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# Prenatal Study of Iraqi Sheep Fetuses Skulls (*Ovis aries*) Through Using Different Techniques: Morphological Study

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## Article Informations

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**ABSTRACT**

This work aims to prepare the skulls of Iraqi sheep fetuses during restricted periods of embryonic development for prenatal morphological study using different techniques.
The skull consists of two parts: the calvarium, which contains the brain, that also called the neurocranium, and the facial part of the skull, that also called the visceral cranium. Thirty five samples of local Iraqi sheep fetuses were taken over regular visits to the slaughterhouses, aged between 72 to 155 days of pregnancy. Maceration, radiography, and fly larvae were the different methods to prepare the fetuses' skull bones for prenatal morphological study.
The method of maceration with sodium hydroxide solution (NaOH) and radiography of embryos heads' showed that the frontal, parietal, inter-parietal, and squamous part of the temporal bone appeared fully ossified at the end of the second trimester of pregnancy, while the occipital, sphenoid, the vertical plate of the ethmoid bone, and the petrous part of the temporal bone appeared fully ossified in the third trimester of pregnancy. While the fly larvae maceration method showed that the bones seemed fully ossified at the end of the second trimester and in the third trimester of pregnancy, but were separated from each other.
In conclusion, the best methods for the prenatal study of fetuses' skulls are the maceration by NaOH and the radiography which allows us to achieve the anatomical measurements of fetuses' skulls. In contrast, the maceration by fly larvae allows us to study the surface area of each bone.

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## Introduction

The skull consists of two parts: the calvarium, which contains the brain, that also called the neurocranium, and the facial part of the skull, that also called the visceral cranium. The skull consists of several bones, that divided according to location: the bones that make up the floor contain the basisphenoid, pre-sphenoid, and basioccipital. The lateral and occipital walls of the skull consist of the temporal and the occipital bones, respectively. The roof in which the brain is located also includes the frontal bone, parietal bone, and inter-parietal bone [1].

Maceration provides bone pieces for morphological and morphometric studies. For this reason, maceration is an important technique to demonstrate the bone component [2]. Different maceration techniques are based on removing soft tissues attached to the bony structures [3]. The most common maceration techniques use inorganic chemicals and insects [4]. The time it takes to perform each technique varies depending on the sample size, environment temperature, and the technique itself [5].

Bone radiography studies are playing a new role in modern medicine and archaeozoology, where most studies focus on the characteristics of bone composition [6]. Head radiography studies are an important tool in Anatomy and diagnostic purposes in humans and animals [7]. However, radiography head Anatomy can be somewhat difficult to understand due to the complex arrangement of many of the structures in the head [8].

[7] conducted a radiography study on the heads of 32 fetuses from one-humped camels (Camelus dromedarius), including radiography evaluation of the calvaria of the skull of different fetuses in 1st, 2nd, and 3rd stages of pregnancy. [9] conducted a study that described the way of ossification in alpaca fetuses (Vicugna pacos) using radiography and diaphanization techniques.

[10] conducted a study to determine the location and time of appearance of ossification centers in the bones of the hind limbs of Awassi sheep using a modified double-staining method to show cartilage with bones, or using radiography. [11] studied the formation of skull bones in single-humped camel fetuses using radiography, after soaking some of the skulls with silver nitrate, all samples were examined with radiography using latero-medial and dorso-ventral projections. [12] conducted a study to determine the location and the sequence of appearance of the ossification centers in the bones of the hind limbs of indigenous black goat fetuses by the double stain method for less than 70 days of pregnancy, and radiography for older ages.
Skulls of advanced ages prepared using the fly larvae method save chemicals and time and provide an opportunity to study each bone individually and compare the parameters of the same bone at different stages of pregnancy [13, 14]. A study conducted by [15] dealt with determining the start time of vomer bone formation in indigenous sheep fetuses, the modified double-staining method was used for young ages, or doping with potassium hydroxide (KOH) or with fly larvae for older ages. [16] studied the development of the lower jaw bone in indigenous sheep fetuses, and the larval maceration method was used for some samples.
Due to the difficulty dealing with fetuses, especially the head, because most of its bones are membranous in origin, this work aims to prepare the skulls of Iraqi sheep fetuses during restricted periods of embryonic development for prenatal morphological study by using different techniques which include maceration, radiography, in addition to fly larvae techniques.

