The Effect of Prenatal Administration of Retinoic Acid on the Survivability and Growth of Chick Embryos.

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ABSTRACT

OBJECTIVE: To determine the effect of retinoic acid on the survivability and growth of chick embryos.
STUDY DESIGN: An experimental study.
PLACE AND DURATION: Department of Anatomy, Islamabad Regional Centre of College of Physicians and Surgeons, Pakistan from 15th of February, 2009 to 15th of February, 2010.
METHODOLOGY: Chicken eggs were divided into an experimental group A and its control group C. Group A was administered 0.3µg of retinoic acid (R2625, Sigma) via yolk sac. The control group was sham injected with saline. The chicks belonging to the experimental embryonic group A1 and its matched control C1 were dissected out at 15th embryonic day and the experimental hatched group A2 and its matched control C2 were dissected out at hatching or day 22 (which ever was earlier). Control and experimental groups were compared for survivability and growth retardation.
RESULTS: At the embryonic stage, in the retinoic acid exposed group A1, 83.3% of chicks were alive while 16.6% were dead. Upon hatching, 80% of the chicks belonging to the experimental group A2 survived, while 20% died. All of the chicks belonging to the control groups were alive. The difference of survivability between the experimental and the control groups was significant at the embryonic stage (p=0.026) as well as at the hatching stage (p=0.011). The mean length and weight of the retinoic acid exposed groups was not significantly different from the control.
CONCLUSION: This study shows that retinoic acid decreases the survivability of chick embryo. Implicating mechanisms include adverse effects on implantation, inner cell mass population and increased permeability of the fetal membranes.
KEY WORDS: Retinoic acid, Chick embryo, Survivability, Growth retardation, Hatching.

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INTRODUCTION

Vitamin A is a known teratogen[1]. Exposure to retinoic acid, a potent form of vitamin A, results in a characteristic pattern of malformations known as "retinoic acid embryopathy".[2] Retinoic acid teratogenicity predominantly manifests itself as defective development of branchial arch derivatives [3];however, some studies have demonstrated its potential to retard embryonic growth and cause fetal death.[4,5] There has been considerable interest in retinoids due to their great promise in stem cell research, cancer therapy and regenerative medicine.[6,7] The discovery of the wide therapeutic applications of retinoic acid has renewed interest in its possible embryotoxic implications. As the pharmacological use of retinoic acid expands, the risk of teratogenicity rises. A number of studies have raised concerns for inducing defects in offspring of women of a child bearing age.[8,9] Despite continued warnings, fetal exposure to retinoids remains high.[10] Although branchial arch teratogenic manifestations of prenatal retinoic acid exposure are well described, [2,3] data regarding its detrimental effects on the growth and survival is comparatively lacking. Keeping this in view, this study was designed to study the effects of prenatal administration of retinoic acid on these parameters. Since body weight and length are effective indicators of growth[11] they could be used to determine any detrimental effects, such as growth retardation. Avian embryos are considered ideal models to study the effects of vitamin A on early development.[12] Additionally there is ample evidence that somite differentiation in birds is similar to that of mammals.[13] Thus any effects on the survivability or growth of chicks may be applicable to humans. Keeping in view the aforementioned this study was designed to determine the effect of retinoic acid on the survivability and growth of chick embryos.

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METHODOLOGY

This was an experimental study which was carried out at the Department of Anatomy, Regional Centre, College of Physicians and Surgeons, Islamabad, from 15th of February, 2009 to 15th of February 2010. To examine the effects of retinoic acid teratogenicity on the survivability and growth of chick embryo, a total of 120 fertilized, Egyptian Fayoumi chicken eggs were purchased. Eggs which were cracked, or more than 24 hours old, were excluded from the study. Simple random tables were used to divide the eggs into an experimental group A and its control group, C. Retinoic acid (Sigma-R2625) was dissolved in ethanol and then dispensed in saline. Experimental group A was injected with a 0.3µm dose of retinoic acid in 0.05ml of saline, via yolk sac.[14] The matched control group C was sham injected with saline. Depending on their day of sacrifice, the chicks in groups A and C were subdivided into subgroups A1, A2, C1, and C2 each comprising 30 eggs. Eggs in the subgroups A1 and C1 were opened on day 15 of incubation while subgroups A2 and C2 were opened at hatching or day 22 (which ever was earlier). The eggs were placed in the incubator and that day was considered as day 1 of incubation. The temperature was maintained at 38±0.5^{0}C. The relative humidity was kept between 60-70% and the eggs were rotated twice daily. On day 15 of incubation, embryos belonging to the groups A1 and C1 were dissected out of the eggs by cutting the membranes and removing the yolks. The 15 day old embryos from the subgroups A1 and C1 were dried on blotting paper and weighed. The length of the embryos was taken from the vertex, defined as the highest point between the eyeballs to the tip of the coccyx. The distance was measured by a thread placed between the vertex and following the curvature of the back reaching the coccyx. Chicks belonging to the groups A2 and C2 were weighed just after hatching. A similar method was adopted to measure their lengths as of the embryonic group. The chicks were allowed to develop and hatch on their own till the day 22 of incubation. Chicks which were not able to hatch till day 22 of incubation were assisted to hatch by dissecting them out of their shells. The number of chicks that survived was noted. The length and weight was measured by the method described previously for the embryonic group.

