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## Identification of primary ossification centers in the forelimbs of buffalo fetus by modified alizarin red – s method

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#### Abstract

In this modified alizarin red-s method, the fetuses were fixed in 10 percent Neutral buffered formalin for 1 week. Potassium hydroxide digestion time was reduced to secure the muscular system and then stained with alizarin red-s. Excess stain was removed by fresh 4 percent potassium hydroxide solution. Stained fetuses were cleared for 2 weeks by using two clearing agents. The primary ossification centers of the forelimbs were clearly visible. The fetuses were preserved in glycerin.

**Keywords:** Alizarin red-s, fetus, primary ossification centers, potassium hydroxide, forelimbs

#### Introduction

Alizarin red-s method was used for fetal skeleton preparations. Alizarin dye has affinity towards calcium, so it binds with bone and stains it red. This modified method of alizarin secures the muscular system so that the skeletal system is clearly visible through the muscular system. This method is useful for the detection of deformities in the skeleton system of the aborted fetuses after the drug trails in the pregnant animals.

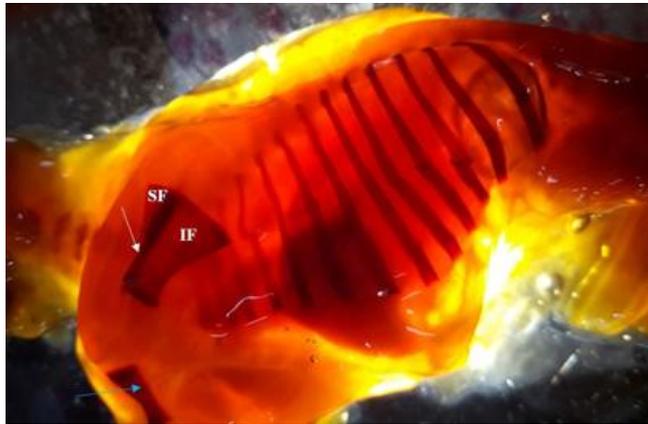
#### Materials and Methods

10 percent Neutral buffered formalin was used as fixative and 10 percent potassium hydroxide was used as digestive agent. Alizarin red-s stain was prepared by 1 ml alizarin red-s solution (Glacial acetic acid 5 ml, glycerin 10 ml, distilled water 85ml, alizarin dye 0.5 gm.) added drop by drop to 4 percent potassium hydroxide solution. For clearing, clearing agent I (glycerin 20 ml, 2 percent potassium hydroxide solution 28 ml, distilled water 52 ml) and clearing agent II (glycerin 50 ml, 2 percent potassium hydroxide solution 3 ml, distilled water 47 ml) were used. Buffalo fetuses were collected from local dairy farm. For clear visibility, the fetuses were de skinned and eviscerated. The fetuses were fixed in 10 percent Neutral buffered formalin for 1 week. For the proper digestion of the tissues, the fetuses were kept in 10 percent potassium hydroxide solution for 2 hours. Then kept the fetus in the alizarin red-s stain for overnight (Rasweiler *et al.* 2009) [5]. Excess stain was removed by fresh 4 percent potassium hydroxide solution. The fetuses were cleared in clearing agent I for 1 week and in clearing agent II for another 1 week. Store the stained fetuses in the pure glycerin and add a thymol crystal as a preservative. For clear visibility of primary ossification centers in photographs, the stained fetal forelimbs have been placed on a transparent glass surface with a light arrangement.

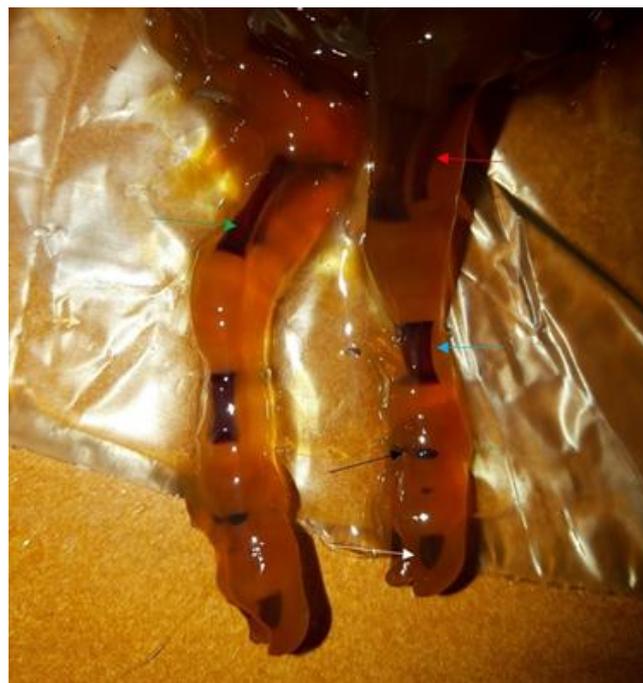
#### Results and Discussion

The primary ossification centers in the shoulder region were scapular spine, supraspinous fossa and infraspinous fossa visible as red colored and the cartilage part was unstained (Fig. 1). The glenoid cavity of scapula was not fully developed and these results were in correlation with Noback (1944) [2]. In the middle of the arm region, primary ossification center of humerus was located. The diaphysis of the humerus was stained red color, the epiphyses of the humerus were cartilaginous, unstained and does not having any primary ossification centers (Fig. 1) and these results were in correlation with Phillips (1976) [3]. The ossification centers of the radius and ulna were located in the forearm region. The ossification center of radius was smaller and located in the anterior part of the forearm (Fig. 2). The ossification center of ulna was larger and located in the posterior part of the forearm (Fig. 2). These results were in correlation with Menegola *et al.* (2001) [1].

Below the forearm region, large ossification center for metacarpal bone was visible (Fig. 2). The unstained space between the ossification centers of the forearm and metacarpal region indicates non-ossified carpals. In the digital region, the ossification centers for phalanges were visible among which the ossification centers of first phalanx and third phalanx were more prominent (Fig. 2). These results were in correlation with Pramod and Vaswani (2011)<sup>[4]</sup>.



**Fig 1:** Photograph showing shoulder region, arm region and thorax of buffalo fetus. SF – Supraspinous fossa, IF – Infraspinous fossa, White arrow – Primary ossification center of scapula, Blue arrow – Primary ossification center of humerus.



**Fig 2:** Photograph showing forelimbs of buffalo fetus. Red arrow - Primary ossification center of radius, Green arrow - Primary ossification center of ulna, Blue arrow - Primary ossification center of metacarpus, Black and White arrows - phalanges.

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