## Materials and Methods

Thirty five samples of local Iraqi sheep fetuses were taken over regular visits to the slaughterhouse Al-Saadoun and neighboring abattoir in Mosul city for 5 months and a half, initially from September 2023 to mid-February 2024, bodily and scientific investigations showed to choose well fetuses and eliminate those having structural malformations and microbial infections. Animal management and sample collecting were completed by following the instructions of the IACUC of the College of Veterinary Medicine, University of Mosul, Mosul, Iraq (UM.VET.2023.082). The measurement of the crown-rump length was done in centimeters using a calculating tape to define gestational ages in days as defined by [17] through Richardson's formulation: Estimated age (in days) = 2.1 (crown-rump length (cm) + 17). This length signifies the space ranging from the coronary rim of the frontal bone and alongside the vertebral column to the root of the tail (Figure 1).

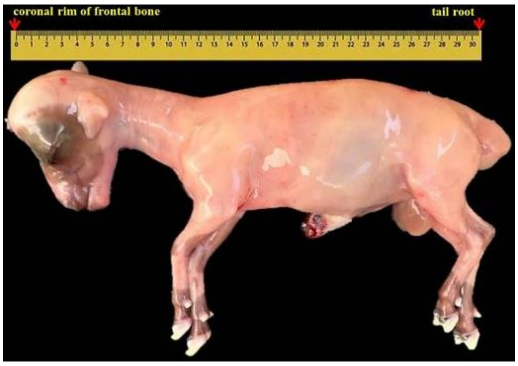


**Figure 1.** A photograph showing the technique for computing the estimated age of the fetus by determining the crown-rump length.

The head of the fetuses were disconnected from their bodies at level of the conjunction between the occipital bone and the atlas vertebra [18]. To confirm the accuracy, effectiveness, and rapidity of the maceration with sodium hydroxide solution (NaOH), the skin, tongue, eyes, and most of the muscles were removed.

**Maceration using sodium hydroxide solution**

To eliminate soft tissue, 28 fetal head samples, representing six age stages were divided into three groups:

**The first group:** consisted of 15 specimens aged between 72-77 days of pregnancy which had crown-rump lengths 17-20 cm. They were soaked in a 2% NaOH solution for 1-5 hours until the soft tissues turned into a gelatinous substance.

**The second group:** consisted of 8 specimens aged between 81-93 days of pregnancy which had crown-rump lengths 22-27.3 cm. They were soaked in 4% NaOH solution for 5-12 hours.

**The third group:** consisted of 5 specimens aged between 98-113 days of pregnancy which had crown-rump lengths 30-37 cm. They were soaked in 8% NaOH solution for 20-24 hours.

After soaking, the specimens were moved from NaOH solution, the mandible was separated from the skull, and the soft tissue remnants were removed using forceps. The samples were then washed and soaked in a 70% ethyl alcohol solution to maintain the moisture and prevent them from fungus [19].

**Radiography examination**

Seven fetuses' ages ranged between 90-155 days were studied using radiography. These samples were photographed using a power of 45 kV, an electricity 20 mA, an exposure time of 0.020 fractions of a second, and a distance of 50 cm using an X-ray machine (Mobile Art Evolution, Shimadzu corporation, Japan) [7, 19].

**Maceration using fly larvae**

After removing what could be removed from the soft tissue, 7 samples whose ages ranged between 90-155 days, which had previously been used in the radiography method, were exposed to fly larvae for two weeks. After the maceration process, the samples were washed with distilled water and placed in 70% ethyl alcohol to keep them moist and free from fungus [2, 20].

## Results

### Maceration using sodium hydroxide solution

In the middle and end of 2nd trimester of pregnancy, and at ages extending from 71-81 days of pregnancy, the frontal and parietal bone had completed their formation and become bone, but there was the fontanel area, which located between the frontal and parietal bones, it was soft, while the rostral end of the frontal bone was ossified, and its articulation with the nasal bone was noted through the frontonasal suture. The opening of the supraorbital foramen located on the dorsal surface of the frontal bone was visible but the channel was not complete yet. The part of the frontal bone that forms part of the orbital wall was ossified except for the caudal part of the orbit, which was not yet completed. The inter-parietal bone was approximately triangular in shape between the parietal and occipital bones was ossified, appearing as a bone divided into two halves by an inter-parietal suture, and the parietal-interparietal suture and the parietal-occipital suture appeared (Figure 2).

