chick mortality provided in the study seems to be of utility for the training of farmers.

CONCLUSION

The role of insulin in developing chick embryo is contentious and warrants further studies with larger number of animal models and extensive histological & histochemical analysis. Keeping in mind the research conducted so far in this regard, we suggest that the use of insulin or any other drug without proper guidance in poultry should be avoided. Further, irrational use of Insulin in human beings should also be avoided as we know that insulin is one of the commonest drugs used by gravid females throughout the world so it should be meticulously administered to them.

REFERENCES

Alzira MFS and López JA (2004). Insulin or insulin-like studies on unicellular organisms: a review. *Brazilian Archives of Biology and Technology* **47**(6).

Anderson WA and Gibson MA (1981). Some effects of glucagon on chick embryo Development. *Journal of Embryology and Experimental Morphology* **62** 95-107

Barron P and Mckenzie J (1962). The Inhibitory Action of Insulin in the Early Chick Embryo. *Journal of Embryology and Experimental Morphology* **10**(1) 88-98.

Bassas L, De PF, Lesniak MA and Roth J (1987). The insulin receptors of chick embryo show tissue-specific structural differences which parallel those of the insulin-like growth factor I receptors. *Endocrinology* **121** 1468-1476

Bokariya P and Umarji BN (2012). The Estimation of Lethal doses for Acyclovir, Insulin and Ondansetron on developing chick embryos. *IOSR Journal of Pharmacy* **2**(4) 29-31.

De PF, Roth J, Hernandez E and Pruss RM (1982). Insulin is present in chicken eggs and early chick embryos. *Endocrinology* **111** 1909- 1916.

Dealy CN and Kosher RA (1995). Studies on Insulin-Like Growth Factor-I and Insulin in Chick Limb Morphogenesis. *Developmental Dynamics* **202** 67-79.

Dealy CN and Kosher RA (1996). IGF-I, insulin and FGFs induce outgrowth of the limb buds of amelic mutant chick embryos. *Development* **122** 1323-1330.

dePablo F, Hernández E, Collía F and Gómez JA (1985). Untoward effects of pharmacological doses of insulin in early chick embryos: through which receptor are they mediated? *Diabetologia* **28**(5) 308-13.

Deprem T and Gülmez N (2007). The Effects of in Ovo Insulin-Like Growth Factor-1 on Embryonic Development of Musculus Longus Colli Dorsalis in Japanese Quail. *Turkish Journal of Veterinary and Animal Sciences* **31**(4) 233-240.

Duraiswami PK (1950). Insulin-induced abnormalities in the skeletal system of chick embryos. *British Medical Journal* **2** 384-90.

Girbau M, Gonzalez GPR, Bassas L and De PF (1992). Insulin receptors and insulin-like growth factor I receptors in embryos from gastrula until organogenesis. *Molecular and Cellular Endocrinology* **90** 69-75.

Guidotti GG, Luneburg B and Borghetti AF (1969). Amino Acid Uptake in Isolated Chick Embryo Heart Cells. Effect of insulin. *Biochemistry Journal* **114** 97-105.

Hughes AF, Freeman RB and Fadem T (1974). The teratogenic effects of sugars on the chick embryo. *Journal of Embryology and Experimental Morphology* **32**(3) 661-674.

Lamošová D and Zeman M.(2001). Effect of Leptin and Insulin on Chick Embryonic Muscle Cells and Hepatocytes. *Physiological Research* **50**: 183-189

Landauer W (1945). Rumplessness of chicken embryos produced by the injection of insulin and other chemicals. *Journal of Experimental Zoology* **98** 65-77.

Landauer W (1947). Insulin-induced abnormalities of the beak, extremities and eyes in chickens. *Journal of Experimental Zoology* **105** 145.

Le RD, Shiloach J, Heffron R, Rubinovitz C, Tanenbaum R and Roth J (1985). Insulin-related material in microbes: similarities and differences from mammalian insulins. *Canadian Journal* of *Biochemistry* and *Cell Biology* **63** 839-849.

Moon Y, Kim HK, Suh H and Chung DH (2007). Toxic Alterations in Chick Embryonic Liver and Spleen by Acute Exposure to *Fusarium*-Producing Mycotoxin Deoxynivalenol. *Biological & Pharmaceutical Bulletin* **30**(9) 1808—1812.

Onoagbe IO (1994). Effects of glucocorticosteroids and insulin on tyrosine aminotransferase activity in isolated chick embryo hepatocytes and in intact embryos *in Ovo*. *Archives of Biochemistry and Biophysics* **309**(1) 58–61.

Patwardhan V, Gokhale M and Ghaskadbi S (2004). Acceleration of early chick embryo morphogenesis by insulin is associated with altered expression of embryonic genes. *International Journal of Developmental Biology* **48**(4) 319-26.

Singh TN, Singh MS and Singh LC (2002). Teratogenic Effect of Maternal Hypoglycaemia: A Study on Newborn Albino Rats. *Journal of the Anatomical Society of India* **51**(2) 216-219.

Widmer U, Schmid C, Zapf J and Froesch ER (1985). Effects of insulin-like growth factors on chick embryo hepatocytes. *Acta Endocrinologica* (Copenh) **108**(2) 237-44.

Wojtczak JA (2000). The hemodynamic effects of halothane and isoflurane in chick embryo. *Anesthesia and Analgesia* **90** 1331–5.