Teratogenic effects of insulin: An experimental study on developing chick embryo

Pradeep Bokariya, Ruchi Kothari, Vijay K. Gujar, M. R. Shende

ABSTRACT

Objective: The objective was to observe the effect of insulin on chick embryos with reference to their growth and developmental defects.

Materials and Methods: An experimental study was performed to assess any abnormal growth pattern caused by insulin. For this, two batches of 100 fertilized eggs were utilized. One batch of 50 was used as a control group and other as an experimental group. Insulin (2 IU) was injected on day 2 of incubation.

Chicken eggs were dissected out on day 19 of incubation and were carefully observed for any congenital abnormalities. The embryos thus dissected out were subjected to measurement of crown-rump length (CRL), changes in weight of egg, volume of embryos were compared in two groups. The embryos were also examined for any congenital anomalies.

Results: No major malformations were observed. Decrease in weight and CRLs was lower in the experimental group as compared to their control counterparts. Values for volume of the embryo were similar in two groups.

Conclusion: No obvious teratogenic effects are observed with insulin in the dose use for the study.

KEY WORDS: Chick embryo, insulin, teratogen

Introduction

Insulin is the mainstay of treatment in all patients with type I diabetes mellitus (DM) and many patients with type II DM. It is one of the most common drugs administered to the gravid women.

Chemically, insulin is a peptide hormone, produced by beta cells in the pancreas and is central to regulating carbohydrate and fat metabolism in the body. Cells in the skeletal muscles, and fat tissue to absorb and utilize glucose from the blood, in the presence of insulin.

Insulin is an old protein that may have originated more than 1 billion years ago.[1] The molecular origins of insulin go at least as far back as the simplest unicellular eukaryotes.[2] Insulin[3] and receptors for insulin[4,5] have been shown to be present in developing chick embryo too.

The role of this drug in early vertebrate embryos is controversial in many of the previous studies and the mechanisms involved are unknown. Some authors[6] have demonstrated the inhibitory action of insulin in the early chick embryo while others[7,8] reported insulin to be nonteratogenic in different experimental animals including the rat. In view of above facts, we planned a study to ascertain the effect of insulin on developing chick embryo.

Materials and Methods

Before the commencement of study, permission from Institutional Animal Ethics Committee was obtained. Easy availability of fertile hen (Gallus domesticus) eggs, low maintenance cost, and easy reproducibility of the experimental conditions are some of the distinct advantages, of this experimental design.

Chick embryo in its early stage is flat, discoid multicellular structure positioned on the yolk at the animal pole of the yellow of the egg which is itself enclosed in a vitelline membrane that is wrapped in sheets of thick albumen and bathed in thin albumin.

A total of 100 fertilized chick eggs were used for the study. Fifty were allotted to the control group and remaining to the experimental group. A set of 10 (5 of the control group and 5 from the experimental group) was incubated simultaneously...
Bokariya, et al.: Effect of insulin on chick embryo

so as to avoid overcrowding of eggs in the incubator. This also nullifies the environmental effect, if any as both groups were incubated simultaneously. The controls were injected with distilled water, and experimental group was injected with 2 IU of rapid-acting insulin on 2nd day of incubation.[6] The parameters studied were mortality, decrease in weight of the egg, crown-rump length (CRL) and volume of the embryo. The data thus obtained were compared statistically.

Embryos were examined for any gross malformations with respect to

- Abnormalities of body and head
- Deformities of limb
- Skeletal deformities (like anencephaly)
- Abnormalities of beak
- Abnormalities like deficient abdominal wall and herniated viscera
- Curvature of the embryo.

Eggs were intermittently checked for mortality using the candle method, and dead embryos were removed. Initial weight of the egg was measured using digital weighing machine on day 1, and final weight was observed on day 19, the difference of two weights were computed as a decrease in weight of the embryo. CRL was measured for each embryo by passing a thread from the root of beak along the back to the tip of coccyx and then measuring the length of thread. Volume of embryos was calculated by using the principle of fluid displacement method. Statistical analysis was done using statistical software EPI Info 6.0 developed by Centers for Disease Control,Atlanta, Georgia (USA) and the World Health Organization.

Results

The percentage of mortality in both groups were compared. The t-test was applied and value of $P < 0.05$ was considered as statistically significant. The mean ± standard deviation values of decrease in weight, CRL, and embryo volume are shown in Table 1. The comparative values for decrease in weight, CRL, and volume are shown in Figures 1-3. The mortality in the experimental group (16%) was not significant as compared to control group (12%). Utmost precautions were taken in measurements though we have observed weight gain in eggs in two cases of the experimental group.

Decrease in weight of the egg which indirectly implies the growth of the embryo was lower in the experimental group, but the results were not statistically significant ($P = 0.212$). CRL was statistically lower in the experimental group ($P = 0.000$). Volume of embryo did not show any statistical difference ($P = 0.11$). No significant malformations (mentioned above) were observed in either of the groups.

Discussion

The basis of drug dose selection in our study was our own previous study.[8] The lowest adverse effect was observed with 2 IU of insulin.