In 3rd trimester of pregnancy, the occipital bone, it was noted that the growth of the squamous part of the occipital bone was not complete, because the growth of the caudal end of it was not fully completed. The occipital crest was observed at the beginning of its formation in the twelfth week of pregnancy, and the external occipital protuberance had not yet appeared. The occipital condyles and jugular process were also ossified. The basilar part of the occipital bone also appeared ossified, while the muscular tubercles had not yet appeared, and it was noted that the occipital condyles and the basilar part of the occipital bone did not merge. The opening of the supraorbital canal and the ethmoid foramen located on the orbital wall of the frontal bone are not yet formed because this wall is incompletely ossified. The caudal part of the basisphenoid bone was ossified, which connects it with the basilar part of the occipital bone, while it was incompletely developed in the rostral end, which connected it with the presphenoid bone, in the middle of 2nd trimester of pregnancy, that is, in the eleventh week of pregnancy, while its growth was completed in the twelfth week. The temporal bone differentiates into two parts, the squamous and petrous parts, forming the squamous part of the body of the zygomatic process, the temporal crest of the zygomatic process, and the temporal wall of the orbit. As for the petrous part of the temporal bone, it appeared incompletely developed, as the papillary process and the external auditory meatus were not yet formed, and the caudal end of the auditory bulla was also not complete, as it had a wide, semi-oval opening on its left lateral surface (Figure 3).

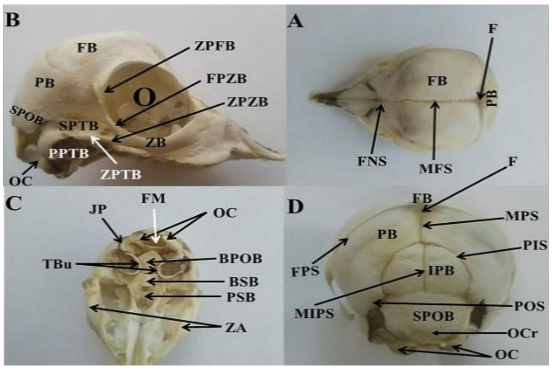


**Figure 2.** Showing skull of indigenous sheep fetus at 75 days, 11 weeks of pregnancy, prepared by maceration with NaOH solution. (A) dorsal view, (B) lateral view (right

side), (C) ventral view, (D) occipital view: (FB) frontal bone, (PB) parietal bone, (F): fontanel area, (FNS): frontonasal suture, (MFS): median frontal suture, (SPOB) squamous part of occipital bone, (SPTB) squamous part of temporal bone, (PPT4) petrous part of temporal bone, (ZPTB) zygomatic process of temporal bone, (OC) occipital condyles, (ZPFB) zygomatic process of frontal bone, (ZB) zygomatic process, (ZPZB) zygomatic process of zygomatic bone, (FPZB) frontal process of zygomatic bone, (O) orbit, (FM) foramen magnum, (JP) jugular process, (BPOB) basilar part of occipital bone, (BSB) basisphenoid bone, (PSB) presphenoid bone , ((TBu) Tympanic bulla, (ZA) zygomatic arch, (IPB) interparietal bone, (MPS): median partial suture, (PIS): parietal-interparietal suture, (MIPS): median interpartial suture, (POS): partio-occipital suture, (OCr): occipital crest, (FPS): frontoparital suture.

