EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

ZOOLOGY

B

ISSN
2090-0759

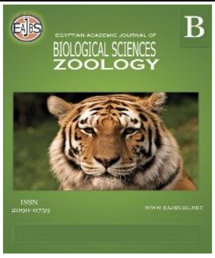
WWW.EAJBS.EG.NET

Vol. 16 No. 1 (2024)

Citation: *Egypt. Acad. J. Biolog. Sci. (B. Zoology) Vol. 16(1) pp:221-227 (2024)*
DOI: 10.21608/EAJBSZ.2024.362383

Egypt. Acad. J. Biolog. Sci., 16(1):221-227 (2024)

Egyptian Academic Journal of Biological Sciences
B. Zoology
ISSN: 2090 – 0759
http://eajbsz.journals.ekb.eg/



Differentiation of the Developing Tooth of Egyptian Cobra, *Naja haje* (Squmata: Serpents: Elapidae) at the Hatching Stage

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ARTICLE INFO
Article History
Received:27/5/2024
Accepted:28/6/2024
Available:30/6/2024

Keywords:
Tooth, Snake,
Naja haje.

ABSTRACT

Vertebrates exhibit a great variety of dental patterns, from completely missing teeth to having several sets of teeth that are replaced over the course of a lifetime. Amniotes carrying teeth unlike other vertebrates have teeth only in the oral cavity and do not have pharyngeal teeth. The majority of what we know about tooth formation comes from research on a small number of model organisms. Furthermore, there are insufficient studies in the literature that provide a detailed description of *Naja haje's* tooth formation at the hatching stage. Thus, we introduce this study as a part of the descriptive work on reptilian dentition. As an additional model, we detailed the tooth formation of a hatching *Naja haje* snake by using serial sections through the head. The histology of these sections demonstrated the histodifferentiation of both ameloblast and odontoblast cells at different developmental stages. All stages of tooth development; dental lamina, bud, cap, bell, implantation, and eruption have been completed. When *N. haje* hatches, its teeth are fully grown and inserted into the corresponding bones.

INTRODUCTION

Vertebrate teeth come in a wide variety of shapes and sizes from simple unicuspid to complex multicuspid teeth; this variation allows them to take many different diets and influence social behavior (Evans *et al*., 2007; Ungar, 2010). The simplest teeth are present in many snakes, monitor lizards, and crocodiles. It may be conical in shape, cylindroconical, flattened, or slightly bent. The most complex teeth are observed in the giant panda. The dentition is formed as a result of interaction between epithelial and mesenchymal layers. The participating epithelial layer may be ectodermal in origin as in the case of oral teeth of reptiles (Buchtová *et al*., 2008; Cobourne *et al*., 2004) or endodermal as in pharyngeal teeth in fishes and amphibians (Fraser *et al*., 2009; Soukup *et al*., 2008; Van der Heyden and Huysseune, 2000).

All snakes have oral teeth only and they do not possess any pharyngeal teeth (Fraser *et al*., 2006a; Wise and Stock, 2006). Teeth are arranged into two rows in the upper jaw and one raw only in the lower jaw. At the upper rows; the marginal row consists of premaxillary teeth rostrally and maxillary teeth caudally and the inner row consists of the palatine and pterygoidal teeth. Between the marginal and the palatine teeth row, there is a deep sulcus called the palatal groove. The single teeth row of the lower jaw is called the dentary row which is carried by the dentary bone (Buchtová *et al*., 2008).

The developing tooth proceeds through the dental lamina, bud, cap, and bell stages. These stages occur in reptiles and are also described in mammals (Luckett, 1993). The epithelial cells of dental lamina invaginate into the oral ectomesenchyme forming a bud. The tooth bud transformed into a cap by the proliferation of bud cells and condensation of mesenchyme around the dental epithelium with instructive signaling to the mesenchyme (Kollar and Baird, 1970).

In the cap stage, the mesenchymal cells form the dental papilla, and the dental epithelium forms a multilayered enamel organ that consists of three morphologically different layers of cells inside the oral epithelium; the inner enamel epithelium (IEE), the outer enamel epithelium (OEE), and the intermediate layer of stellate cells (Buchtova *et al*., 2007, 2008). The IEE and OEE are associated with each other by epithelial cells of developing cervical loops that grow down around the dental papilla (Zahradnicek *et al*., 2008).

