General Discussion
Embryonic development occurs by precisely regulated biochemical changes accompanying cell proliferation and morphogenesis. The necessary components required for development preexist in unfertilized egg in quantities, sufficient for the needs of the developing embryo (Mes-Hartree & Armstrong, 1976). In a wide variety of organisms; during the course of development, the yolk deposition in the egg plays a vital role. However, one of the distinguishing characteristics in vertebrates is the amount and distribution of yolk in the eggs, which may vary widely both within and among species. Yolk stored ultimately determines the embryo’s ability to survive beyond depository (Browder, et al., 1991).

A lot of research had been done to determine the lipid composition of yolk in different organisms. Whole body composition and its changes with the progressive development have been extensively investigated in fish embryos (a review by Heming & Buddington, 1988) and chick embryos (a review by Romanoff, 1967). However, none of the studies gave complete account of quantitative lipid profile in each developmental stage in the amphibian species under study. Rather, excluding some of the developmental stages; they used major stages as representative of the whole embryonic development.

In the present study we established the quantitative profile of cholesterol and phospholipids using highly sensitive technique of HPLC in the complete embryonic development of frog Microhyla ornata. From the results of the present study, it was evident that concentration of lipids does not change significantly, except during gastrulation of Microhyla ornata as reported in most of the similar type of studies. However, on the contrary cholesterol and phospholipids levels vary according to the developmental stages. It was also evident from the results that amphibians employ different lipid-utilization strategies at different stages of embryonic development.

Cholesterol is an essential lipid, found in all the mammalian cells as a major lipid component of the cell membrane. In addition to its role as an structural component of the cell membranes, it also acts as a precursor molecule for the sterol based compounds including bile acids, oxysterols, neurosteroids, glucocorticoids, mineralocorticoids and sex steroids such as estrogen and testosterone (Correa-Cerro and Porter, 2005). Cholesterol is emerging as an important molecule in many aspects of cell biology and developmental biology; including related genetic disorders arising out of defects in its biosynthetic enzymes. Cholesterol is a remarkably versatile
molecule (Gibbons, 1982). It determines the biophysical properties of the cellular membranes (Yeagle, 1985), serves as precursor for steroid hormones and regulates the function of signaling molecules like hedgehog (Mann and Beachy, 2000). Given this multitude of functions, it is not surprising that acquired or genetic defects in cholesterol metabolism cause severe diseases (Farese and Herz, 1998; Moebius et al., 2000; Roux et al., 2000; Kelly and Herman, 2001; Nwokoro et al., 2001) including arteriosclerosis (Sacks, 1998; McNamara, 2000) Smith–Lemli–Opitz syndrome (Opitz et al., 2002) and Niemann–Pick type C disease (Vanier, 1999).

In the present study the effect of cholesterol inhibition was studied using early developmental stages of frog and chick embryos. It was also found out that inhibition of cholesterol biosynthesis and depletion of cholesterol in frog and chick embryos lead to defects in the length of AP axis of the embryos, axis associated structures, somites and brain indicating possible altered hedgehog and Gli signaling and the effects thereof.

Why the unimpaired supply of cholesterol to the developing embryo is so important and why does it affect primarily the development of the central nervous system? Although it is not possible to give a complete answer to these questions at present, a possible picture is starting to emerge. The central nervous system undergoes an enormous cellular expansion at this critical time of embryonic development. Cholesterol is a crucial component of the plasma membrane of all cells, and the proper ratio of cholesterol and phospholipids determines their physiochemical characteristics.

Thus, it is conceivable that during evolution, a checkpoint was established that determines whether sufficient cholesterol is available to proceed with the expansion of the developing brain or whether cell division should slow down. The recent discovery of the cholesterol-mediated activation of the hedgehog protein family of signaling molecules (Porter et al., 1996) and demonstration that sonic hedgehog is required for the development of the mouse brain (Chiang et al., 1996) support this hypothesis and suggest that hedgehog protein may be involved in the control of this checkpoint (Herz and Farese Jr., 1999).

Phosphatidylcholine is implicated in cell proliferation through activation of intranuclear phosphatidylcholine dependent phospholipase C and diacylglycerol
production. An inhibition of phosphatidylcholine synthesis is responsible for initiation of apoptosis (Albi & Viola Magni, 2004). Many agents that perturb phosphatidylcholine homeostasis in mammalian cells leading to cell death have been identified, but the signaling pathway that mediate this cell death have not been well-defined (Cui & Houweling, 2002).

The role of phosphatidylcholine in the embryonic development of frog Microhyla ornata and chick Gallus domesticus was undertaken for the present study after inhibiting phosphatidylcholine synthesis by treating gastrulating frog embryos with its inhibitor. In M. ornata it caused shortening of the anterio-posterior axis and underdeveloped craniofacial features (reduced head size, absence of optic vesicle). In G. domesticus the treatment with the PC synthesis inhibitor resulted in the shortening of anterio-posterior axis and failure of the brain differentiation. It has also been found that inhibition of phosphatidylcholine resulted in neural tube defects, reduced craniofacial development and growth retardation in frog as well in chick embryos.