Data analysis: The Fisher exact test was used to compare any significant differences between the survivability of the hatched groups, A2 and C2. The unequal variances t-test was applied to compare the lengths and weights of the embryonic and the fully hatched experimental and control groups. Values of $P < 0.05$ were considered significant.

RESULTS

Out of a total of 30 embryos belonging to the retinoic acid exposed embryonic group A1, 83.3% (n=25) were dissected alive while 16.6% (n= 5) were dead and macerated. From the chicks belonging to the age matched control group C1 100% (n=30) of the chicks were dissected alive. This difference of survivability at the embryonic stage between the groups A1 and C1 was statistically significant (p=0.02). Regarding the fully hatched group 80% (n-=24) of the chicks from the experimental group A2 were hatched alive and 20% (n= 6) did not survive. From the age matched control group C2, 100% (n=30) of the chicks survived. The difference of survivability between the fully hatched experimental and the control groups A2 and C2 was significantly different (p=0.01) (Table-I). Regarding the growth parameters, the mean length of embryonic group A1 (7.312±0.003) was slightly less than the control group C1 (7.356±0.003) but the difference was not statistically significant (p=0.07). The mean length of the fully hatched experimental group A2 was 8.39±0.041 and that of the age matched control group C2 was 8.44±0.004. This difference in their lengths was not significantly different (P=0.06). Similar findings were encountered regarding the weight of the two groups. The mean weight of the embryonic group A1 was 33.266±0.028 while the

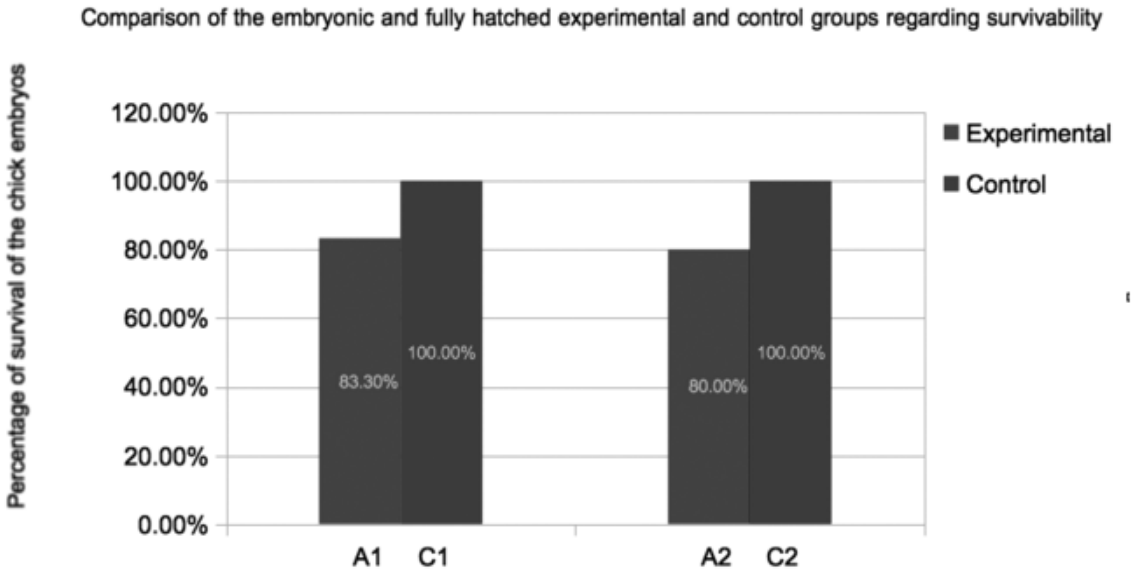

Figure – 1 : Graphic representation of difference in survivability of retinoic acid exposed experimental groups A1(n=25) and A2 (n=24) as compared to the control groups C1(n=30)and C2 (n=30)

mean weight of the embryos belonging to the control group C1 was 33.88±0.035. This difference was not significantly different (p=0.01). Similarly the difference of mean weights between the fully hatched experimental group A2 (37.6±0.026) and its matched control C2 (37.9 ±0.0185) was not statistically significant (p=0.07) (Table II).