During the bell stage, the differentiation of layers occurs and the crown will is formed. The inner enamel epithelium (IEE) forms the ameloblasts while the outer enamel epithelium (OEE) gives rise to the successional dental lamina (Richman and Handrigan, 2011). Both of the formed odontoblasts and ameloblasts secret dentin and enamel respectively (Richman and Handrigan, 2011).

Herein, we provide the cobra (*Naja haje* Linnaeus, 1758) as an example of Elapids. *N. haje* (Family: Elapidae) has a body characterized by a short tail, a distinct head, and moderate eyes with round pupils. When this snake is threatened, it raises the anterior part of the body by flatting the neck; it is the characteristic feature of elapids (El Dine, 2006). This species is distributed in Africa and South Asia. *N. haje* reaches sexual maturity after 27 months. After copulation in early summer, females can lay eggs in loose sand, abandoned rodent burrows, and under rocks (Schleich *et al*. 1996). We provide the cobra (*N. haje*) as an example of *Naja* genus for the description of developing teeth at the hatching stage.

MATERIAS AND METHODS

A mature gravid female was obtained for the study after copulation, and the fertilized eggs were carefully gathered and chosen to be incubated in plastic boxes filled with perlite that had been vented and wet. At hatching, embryos were removed from the eggs. Using forceps, the embryos were extracted from the surrounding extra embryonic membranes after the eggs were opened in Petri dishes containing phosphate-buffered saline (PBS).

Newborn specimens were then preserved in paraformaldehyde (4%). After that, the specimens were cleaned (in PBS) and dried using a series of ascending ethanol concentrations. Before being sectioned, samples were cleared in xylene (Sigma-Aldrich), infiltrated, and embedded in paraplast. Samples were cut frontally and sagittally. The sections were 7–9 µm thickness using Slee Cut 5062 microtome. Some Sections were stained in hematoxylin and eosin (Sigma-Aldrich) and others in Mason's trichrome.

Two times xylol was used to deparaffinize paraffin sections. Sections were hydrated using descending concentrations of ethanol series up to H_2O. Some Sections were stained in hematoxylin and eosin (Sigma-Aldrich) and others in Mason's trichrome. After staining, the sections were mounted in DPX (Sigma-Aldrich) and the slides were covered after being dried using increasing ethanol concentrations.

RESULTS

The serial sections showed that the teeth of *N. haje* at hatchling are established in two rows in the upper jaw and one row in the lower jaw. All teeth in the upper and lower jaws are unicuspid, conical in shape, and directed backward. The results showed full formation of teeth. All stages of tooth development (dental lamina, bud, cap, and bell) had been completed. The histodifferntation of cells that occurred in the bell stage is demonstrated. The cells that formed the enamel (ameloblasts) are columnar with basal and oval nuclei, and also the polygonal odontoblasts cells that formed the dentine (Fig. 1). There are three to four teeth generations in both the upper and lower jaws (Fig. 2). In all teeth of the first generation, the dentin reaches the bone and the tooth becomes ankylosed to its related bone (Figs. 3 & 4).