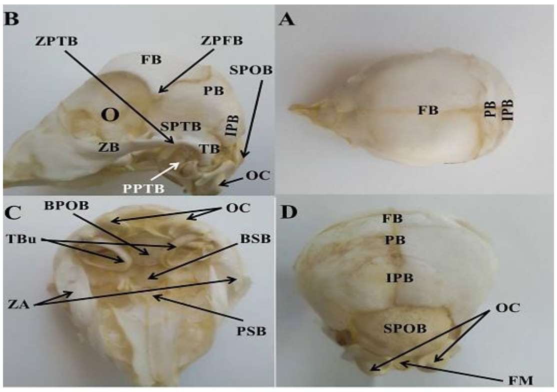


**Figure 3.** Showing skull of indigenous sheep fetus at 81 days, 12 weeks of pregnancy, prepared by maceration with NaOH solution. (A) dorsal view, (B) lateral view (left side), (C) ventral view, (D) occipital view: (FB) frontal bone, (PB) parietal bone, (IPB) interparietal bone, (SPOB) squamous part of occipital bone, (OC) occipital condyle, (TB) temporal bone, (SPTB) squamous part of temporal bone, (PPT4) petrous part of temporal bone, (ZPTB) zygomatic process of temporal bone, (ZPFB) zygomatic process of frontal bone, (ZB) zygomatic bone, (O) orbit, (BPOB) basilar part of occipital bone, (BSB) basisphenoid bone, (PSB) presphenoid bone, ((TBu) tympanic bulla, (ZA) zygomatic arch.

At the end of 2nd trimester and the beginning of 3rd trimester of pregnancy, at ages 84-113 days of pregnancy, that is, in the twelfth week to sixteenth week of pregnancy, the entire frontal and parietal bones were ossified and hard, and the supraorbital foramen and canal were also fully developed and became clear. The frontal part, which forms part of the orbital wall, was ossified. It was also noted that the posterior part of the orbit had been completed, as all the orbital rims appeared fully ossified at age 113 days of pregnancy, while they were incomplete at age 93-98 days of pregnancy. The ethmoid foramen was not yet formed at age 93-98 days of pregnancy, then it appeared as a small hole at age 113 days of pregnancy. There was complete ossification and partial articulation of the frontal process of the zygomatic bone and the zygomatic process of the frontal bone at age 113 days of pregnancy, while at age 93-98 days of pregnancy they were not ossified and completely articulated. Sutures were evident between the cranium bones. The occipital bone appeared as a bone composed of many bony parts. The occipital suture that distinguishes the occipital condyle and the squamous part of the occipital bone was visible and clear. The occipital condyles appeared fully developed and ossified, and the opening of the hypoglossal canal and the oval foramen were visible and clear also, the jugular process appeared fully developed, and the caudal end of the squamous part of the occipital bone continued to grow, as the occipital crest appeared at the beginning of its formation and the external occipital protuberance had not yet formed. The basilar part, which is the cartilaginous part of the occipital bone, was ossified, while the muscular tubercles had not yet appeared. The auditory bulla was ossified, but had a wide, semi-oval opening on its left and right lateral surfaces at age 113 days of pregnancy, while it had a wide, semi-oval opening only on the left side at age 93-98 days of pregnancy. The squamous part of the temporal bone, which includes the temporal crest of the zygomatic process and the temporal wall of the orbit, was fully developed at these ages, while the petrous part of the temporal bone, which included the papillary process and the external auditory meatus, was not yet fully developed, and the caudal end of the auditory bulla was not yet fully developed. It was noted that the presphenoid and basisphenoid bones were ossified along with the wing at these ages. The optical foramen was not yet visible. The medial ridge, which is positioned on the outer surface of the basisphenoid bone, had begun to grow at the age of 113 days of pregnancy. As for the ethmoid bone, the formation of the ethmoid plate (cribriform plate) was observed, with some holes in it. As for the Crista Galli which separates the ethmoid bone into two halves, was observed to ossify at the age of 113 days of pregnancy (Figure 4-9).

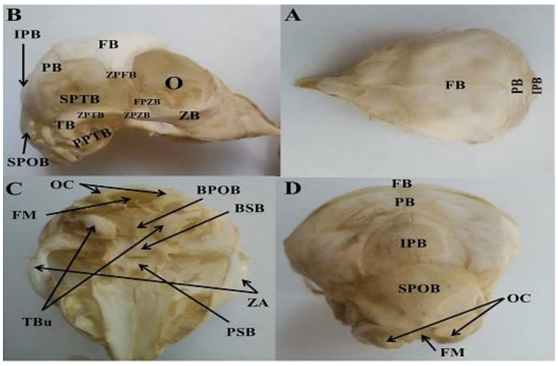


**Figure 4.** Showing skull of indigenous sheep fetus at 93 days, 13 weeks of pregnancy, prepared by maceration with NaOH solution. (A) dorsal view, (B) lateral view (right side), (C) ventral view, (D) occipital view: (FB) frontal bone, (PB) parietal bone, (IPB) interparietal bone, (SPOB) squamous part of occipital bone, (TB) temporal bone, (SPTB) squamous part of temporal bone, (PPT4) petrous part of temporal bone, (ZPTB) zygomatic process of temporal bone, (ZPFB) zygomatic process of frontal bone,