Table I: Percentage of dead and alive embryos and chicks in retinoic exposed and control subgroups regarding survival. (n=120)

Subgroup	n	Percentage of alive embryos and chicks	Percentage of dead embryos and chicks	P-value
A1	30	83.3%	16.6%	0.0260*
C1	30	100%	0%	
A2	30	80%	20%	0.01186*
C2	30	100%	0%	

Table-II: Comparison of the length and weight of retinoic acid exposed embryonic group and fully hatched groups with their age matched controls. (n=109)

Subgroup	n	Length (cm) Mean ± SE	P-value	Weight (g) Mean ± SE	P-value
A1	25	7.312±0.003	0.07	33.266±0.028	0.10
C1	30	7.356 ±0.003		33.88±0.035	
A2	24	8.39±0.0416	0.06	37.6±0.026	0.07
C2	30	8.44 ±0.004		37.9±0.0185	

DISCUSSION

Retinoic acid is the most widely used teratogenic drug during pregnancy.[10] Maternal use of retinoic acid has been shown to affect the fetus, causing embryo-lethality and growth retardation. Studies document that exogenous retinoid administration during pregnancy has also led to substantial increase in the rate of fetal resorptions and still births.[15] Our study was undertaken to determine the effects of prenatal retinoic acid administration on the survival and growth of the chick embryo. The results of the study show that retinoic acid administration resulted in significantly decreased embryonic survival. These findings are in accordance with previous studies supplementing the embryo-lethal effects of early exogenous retinoic acid exposure. There are multiple possible mechanisms which might have led to increased embryonic deaths in our study. Firstly, retinoic acid is a morphogen that directly impacts the implantation and disrupts the germ layer specific gene activities at the stage of morula during embryonic development.[16] Retinoic acid accumulation in the stroma during implantation has been shown to inhibit the expression of three implantation essential genes, LIF, HB-EGF and CSF-1. [17] In our study interruption of developmental stage probably disrupted implantation and significantly increased rate of fetal resorptions. Moreover, in addition to disrupting the implantation, retinoic acid also induces the apoptosis of the inner cell mass of the blastocyst. In the present study, this could also have played a role in decreasing the survival. This induction of apoptosis results from the preferential up-regulation of the gene expression of retinoic acid receptors which, in turn, induce cell cycle arrest. A number of scientists have demonstrated that retinoic acid administered during the peri-implantation period can cause excessive reduction in the inner cell mass by this signalling pathway. Infact, apoptosis, due to excessive retinoic acid, has shown to reduce the normal population of inner cell mass by 70% therefore leading to decreased viability and causing a high incidence of fetal loss.[18,19] Another factor leading to decreased survivability in our study could be due to the detrimental effects of exogenous retinoic acid during the decidua formation. Normally, decidualization alters the cellular retinoic acid signalling by decreasing the expression of retinoic acid receptors. Exogenous retinoic acid can cause the failure of this normal attenuation of the retinoic acid pathway. This leads to altered decidualization. Since decidual cells are crucial for survival of the embryo, their alteration can lead to pregnancy failure and fetal loss. [20] Additionally decreased survivability in our study could be as a result of increased expression of aquaporins in the developing placenta and amniotic membrane. Endogenous retinoic acid is essential for the normally expressed aquaporins which are the channels involved in transport of water across fetal membranes. Studies have shown that exogenous administration of retinoic acid disrupts the retinoic acid signalling pathway, resulting in enhanced expression of these channels. This increases cellular permeability in the epithelial amniotic environment

causing hydremia and fetal death.[21] All the above mentioned mechanisms might have played a role in decreasing survival of the retinoic acid exposed chick embryos in the present study. No significant difference between the lengths and weights of the experimental and the control groups in this study depicts the absence of any growth retardation. This can be explained by the fact that the growth retarding effects of retinoic acid are stage and dose dependant. [22] Scientific studies document that retinoic acid retards the growth of the embryo only when administered at a specific stage of development. On the other hand retinoic acid administered before or after this stage can cause internal malformations without any deleterious effects on external growth.[22,23] The stage at which the chick embryos were exposed to retinoic acid in this study affected internal growth. However, it was probably too early to cause retardation of external growth. [22,23] Such effects, on internal rather than external development, lead to embryo-lethality without affecting the external growth of the embryo.[22]

CONCLUSION

This study shows that retinoic acid decreases the survivability of chick embryo. Implicating mechanisms include adverse effects on implantation, inner cell mass population and increased permeability of the fetal membranes.

CONTRIBUTION OF AUTHORS

Haque A: Conceived Idea, Data Collection, Designed Research Methodology, Literature Review
Data Interpretation, Manuscript Writing.
Khan MY: Conceived Idea, Manuscript final reading and approval

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