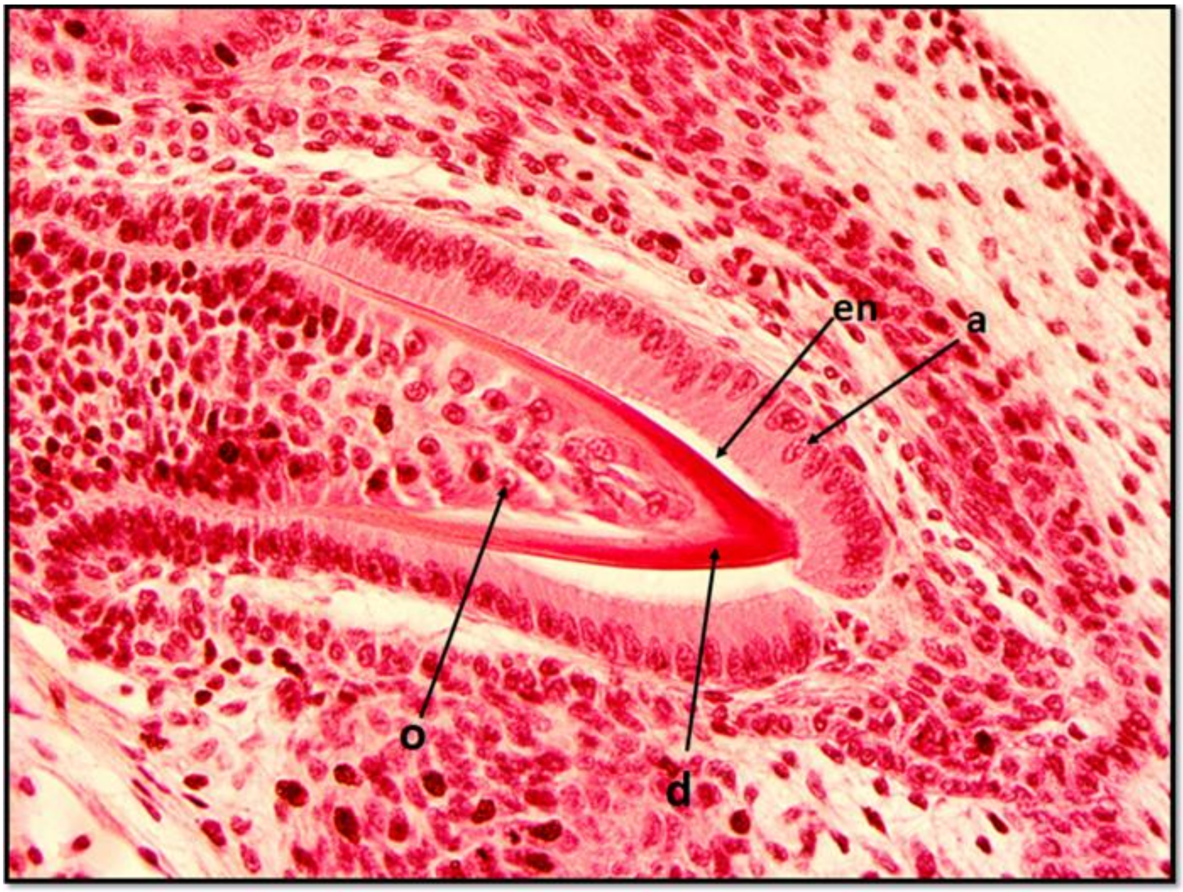

Fig. 1: Sagittal section of hatchling in *N. haje* shows histodifferentation of cells; columnar cells are ameloblasts that forming enamel and polygonal cells are odontoblasts that forming dentine. Abbreviation; d: dentine, en: enamel, o: odontoblasts & a: ameloblasts.

Fig. 2: frontal section of hatchling in *N. haje,* shows different generations of teeth. Abbreviation; g1, g2, g3 &g4 are the four generations of teeth.