(ZB) zygomatic bone, (ZPZB) zygomatic process of zygomatic bone, (FPZB) frontal process of zygomatic bone, (O) orbit, (OC) occipital condyle, (FM) foramen magnum, (BPOB) basilar part of occipital bone, (BSB) basisphenoid bone, (PSB) presphenoid bone, ((TBu) tympanic bulla, (ZA) zygomatic arch.

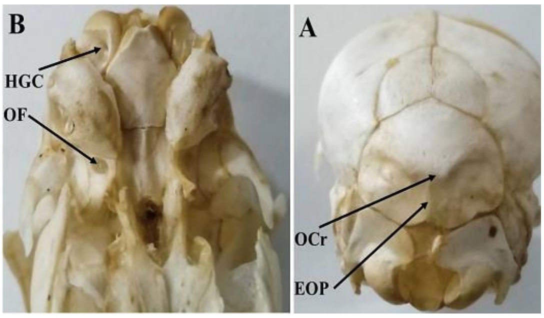


**Figure 5.** Showing skull of indigenous sheep fetus at 95 days, 13 weeks of pregnancy, prepared by maceration with NaOH solution. (A) occipital view, (B) ventral view: (OCr) occipital crest, (HGC) hypoglossal canal, (OF) oval foramen.

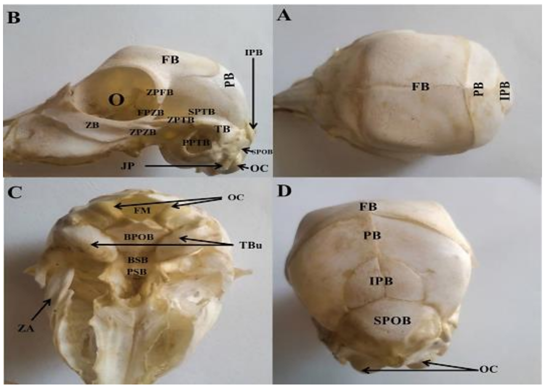


**Figure 6.** Showing skull of indigenous sheep fetus at 98 days, 14 weeks of pregnancy, prepared by maceration with NaOH solution. (A) dorsal view, (B) lateral view (left side), (C) ventral view, (D) occipital view: (FB) frontal bone, (PB) parietal bone, (IPB) interparietal bone, (SPOB) squamous part of occipital bone, (OC) occipital condyle, (TB) temporal bone, (SPTB) squamous part of temporal bone, (PPT4) petrous part of temporal bone, (ZPTB) zygomatic process of temporal bone, (ZPFB) zygomatic process of frontal bone, (ZB) zygomatic bone, (ZPZB) zygomatic process of zygomatic bone, (FPZB) frontal process of zygomatic bone, (O) orbit, (JP) jugular process, (FM) foramen magnum, (BPOB) basilar part of occipital bone, (BSB) basisphenoid bone, (PSB) presphenoid bone, (TBu) tympanic bulla, (ZA) zygomatic arch.

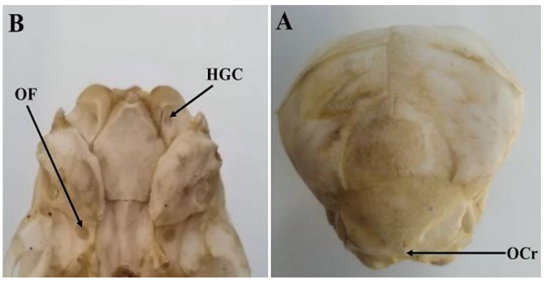


**Figure 7.** Showing skull of indigenous sheep fetus at 98 days, 14 weeks of pregnancy, prepared by maceration with NaOH solution. (A) occipital view, (B) ventral view: (OCr) occipital crest, (EOP) external occipital protuberance, (HGC) hypoglossal canal, (OF) oval foramen.

