

# SKULL ABNORMALITIES ASSOCIATED WITH ANOPHTHALMOS CONDITION IN CHICKS

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## ABSTRACT

Orbital asymmetry and the skeletal abnormalities associated with anophthalmos condition was studied using six each of hatched and unhatched chicks, using Toluidine Blue - Alizarin Red S staining of cartilage and bone protocol. Of these birds, one each of unilateral anophthalmos was also associated with beak deformities. Orbital part of the frontal bone, interorbital septum, horizontal plate of ethmoid bone and lacrimal bone were found defective in the affected side. Jugal bone was absent on the affected side and all these deformities collectively contributed to the deformed orbit. Etiology of these skeletal deformities and anophthalmos may be attributed to spontaneous and genetic aberrations, indicated by the mismatching between nucleotides at various locations in the ovomucoid gene.

**Key Words:** Anophthalmos, chicks, skull abnormalities.

## INTRODUCTION

Anophthalmos or anophthalmia is the congenital lack of one or more eyes in chicks. Anophthalmus is an animal which lacks one or more eyes. True or primary anophthalmos, with complete absence of the ocular tissue within the orbit is very rare. Extreme microphthalmos is more common,

where a very small globe is present within the orbital soft tissue, which is not visible on initial examination. Anophthalmos in chicks presents not only the absence of an eye but also the secondary disfigurement of the orbit, the lids, and the eye socket. The present study was undertaken to describe the skeletal anomalies associated with anophthalmos in chicks.

## MATERIALS AND METHODS

The orbital asymmetry was studied using six hatched and six unhatched chicks with anophthalmos condition collected from Hatchery units of AICRP poultry, University Poultry Farm and Revolving fund Hatchery, Mannuthy. In all cases the birds showed orbital asymmetry. After recording the gross abnormalities the birds were sacrificed and the skeletal abnormalities were studied using Toluidine Blue - Alizarin Red S staining of cartilage and bone protocol (Alphonse, 1965). Cartilage and bone were differentiated in whole-mount preparations with toluidine blue-alizarin red S staining after formalin, acetic acid and alcohol (FAA) fixation. Specimens were fixed in FAA solution having the ratio of three components as 1:1:8 for approximately 40 minutes. Then they were stained in 0.06 per cent toluidine blue made in 70 per cent ethyl alcohol for 48 hours at room temperature. 20 volumes of stain solution to the estimated

volume of the specimen were used. Soft tissues were destained in 35 per cent ethyl alcohol for 20 hours; 5 per cent for 28 hours and 70 per cent for 8 hours, respectively. The specimens were counterstained in a freshly prepared 1 per cent aqueous solution of KOH to which was added 2-3 drops of 0.1 per cent alizarin red S per 100 ml of solution. The specimens were transferred into the fresh 1 per cent KOH-alizarin mixture daily for 3 days, or until the bones had reached the desired intensity of red and soft tissues. The specimens were rinsed in water, placed in a 1:1 mixture of glycerol and ethyl alcohol for 1-2 hours and then transferred into fresh glycerol-alcohol for final clearing and storage.

## RESULTS AND DISCUSSION

Of the twelve birds studied, eight were with bilateral anophthalmos, two with unilateral anophthalmos of left side (Fig. 1) and two with unilateral anophthalmos of right side. Of these birds, one each of unilateral anophthalmos was also associated with beak deformities. The beak as such was not deformed, but the upper beak crossed the lower beak so that the tip of the upper beak was deviated to the affected side with anophthalmos and the lower beak was normal (Fig. 1).

Among the bones of the neurocranium, frontal bone, especially its orbital part of the affected side showed deformities (Figs. 2&3). The interorbital septum formed by the orbital wings of sphenoid together with the more rostrally situated vertical plate of ethmoid bone, was also deformed. The horizontal plate of ethmoid bone lay below the processes of the frontal and nasal bones and corresponded to the lamina cribrosa of mammals and separated the orbit from the nasal cavity in normal birds (Fig. 4). It was also found defective in the affected side in the present study. Among the bones of the splanchnocranium, lacrimal bone was defective and reduced on the affected side. Zygomatic or jugal bone was absent on the affected side, collectively contributing

the deformed orbit. Anophthalmia and microphthalmia (Fig. 5) may occur secondary to the arrest of development of the eye at various stages of growth of the optic vesicle during the embryonic period. It is important to recognize microphthalmia because the development of the orbital region, as well as the lids and the fornices, is dependent on the presence of a normal-sized eye *in utero*.

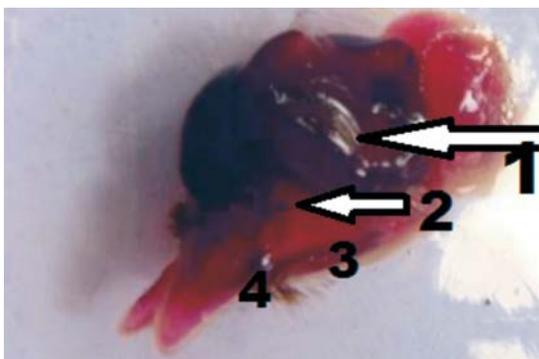
Pourlis (2011) opined that the etiology of these skeletal deformities and anophthalmos may be contributed to spontaneous and genetic aberrations. In the present study, as a means to detect the reduced hatchability of chicks, the genomic DNA samples of the affected chicks with structural anomalies were PCR-amplified for the ovomucoid gene, the PCR products sequenced, the results aligned and blast in the NCBI DNA database and compared



**Fig.1.** Day – old chick with anophthalmos condition on left side

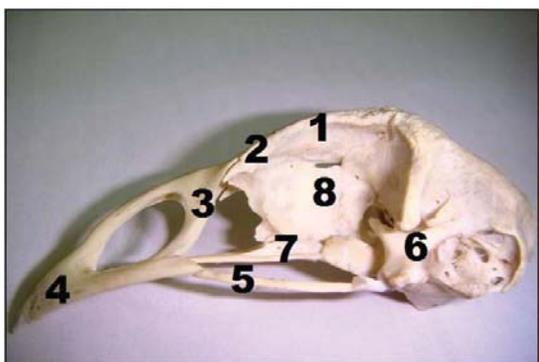


**Fig. 2.** Head of day-old chick with anophthalmos condition on left side stained with Toluidine Blue - Alizarin Red S Staining. Dorso- frontal view.



**Fig.3.** Head of day -old chick with anophthalmus condition on left side stained with Toluidine Blue - Alizarin Red S Staining. Left view.

1. Frontal 2. Lacrimal 3. Mandible 4. Maxilla



**Fig 4.** Upper skull of normal adult fowl. Left view.  
1. Frontal 2. Lacrimal 3. Maxilla 4. Premaxilla  
5. Jugal 6. Quadrate 7. Palatine 8. Interorbital septum

with those in the literature. Even though TAT deletion as noted in the ovomucoid gene (GenBank accession: HM776315) in ducks of low-hatchability group was not detected in the present study, the nucleotide sequences showed mismatching between nucleotides at various locations varying in number from 1-



**Fig. 5.** Day -old chick with microphthalmos condition on both sides.

12, which might be contributed one reason for the deformed nature of the chicks.

#### ACKNOWLEDGEMENT

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