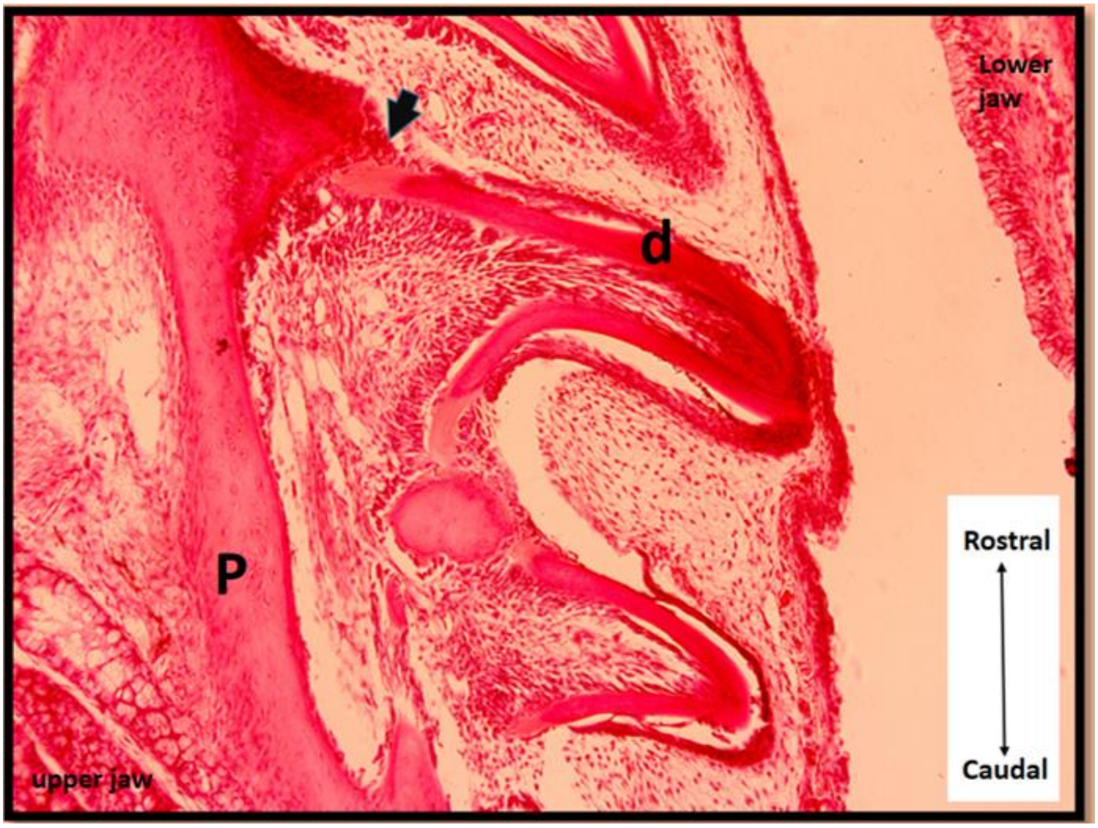

Fig.3: Sagittal section of hatchling in *N. haje* shows the implantation of palatal teeth and its direction. Abbreviation; p: palatal bone, d: dentine.

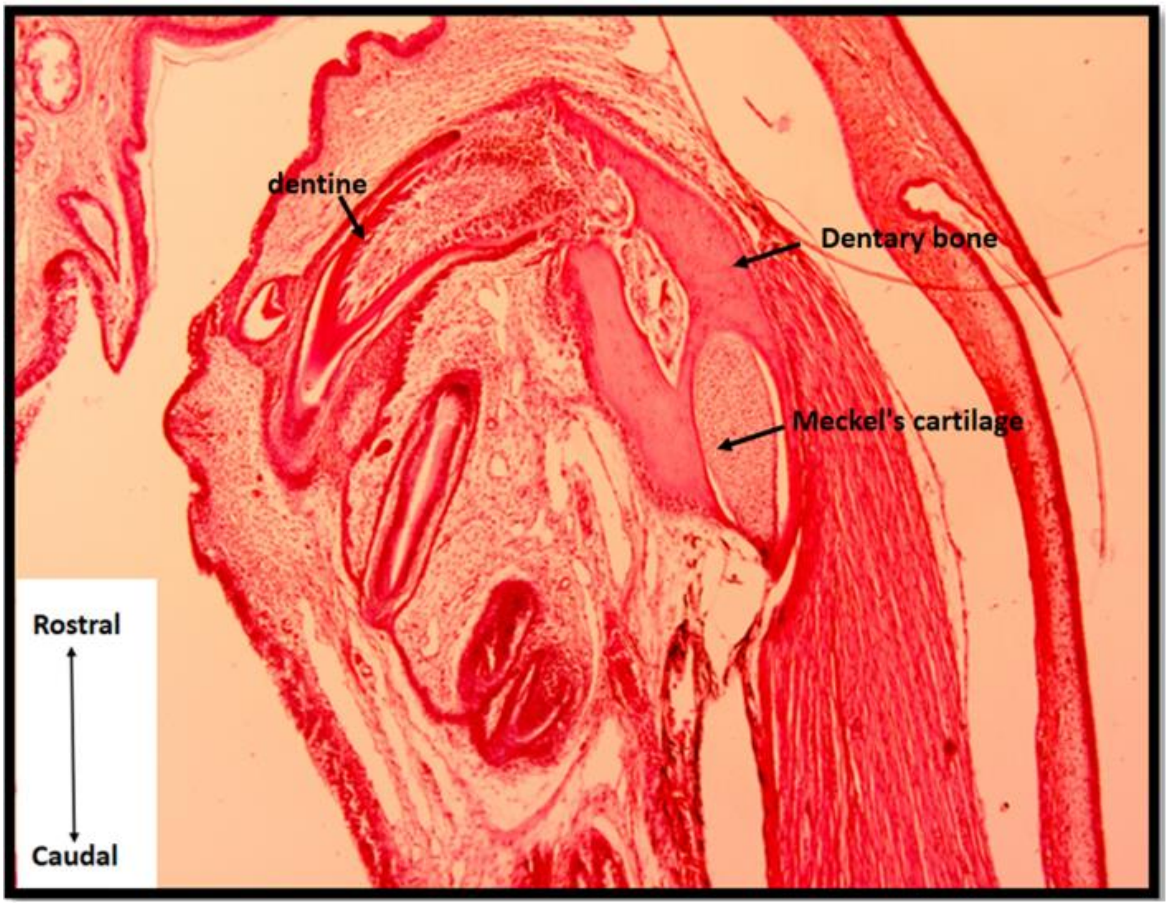

Fig. 4: sagittal section of hatchling in *N. haje* shows, the dentery teeth.

