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Didactic model of the chicken embryo development using modified Dawson's diaphanization and staining technique¤

Modelo didáctico del desarrollo embrionario del pollo usando la técnica modificada de Dawson para transparentación y tinción

Modelo didático do desenvolvimento embrionário do frango usando a técnica modificada de Dawson para diafanização e coloração

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Summary

Background: didactic models are a very useful tool for pedagogy in Agrarian Science careers (i.e. Veterinary Medicine and Animal Science). **Objective:** the aim of this work was to create a didactic model of the chicken embryo development using modified Dawson´s diaphanization and staining technique, which allows the centers of ossification to be viewed. **Methods:** chick embryos from day 5 to day 21 were diaphanized with KOH, stained with Alizarin Red, and stored in glycerol. **Results:** growth of primary ossification centers during embryonic development was easily visualized. **Conclusion:** to our knowledge, this is the first literature report showing an anatomical model of all embryonic stages of chicken development. Impact of this model in pedagogy must be evaluated in future works.

Key words: *anatomy, cartilage, embryology, organic system, pedagogy.*

Resumen

Antecedentes: los modelos didácticos son una muy buena herramienta para el proceso de enseñanza-aprendizaje en los cursos de Ciencias Agrarias (Medicina Veterinaria y Zootecnia, entre otros). **Objetivo:**

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hacer un modelo didáctico del desarrollo embrionario del pollo usando la técnica modificada de Dawson para transparentación y tinción, que permite visualizar los centros de osificación. **Métodos:** embriones de pollo del día 5 al 21 fueron transparentados con KOH, teñidos con Rojo de Alizarina y almacenados en glicerina. **Resultados:** se pudo visualizar el crecimiento de los centros primarios de osificación durante el desarrollo embrionario. **Conclusión:** este es el primer reporte en literatura que muestra un modelo anatómico de todas las etapas del desarrollo embrionario del pollo. En futuras investigaciones se debe evaluar el impacto que tiene este modelo en el proceso enseñanza-aprendizaje.

Palabras clave: *anatomía, cartílago, embriología, pedagogía, sistemas orgánicos.*

Resumo

Antecedentes: os modelos didáticos são uma boa ferramenta para o processo ensino-aprendizado nos cursos de Ciências Agrárias (Medicina Veterinária, Zootecnia, entre outros). **Objetivo:** fazer um modelo didático do desenvolvimento do frango usando a técnica modificada de Dawson para diafanização e coloração, que permite visualizar os centros de ossificação. **Métodos**: Embriões de frango do dia 5 ao 21 foram transparentados com KOH, corados com Vermelho de Alizarina e armazenados em glicerina. **Resultados:** foi possível visualizar o crescimento dos centros primários de ossificação durante o desenvolvimento embrionário. **Conclusão:** este é o primeiro reporte na literatura de um modelo anatômico do desenvolvimento embrionário do frango. Futuras pesquisas devem ser focadas no impacto que tem este modelo no processo ensino-aprendizado.

Palavras chave: *anatomia, cartilagem, embriologia, pedagogia, sistemas orgânicos.*

Introduction

Didactic models are tools used to improve analytic and argumentative capacity, imagination, and innovation skills in students in order to enrich their learning process in agrarian careers. The integrative course in Veterinary Medicine and Animal Science at the Faculty of Agrarian Sciences of the University of Antioquia is titled Organic Systems. Aiming to improve the integration of anatomy, histology and physiology concepts, students have to develop a didactic model and present it in an exhibition during this course (Lenis and Tamayo, 2011).

Avian embryo development is of great economic importance because it is during this time that the genetic basis of the line or breed to be produced is consolidated. Nowadays, it is very common to use animal didactic models for improving pedagogy in agrarian science careers (i.e. Veterinary Medicine and Animal Science).

Dawson´s technique is an anatomical technique which diaphanizes soft tissues and stains embryo bones (Dawson, 1926). Several modifications have been made since the first description of the technique (Randle, 1969; Tipton and Burt, 1977; Sanchez *et al*., 1996; Staples and Schnell, 1964; Jensh and Brent, 1966; Kawamura *et al*., 1990), and it is considered as a very useful technique for studying the embryological development of the skeletal system. Essentially, the method consists of macerating the fetus with alkali, followed by staining it with a dye, which has a strong affinity for calcium salts, such as Alizarin Red (a dye derived from anthraquinone). The resulting specimens allow the visualization of the stained red bone beneath the transparent soft tissues.

It is necessary for Animal Science and Veterinary Medicine students to develop and have access to didactic models for studying skeletal system ossification. The study of this important process allows the students to better understand some of the skeletal problems that animals can encounter, especially those derived from developmental impairments. The aim of this work was to describe the diaphanization and stain of the skeletal system of chicken embryos aged 5 to 21 days for its use as a didactic model for teaching purposes.

Material and methods

Animals

Chicken embryos provided by Marruecos poultry farm (Medellín, Colombia) from 5 to 21 days of incubation were used. Day 5 was chosen because skeletal system development (endochondral and intramembranous ossification) starts at that day, with small ossification centers in the skull, limb buds and prevertebrae (Sawad *et al.,* 2009). Each embryo was separated from the attached structures of the egg and placed in individual 250 mL bottles.