The mortality in both groups is higher but insignificant statistically. The rumplessness was observed in chicks by injecting insulin into the yolk sac at 24 h of incubation.[9] Same authors have observed abnormalities of the beak, extremities, and eyes after administering it at 5–6 days of incubation.[10] Another study observed abnormalities of the beak, eyes, and limbs with insulin treatment of the chick embryo.[11] Insulin was reported to be nonteratogenic in different experimental animals including rats.[7,12]

We dissected out chicks on 19th day, and this may be one of the reasons that we have observed no major malformations. Had there been any congenital malformations, the embryo might have not survived since developmental defects are usually associated with mortality.

Good hatchability is dependent on meeting all crucial incubation parameters and conditions. One of these important parameters is weight loss. In general, eggs should lose 11–13% of initial weight during the first 18 days of incubation. Weight loss in hatching eggs is caused by the regular evaporation of water from the eggs and inseparably

| Parameter | Control | Experimental | P |
|-----------|---------|--------------|---|
| Mortality | 12%     | 16%          | Not significant |
| Decrease in weight (g) | 3.92±0.92 | 3.81±1.12 | 0.212 ($P>0.05$) |
| CRL (cm)  | 5.93±0.36 | 5.59±0.40 | 0.000 ($P<0.05$) |
| Volume of embryo (ml) | 5.67±0.62 | 5.51±0.61 | 0.11 ($P>0.05$) |

Student’s t-test was applied and $P<0.05$ was considered as statistically significant.

CRL=Crown-rump length

Figure 1: Effect of insulin on decrease in weights in control and experimental groups of developing chick embryos

Figure 2: Effect of insulin on crown-rump lengths in control and experimental groups of developing chick embryos

Table 1: An evaluation of possible teratogenic effects of insulin on developing chick embryo
linked to achieving optimum embryonic development during incubation.

The weight loss from the egg is essential for the formation of the air cell and at the same time, the evaporation of water from the egg facilitates optimized water and mineral balances in the different embryonic compartments formed during embryonic development.

As soon as internal egg temperature rises, evaporation through the shell and the transport of water from the albumen to sub-embryonic cavity increase. The reasons of weight gain in two cases are beyond our knowledge and an extensive literature search for this observation did not yield any explanation.

The results in our study contradict with previous studies where teratogenicity is observed with insulin. Insulin has found to be a safe drug in this study. However, a note of caution is yet warranted against the irrational use of insulin by pregnant women until similar reports are obtained from larger studies with a bigger sample size.

### Conclusion

Though similar studies have been conducted by various researchers and findings are contradictory. Insulin (2 IU) has been found to be a safe drug in developing chick embryos in this study.

### References

1. Alzira MF, López JA. Insulin or insulin-like studies on unicellular organisms: A review. Braz Arch Biol Technol 2004;47:973-81.
2. LeRoith D, Shiloach J, Heffron R, Rubinovitz C, Tanenbaum R, Roth J. Insulin-related material in microbes: Similarities and differences from mammalian insulins. Can J Biochem Cell Biol 1985;63:839-49.
3. De Pablo F, Roth J, Hernandez E, Pruss RM. Insulin is present in chicken eggs and early chick embryos. Endocrinology 1982;111:1909-16.
4. Bassas L, de Pablo F, Lesniak MA, Roth J. The insulin receptors of chick embryo show tissue-specific structural differences which parallel those of the insulin-like growth factor I receptors. Endocrinology 1987;121:1468-76.
5. Girbau M, González-Guerrero PR, Bassas L, de Pablo F. Insulin receptors and insulin-like growth factor I receptors in embryos from gastrula until organogenesis. Mol Cell Endocrinol 1992;90:69-75.
6. Barron P, McKenzie J. The inhibitory action of insulin in the early chick embryo. J Embryol Exp Morphol 1962;10:88-98.
7. Sadler TW, Horton WE Jr. Effects of maternal diabetes on early embryogenesis. The role of insulin and insulin therapy. Diabetes 1983;32:1070-4.
8. Bokariya P, Umarji BN. The estimation of lethal doses for acyclovir, insulin and ondansetron on developing chick embryos. IOSR Journal of Pharmacy 2012;2:29-31.
9. Landauer W. Rumpleness of chicken embryos produced by the injection of insulin and other chemicals. Exp Zool 1945;98:65-77.
10. Landauer W. Insulin-induced abnormalities of beak, extremities and eyes in chickens. J Exp Zool 1947;105:145-72.
11. Duraiswami PK. Insulin-induced abnormalities in the skeletal system of chick embryos. Br Med J 1950;2:384-90.
12. Muhammad M, Muhammad LU, Ambai AG, Mani AU. A survey of early chick mortality on small-scale poultry farms in Jos, central Nigeria. Int J Poult Sci 2010;9:446-9.

Cite this article as: Bokariya P, Kothari R, Gujar VK, Shende MR. Teratogenic effects of insulin: An experimental study on developing chick embryo. Indian J Pharmacol 2015;47:212-4.

Source of Support: Nil. Conflict of Interest: No.