ABSTRACT

Lipids are a diverse group of compounds with many key roles enabling them to serve as forms of energy storage, structural moieties and signaling molecules. During embryonic development the early embryonic cells undergo repeated cycles of division, migration and apoptosis followed by terminal differentiation. All these processes involve generation and breakdown of transient specialized membranes by virtue of contributions from lipids. Lipid plays multiple roles during embryonic development. Deficiency of different lipids causes different diseases and disorders.

The proposed study holds the importance of identifying a profile of lipid composition in normal embryonic development and will serve as a basis for understanding the role of lipid signaling in embryonic development.

In the present study the quantitative profiles of cholesterol and phospholipids were established using highly sensitive technique of HPLC in the complete embryonic development of frog Microhyla ornata. It was found that concentration of lipids does not change significantly during complete embryonic development, except during gastrulation of Microhyla ornata. However, cholesterol and phospholipids levels vary according to the developmental stages. The levels of cholesterol and phospholipids are regulated throughout the embryonic development and amphibians employ different lipid-utilization strategies at different stages of development. This finding in the present study gave us a platform to study the regulation of cholesterol and phospholipids during embryonic development by using different biosynthesis inhibitors.

This is the first study using frog Microhyla ornata as an animal module to determine the quantitative changes in cholesterol and phospholipid contents at early and late embryonic developmental stages until hatching.

We studied the effect of the cholesterol inhibition and depletion on embryonic development using Microhyla ornata and Gallus domesticus early embryos as model organisms. It was found that unavailability of cholesterol during early embryonic development perturbed the normal lay down of AP body plan and brain development. It was also found that supplementation of cholesterol helped embryos to recover from the effect of inhibitors, when the cholesterol biosynthesis was blocked at distal end.
Histological studies further confirmed the occurrence of the development of abnormalities in the treated embryos at tissue level. The overall results clearly indicate influence of cholesterol biosynthetic inhibition on the axial structures, which is known to be under regulation of Hedgehog and Wnt protein gradient.

These findings are well in concordance with the current understanding of the cholesterol modification of hedgehog protein, inborn disorders of cholesterol deficiency and possible involvement of cholesterol signaling in the embryonic development. The finding that inhibition of cholesterol biosynthesis and depletion of cholesterol in frog and chick embryos lead to defects in the AP axis, in the development of the brain and somites indicating possible altered hedgehog and Gli signaling.

We also studied the effect of inhibition of phosphatidylcholine biosynthesis in the early gastrulating embryos of *Microhyla ornata* and *Gallus domesticus* by treating them with PC biosynthesis inhibitor ET18-O-CH$_3$.

Embryos of both the animal modules showed neural tube defects, reduced craniofacial development and growth retardation. The frog embryos clearly showed hypoplasia of brain, underdeveloped eyes, and distension of notochord and undeveloped pharynx of the embryo. The whole mount preparation of the treated chick embryos showed open brain, distended neural tube and development of the diffused somites. Histological study of the treated embryos further confirmed the development of abnormalities at the tissue level.

The inhibition of phosphatidylcholine resulted in neural tube defects, reduced craniofacial development and growth retardation in frog as well chick embryos at early stages of development. This is probably due to the decreased level of choline and its metabolites, which led to the decreased level of s-adenosylmethionine. This might have resulted in hypo-methylation and altered gene function and thus have possible effect on the neural tube closure and brain development.

**Important findings of the present study:**

This is the first study to determine the quantitative changes in cholesterol and phospholipid contents at each embryonic developmental stage. We found that the levels of cholesterol and phospholipids are regulated throughout the embryonic
development and amphibians employ different lipid-utilization strategies at different stages of development.

We also found out that inhibition of cholesterol biosynthesis and depletion of cholesterol in frog and chick embryos leads to defects in brain, axis and somites indicating possible altered hedgehog and Gli signaling.

It has also been found that inhibition of phosphatidylcholine resulted in neural tube defects, reduced craniofacial development and growth retardation in frog as well chick embryos. This is possibly because decreased choline and its metabolites leads to decreased s-adenosylmethionineresulted in hypomethylation and altered gene function and thus have possible effect on neural tube closure and brain development.