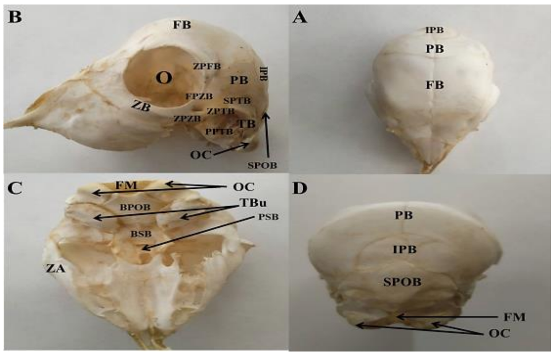


**Figure 8.** Showing skull of indigenous sheep fetus at 113 days, 16 weeks of pregnancy, prepared by maceration with NaOH solution. (A) dorsal view, (B) lateral view (left side), (C) ventral view, (D) occipital view: (FB) frontal bone, (PB) parietal bone, (IPB) interparietal bone, (SPOB) squamous part of occipital bone, (OC) occipital condyle, (TB) temporal bone, (SPTB) squamous part of temporal bone, (PPT4) petrous part of temporal bone, (ZPTB) zygomatic process of temporal bone, (ZPFB) zygomatic process of frontal bone, (ZB) zygomatic bone, (ZPZB) zygomatic process of zygomatic bone, (FPZB) frontal process of zygomatic bone, (O) orbit, (FM) foramen magnum, (BPOB) basilar part of occipital bone, (BSB) basisphenoid bone, (PSB) presphenoid bone, ((TBu) tympanic bulla, (ZA) zygomatic arch.

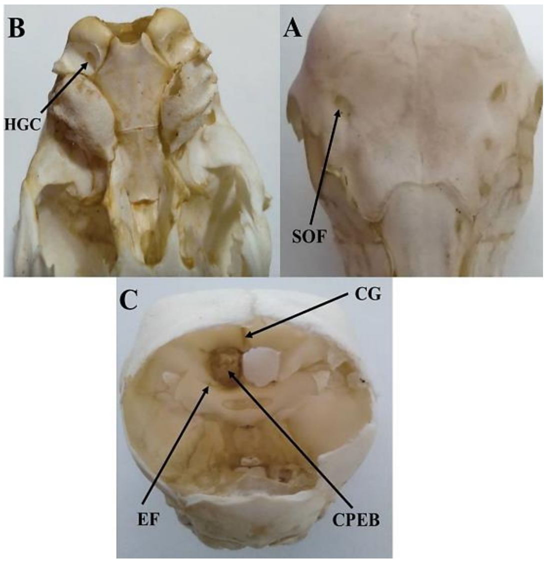


**Figure 9.** Showing skull of indigenous sheep fetus at 113 days, 16 weeks of pregnancy, prepared by maceration with NaOH solution. (A) dorsal-frontal view, (B) ventral view, (C) dorso-occipital view: (SOF) supraorbital foramen, (HGC) hypoglossal canal, (CG) crista galli, (EF) ethmoid foramen, (CPEB) cribriform plate of ethmoid bone.

## Radiography examination

The dorsal and lateral radiographs of the head of an indigenous sheep fetus at age 90 days, the thirteenth week of pregnancy showed that thin areas of growth and ossification of the frontal bone appeared, but not being fully completed, as areas transparent to radiolucent appeared in it. The parietal bone was growing on the right side rather than on the left side, and the inter-parietal bone was incomplete. The zygomatic process of the temporal bone was noted that the growth of the left side was complete rather than the right side, and the continued growth of the temporal bone caudally, the completion of ossification of the tympanic bulla and the external auditory meatus. The ossification of the squamous part of the occipital bone and the occipital condyles was observed, and the basilar part of the occipital bone was mostly completely ossified. The rostral, dorsal, and ventral rim of the orbit was completed, but the caudal rim was incomplete, and the sutures between some bones were not clear at this age (Figure 10).

