BONE DEVELOPMENT IN THE CHICKEN EMBRYO: MACROSCOPIC AND MICROSCOPIC ANALYSES

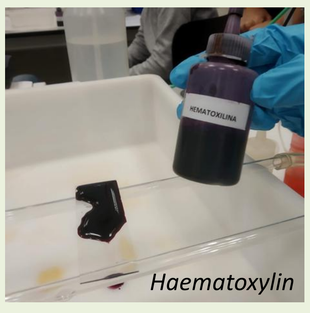
L. Aguilar Garrido[1], C. Cobo López[1], M. Cobo López[1], J.C. Cobo Planet[1], M. Dávila Romero[1], A. Fernández Cámara[1], A. Jiménez López[1], I. Marín Salido[1], S. Perandones Rosa[1], P. Soria Gámez[1], P. Colmenero Varea[1], I. García Valencia[2], R. Hernández Cobo[2], S. Blanco Ruiz[2]

[1] IES Sierra Magina, Tetuán s/n, 23100 Mancha Real, Jaén, Spain
[2] Departament of Expiremental Biology, Universidad de Jaén, Campus de Las Lagunillas s/n, 23071 Jaén, Spain

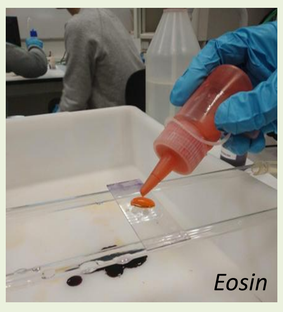
INTRODUCTION

Bone formation occurs by two main mechanisms: **endochondral**, in which cartilage turns into bone, and **intramembranous** in which bone is formed directly independent of cartilage. These processes can be observed microscopically and macroscopically with the *haematoxylin/eosin* and *alcian blue/alizarin red* staining techniques, respectively.

MATERIAL & METHODS



Haematoxylin

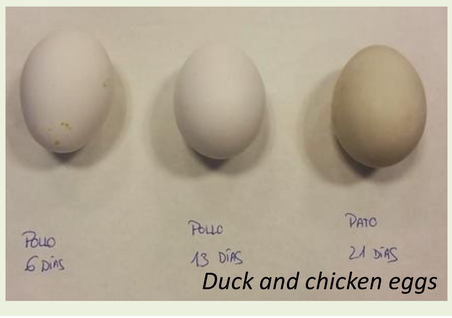

Eosin

MICROSCOPIC STUDY

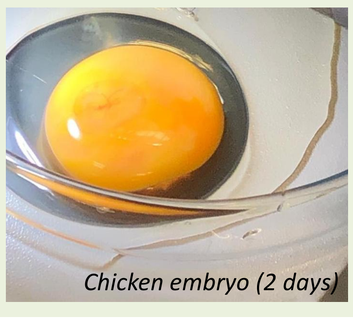
Rat cartilage samples were obtained using a cryostat and further stained with *haematoxylin/eosin*. Haematoxylin is a basic dye that binds to acid structures, staining the cell nuclei in blue, whereas eosin is an acid dye that stains the cytoplasm in red. Bone samples, previously prepared by the members of the Research Group BIO-184 using the same staining were observed as well.

MACROSCOPIC STUDY

Duck (day 21) and chicken (various stages) embryos were obtained directly from eggs. The embryos were collected, cleaned and rinsed prior to the staining protocol as described by Rigueur and Lyons (2014). *Alcian blue* (cationic dye) binds strongly to the glycosaminoglycans (GAGs) of the cartilage, while the anionic dye *alizarin red* binds specifically to cationic metals, such as the calcium found in bones.



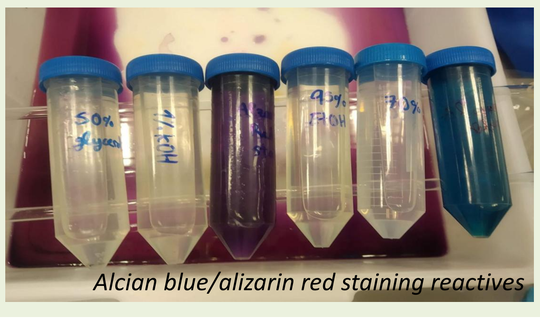
Duck and chicken eggs


Chicken embryo (2 days)


Chicken embryo (6 days)


Chicken embryo (13 days)


Duck embryo (21 days)

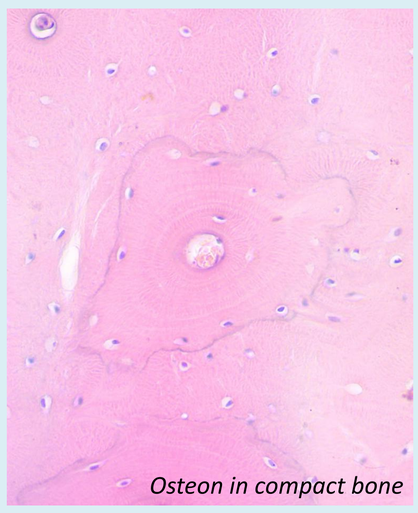

Alcian blue/alizarin red staining reactives

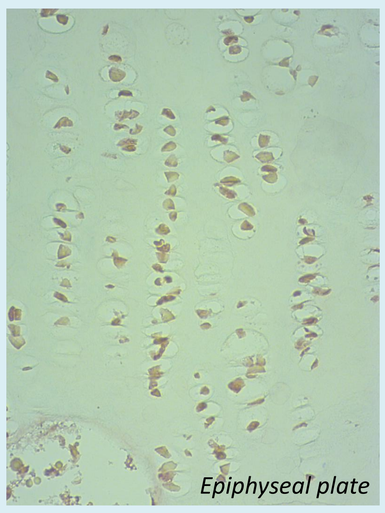
RESULTS AND DISCUSSION

MICROSCOPIC OBSERVATION

We could observe chondrocytes within lacunae surrounded by territorial and interterritorial matrixes in the cartilage, and the Haversian systems (osteons) formed by Haver's canal, lamellae, and osteocytes in lacunae in the compact bone. We have observed epiphyseal plate preparations as well, where we have identified all the stages of the endochondral bone formation process.


Cartilage


Osteon in compact bone


Epiphyseal plate

MACROSCOPIC OBSERVATION

We have confirmed that at the earliest stages of development, cartilage is formed first, whereas bone begins to develop later.


Duck embryo (21 days)


Chicken embryo (13 days)

CONCLUSIONS

- Cartilage and bone histology share a common layout of cells within lacunae immersed in a matrix. The main difference between both tissues is found in the distribution and organization of the lacunae and in the composition, consistency and vascularisation of the matrix.
- During endochondral bone formation, cartilage is successively replaced by bone following several processes involving cell hyperplasia and programmed death, and matrix calcification.
- At the earliest stages of development, cartilage is formed first. Afterwards, bone begins to develop in long bones by the endochondral mechanism.

ACKNOWLEDGEMENTS

We want to thank the Project Science-IES, which has facilitated our participation in this project. We want to express our gratitude to the Department of Experimental Biology of the University of Jaén, specifically to the Drs. Raquel Hernández and Santos Blanco, who have taught us many things, and have allowed us to use novel labware. Finally, we want to express thanks to our high school, which has allowed us to participate in this project.

REFERENCES

- https://mmegias.webs.uvigo.es/6-tecnicas/protocolos/p-tincion-h-e.php
- Rigueur & Lyons, 2014. *Whole Mount Skeletal Staining*. Methods Mol Biol. 2014 ; 1130: 113–121.