DISCUSSION

As we know tooth development results from interaction between the oral ectoderm and neural crest-derived mesenchyme with epithelial instructive signal (Lumsden, 1988; Mina and Kollar, 1987). The tooth-forming region forms the odontogenic band within the dental epithelium. In squamates (snakes and lizards), this band is homogeneous along tooth row length. After that, this band increases in thickness and forms the dental lamina, a simple ribbon of epithelial cells that grow inside the neural crest-derived mesenchyme (Buchtova´ *et al*., 2008; Handrigan and Richman, 2010a). After the earliest stages of the odontogenic band and fully formed dental lamina, the tooth germ passes through the bud, cap, and bell stages. These stages occur in reptiles and are also described in mammals (Luckett, 1993). During the bell stage, odontoblast cells start in dentin formation (Richman and Handrigan, 2011). This histological study has shown that the tooth of the hatchling *N. haje* snake completed its developmental stages. The morpho-differentiations of inner enamel epithelium and mesenchymal cells that occurred at the bell stage are completed and shown in tooth formation. The columnar cells of ameloblasts and the polygonal cells of odontoblasts are demonstrated. The formation of the two main hard tissues of the tooth, enamel and dentin, is done. The dentin of the first generation reaches the bone and the tooth becomes ankylosed to its related bone. Implantation of a tooth in the corresponding bone is a late stage in tooth development. There are three to four tooth generations at the hatching stage. This was also found in the python (Buchtova *et al*., 2007). According to this study, the hatching of *N. haje* has unicusped and a conical tooth, which verifies earlier findings (Vaahtokari *et al*., 1996) and helps us understand how they differ from multicusped mammalian teeth in having a simpler conical shape.

Declarations:

Ethical Approval: All of the animal work was approved by the Committee on the Ethics of Animal Experiments of the Zoology Department, Faculty of Science, Fayoum University

Conflicts of Interest: There is no conflict of interest.

Informed consent: All the authors of this manuscript accepted that the article is submitted for publication in the Egyptian Academic Journal of Biological Sciences, B. Zoology, and this article has not been published or accepted for publication in another journal, and it is not under consideration at another journal.

Authors Contributions: All authors are equal in contribution.

Funding: None.

Acknowledgements: We deeply acknowledge Zoology Department, Faculty of Science, Fayoum University, Fayoum, Egypt.

REFERENCES

Buchtová, M.; Boughner, J. C.; Fu, K., Diewert, V. M. and Richman, J. M. (2007). Embryonic development of Python sebae–II: Craniofacial microscopic anatomy, cell proliferation and apoptosis. *Zoology*, *110*(3), 231-251.

Buchtová, M.; Handrigan, G. R.; Tucker, A. S.; Lozanoff, S.; Town, L., Fu, K. ... and Richman, J. M. (2008). Initiation and patterning of the snake dentition are dependent on Sonic hedgehog signaling. *Developmental biology*, *319*(1), 132-145.

Cobourne, M. T.; Miletich, I. and Sharpe, P. T. (2004). Restriction of sonic hedgehog signalling during early tooth development. *Development*, 131 (1),2875-2885.

El Din, S. B. (2006). *A Guide to the Reptiles and Amphibians of Egypt*. Oxford University Press.

Evans, A. R.; Wilson, G. P.; Fortelius, M. and Jernvall, J. (2007). High-level similarity of dentitions in carnivorans and rodents. *Nature*, *445* (7123), 78-81.

Fraser, G. J.; Berkovitz, B. K.; Graham, A. and Smith, M. M. (2006a). Gene deployment for tooth replacement in the rainbow trout (Oncorhynchus mykiss): a developmental model for evolution of the osteichthyan dentition. *Evolution & development*, *8*(5), 446-457.

Fraser, G. J.; Hulsey, C. D.; Bloomquist, R. F.; Uyesugi, K.; Manley, N. R.; and Streelman, J. T. (2009). An ancient gene network is co-opted for teeth on old and new jaws. *PLoS biology*, *7*(2), e1000031.