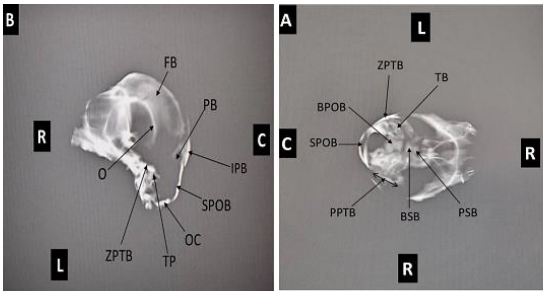


**Figure 10.** Radiographs of the skull of an indigenous sheep fetus, aged 90 days of pregnancy. (A) (dorsal view): TB: Tympanic bullae, ZPTB: Zygomatic process of the temporal bone, PPOB: Basilar part of the occipital bone, SPOB: Squamous part of the occipital bone, PPTB: Petrous part of the temporal bone, BSB: Basisphenoid bone, PSB: Presphenoid bone. (B) (lateral view) FB: Frontal bone, PB: Parietal bone, IPB: Inter-parietal bone, SPOB: Squamous part of the occipital bone, OC: Occipital condyles, ZPTB: Zygomatic process of the temporal bone, EAM: External auditory meatus, O: Orbit.

The radiographs of the head of an indigenous sheep fetus at age 105 days, fifteenth week of pregnancy, showed that the frontal bone and the inter-parietal bone were completely ossified, as these bones appeared radiopaque. In the parietal bone and the squamous part of the occipital bone, an increase and extension of ossification was observed, although it was not completely completed. The bones of the base of the skull, represented by the basilar part of the occipital bone, the occipital condyles, and the sphenoid bone, their growth has been completed, with the appearance of sutures between them, along with the completion of the caudal rim of the orbit. The zygomatic process of the temporal bone was completed, thus completing the squamous part and the petrous part of the temporal bone along with the external auditory meatus and the tympanic bulla (Figure 11).

The radiographs of the heads of indigenous sheep fetuses at ages 117-126-130 days, seventeenth, eighteenth, and nineteenth week of pregnancy showed an increase in the thickness of the bone material and the size of the cranium bones, represented by the frontal bone, the parietal bone, and the inter-parietal bone. The bones of the base of the skull, represented by the basilar part of the occipital bone, the sphenoid bone; and the sutures between them, have completed their growth. At this age, the temporal bone with its squamous and petrous parts is also completed (Figure 12).

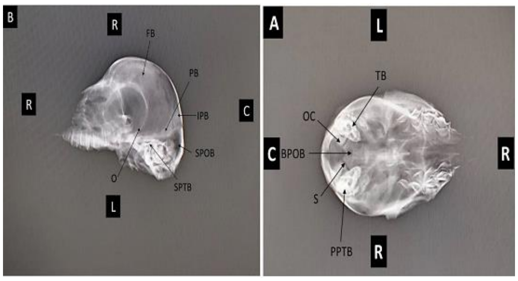


**Figure 11.** Radiographs of the skull of an indigenous sheep fetus, aged 105 days of pregnancy. (A) (dorsal view): TB: Tympanic bulla, PPTB: Petrous part of the temporal bone, OC: Occipital condyle, BPOB: Basilar part of the occipital bone, S: Suture. (B) (lateral view) FB: Frontal bone, PB: Parietal bone, IPB: Inter-parietal bone, SPOB: Squamous part of the occipital bone, SPTB: Squamous part of the temporal bone, O: Orbit.

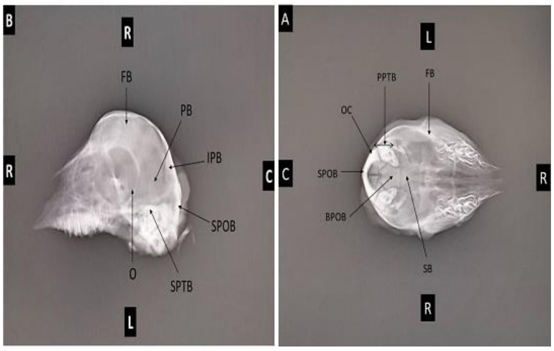


**Figure 12.** Radiographs of the skull of an indigenous sheep fetus, aged 126 days of pregnancy. (A) (dorsal view) FB: Frontal bone, PPTB: Petrous part of the temporal bone, OC: Occipital condyles, SPOB: Squamous part of the occipital bone, BPOB: Basal part of the occipital bone, SB: Sphenoid bone. (B) (lateral view) FB: Frontal bone, PB: Parietal bone, IPB: Inter-parietal bone, SPOB: Squamous part of the occipital bone, SPTB: Squamous part of the temporal bone, O: orbit.