Specimen processing

In order to avoid excessive manipulation and damage the embryos were not transferred from the bottles. Taking into account the small size of the specimens, it was not considered necessary to eviscerate or skin the embryos. They were fixed in 10% formalin (Protokimica, Medellín, Colombia) for eight days to attain an adequate fixation, then dehydrated in 70% ethyl alcohol (Protokimica, Medellín, Colombia) mixed with molecular iodine (Carlo Erba, Val-de-Reuil, France) for two days. Molecular iodine was used as a mordant to potentiate fixation of Alizarin Red in bone calcium. Next, specimens were transferred to a 2% KOH solution (Protokimica, Medellín, Colombia) added to 1% Alizarin Red (Merck, Parkville, Australia) for 24 hours. The embryos were transferred to a 2% KOH solution for eight days for clearing. At this time, embryos were completely stained by Alizarin Red, and no difference between muscle and bone was observed. For an additional clearing, a 1:1:1 2% KOH solution, ammonia (Protokimica, Medellín, Colombia) and glycerol (Protokimica, Medellín, Colombia) was used for eight days. Finally, glycerol was used to store the specimens.

Results

Embryos were successfully diaphanized, yielding the ossification centers in red (Figure 1). To attain the main objective of this work—that is, the process of forming a didactic model—all the bottles were placed in a specially designed shelf including photos of the fresh specimen in all the stages to enhance the steps of the embryologic development process, as shown in figure 2.

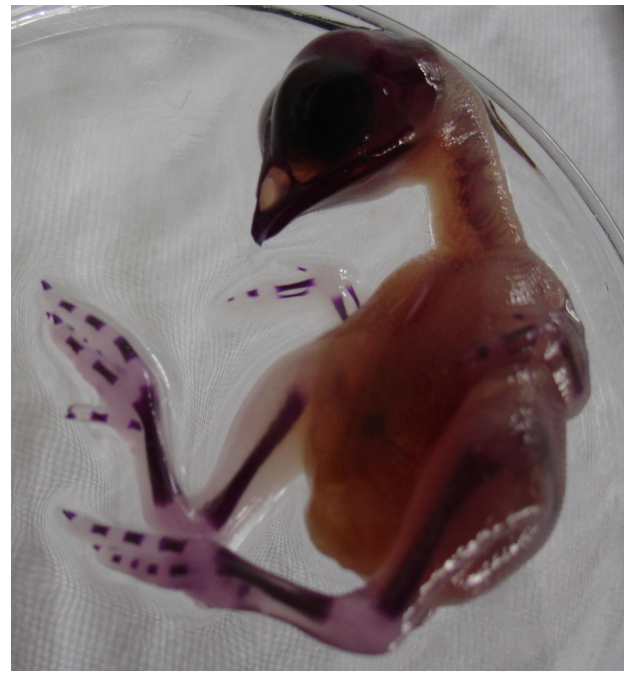

Figure 1. 16-day-old chick embryo. Primary centers of ossification are clearly seen in red at bone diaphysis, while cartilage is seen in white at bone extremities.

Figure 2. Final aspect of the didactic model displaying chicken development stages from days 5 to 21. Fetuses were placed in individual bottles. Photographs were placed in the central panel for additional explanations.

The resulting didactic embryo model was as the final project of the Organic Systems course at the Faculty of Agrarian Sciences-University of Antioquia (Colombia), exhibited during the First Undergrad Students Exhibition of Animal Didactic Models (Lenis and Tamayo, 2011).

Discussion

To our knowledge, this is the first literature report showing an anatomical model of all the embryonic stages of chicken development. The modified Dawson´s technique stained the calcified bone and allowed the visualization of the primary ossification center development.

Given multiple factors such as species, development stage, size, and evisceration, experience is needed to optimize diaphanization and staining of ossification processes. Several modifications of Dawson´s technique have been proposed to optimize the diaphanization process. Sanchez *et al*. (1996) used a higher KOH concentration (15%) to decrease the time spent in diaphanization from one month to one week in human fetuses. Betti (2005) used 2-4% KOH to diaphanize fish larvae but only for 10 minutes, while Maiolino *et al*. (2009) used 6% KOH, under an unspecified period of time. Care must be taken when using higher KOH concentrations due to the risk of tissue disintegration. When pigmentation is present, H_20_2 10 V solution must be used for 3 to 5 minutes to clarify the specimen (Maiolino *et al.*, 2009).

Sanchez *et al*. (1996) changed the Alizarin Red to 20% alcoholic carmine, with good results. Rodrigues (2005) recommends 0.05% methyl green in 70% ethylic alcohol for additional cartilage staining (Lundwall method) (Da Rocha, 2005). This can also be achieved using Alcian blue (Maiolino, 2009; Sawad *et al*., 2009; Whitaker and Dix, 1979; McLeod, 1980; Webb and Byrd, 1994; Depew, 2000; Narotsky and Rogers, 2000).

We want to highlight the particularities of our method, which are the use of molecular iodine and ammonia. Molecular iodine acts as a mordant, avoiding discoloration of the specimen with time and guaranteeing that the specimen will withstand time while remaining in good condition. The addition of ammonia makes specimens more transparent than only KOH and has the advantage of being less corrosive to the delicate tissues of the fetus.

Although diaphanization protocols are widely used for research, they are poorly used as didactic models for the explanation of basic science topics in agrarian careers. With this model we obtained a useful tool for the pedagogy of avian embryology.

Prospective future works include constructing didactic embryological models for other species and evaluating the impact that these models have upon pedagogy. We want to stain not only the bone but also the cartilage using Alcian blue in chickens and other animal species to study the musculoskeletal system at earlier stages.

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