Handrigan, G. R. and Richman, J. M. (2010b). A network of Wnt, hedgehog and BMP signaling pathways regulates tooth replacement in snakes. *Developmental biology*, *348*(1), 130-141.

Kollar, E. J. and Baird, G. R. (1970). Tissue interactions in embryonic mouse tooth germs: II. The inductive role of the dental papilla. *Development*, *24*(1), 173-186.

Linnaeus, C. (1758). Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus I. Editio decima, reformata, 10th Edition. Laurentii Salvii, Stockholm.

Luckett, W. P. (1993). Ontogenetic staging of the mammalian dentition, and its value for assessment of homology and heterochrony. *Journal of Mammalian Evolution*, *1*, 269-282.

Lumsden, A. G. S. (1988). Spatial organization of the epithelium and the role of neural crest cells in the initiation of the mammalian tooth germ. *Development*, *103*(Supplement), 155-169.

Mina, M. and Kollar, E. J. (1987). The induction of odontogenesis in non-dental mesenchyme combined with early murine mandibular arch epithelium. *Archives of oral biology*, *32*(2), 123-127.

Richman, J. M. and Handrigan, G. R. (2011). Reptilian tooth development. *genesis*, *49*(4), 247-260.

Schleich, H. H.; Kästle, W. and Kabisch, K. (1996). *Amphibians and reptiles of North Africa* (Vol. 63). Germany: Koeltz scientific books, Koenigstein.

Soukup, V.; Epperlein, H. H.; Horácek, I. and Cerny, R. (2008). Dual epithelial origin of vertebrate oral teeth. *Nature, 455*(7214), 795-798.

Ungar, P. S. (2010). *Mammal teeth: origin, evolution, and diversity*. Johns Hopkins University Press.

Vaahtokari, A.; A° berg, T.; Jernvall, J. and Thesleff, I., (1996). The enamel knot as a signalling centre in the developing mouse tooth. *Mechanisms of development*,54(1), 39–43.

Van der heyden, C. and Huysseune, A. (2000). Dynamics of tooth formation and replacement in the zebrafish (Danio rerio) (Teleostei, Cyprinidae). *Developmental dynamics: an official publication of the American Association of Anatomists*, *219*(4), 486-496.

Wise, S. B. and Stock, D. W. (2006). Conservation and divergence of Bmp2a, Bmp2b, and Bmp4 expression patterns within and between dentitions of teleost fishes. *Evolution & development*, *8*(6), 511-523.

Zahradnicek, O.; Horacek, I. and Tucker, A. S. (2008). Viperous fangs: development and evolution of the venom canal. *Mechanisms of development*, *125*(9-10), 786-796.

ARABIC SUMMARY

تمايز السن النامى للكوبرا المصرية في مرحلة الفقس

هاجر ابراهيم بيومى , نجلاء رفعت اسماعيل ,أحمد على قنديل
قسم علم الحيوان كلية العلوم جامعة الفيوم

تظهر الفقاريات تنوعا كبيرا في أنماط الأسنان, بدءًا من الفقاريات عديمة الأسنان إلى وجود عدة مجموعات متنوعة من الأسنان التي يتم إستبدالها على مدار العمر. توجد بالزواحف والثدييات أسنان في التجويف الفمي فقط بينما تفتقر للأسنان البلعومية على عكس الفقاريات الاخرى. إن أغلب ما نعرفه عن تكوين الأسنان يأتي من الأبحاث التي أجريت على بعض الزواحف والثدييات. في هذه الدراسة تم إستخدام *Naja haje* (Elapidae) كنموذج للثعابين فى الدراسة الوصفية لأسنان الزواحف. في هذه الدراسة تم وصف الجوانب المورفولوجية والنسيجية لنمو الأسنان عند الفقس, من خلال عمل قطاعات (سهمية وعرضية) تسلسلية لرأس الثعبان. أظهرت النتائج التمايز النسيجي لكل من الخلايا المكونة للعاج odontoblast والخلايا المكونة للمينا ameloblasts . وأظهرت الدراسة أيضا إكتمال تكوين مادة العاج والمينا وإتصال عاج الاسنان بالعظام المصاحبة لها وأن أسنان *N. haje* عند الفقس تكون قد أتمت جميع مراحل نموها المتعارف عليها.