The radiographs of the heads of indigenous sheep fetuses at ages 147 and 155 days, twenty-one week and twenty-two week of pregnancy, showed that growth with an increase in the thickness of the frontal bone, the parietal bone, the inter-parietal bone, the squamous part of the occipital bone, and the occipital condyles, were completed which appeared radiopaque with the increase in the size of the skull and an increase convexity of the frontal, parietal, and inter-parietal bones gave these bones a dome-like appearance to the skull. The growth of the temporal bone, with its squamous and petrous parts, was completed, along with the completion of the orbital rims, and the completion of the growth of the bones of the base of the skull (the basilar part of the occipital bone and the sphenoid bone, with its two parts) (Figure 13).

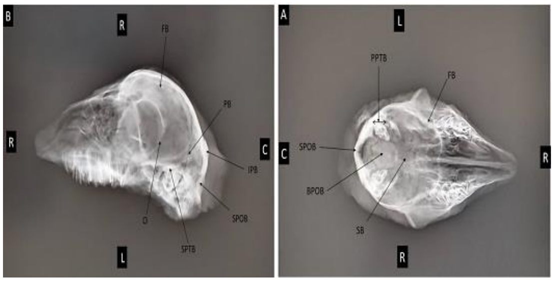


**Figure 13.** Radiographs of the skull of an indigenous sheep fetus, aged 147 days of pregnancy. (A) (dorsal view) FB: Frontal bone, PPTB: Petrous part of the temporal bone, SPOB: Squamous part of the temporal bone, BPOB: Basilar part of the occipital bone, SB: Sphenoid bone, (B) (lateral view) FB: Frontal bone, PB: Parietal bone, IPB: Inter-parietal bone, SPOB: Squamous part of the occipital bone: SPTB: Squamous part of the temporal bone, O: Orbit.

### Maceration using fly larvae

Fly larvae were used as an alternative to traditional methods that may take longer or require devices or chemical materials. After moistening them, the samples were placed in a container in the open air and exposed to flies. After detecting the presence of small larvae, the sample was kept in an incubation box for safekeeping in cold weather, and a lighting source was also been placed for heating in the evening. The wetting stage, where the skull was immersed in water in the first stage, and the first wetting stage had to be repeated for some of them if the sample was noticed to have dried out. In the first examination, eight days after the samples were exposed to flies, it was noted that samples of small-sized fetuses aged 90 days, at the end of 2nd trimester of pregnancy; and samples of medium-sized fetuses aged 105-117-126-130-147 days, in 3rd trimester of pregnancy (Figure 14), the bones separated from each other; and the fetus that was 155 days old, at the end of 3rd trimester of pregnancy (Figure 15&16), was noted that the facial bones separated and the cranium bones remained connected after two weeks, but some of the bones of the skull base separating after the washing process. At the end of the maceration process, clean bones were obtained, but they were not articulated, that is, separated from each other. This allowed these bones to study them separately, measuring bone surface area, and comparing the features and details of each bone at different stages of pregnancy.

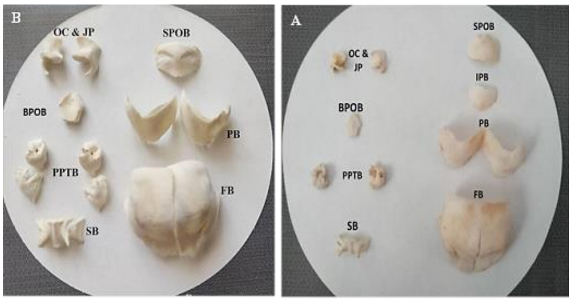


**Figure 14.** Showed the cranium and skull base bones of an indigenous sheep fetus (A) at thirteen weeks of pregnancy, (B) at eighteen weeks of pregnancy prepared by maceration with fly larvae method. SPOB: Squamous part of the occipital bone, IPB: Inter-parietal bone, PB: Parietal bone, FB: Frontal bone, OC&JP: Occipital condyles & jugular process, BPOB: Basilar part of the occipital bone, PPTB: Petrous part of the temporal bone, SB: Sphenoid bone.

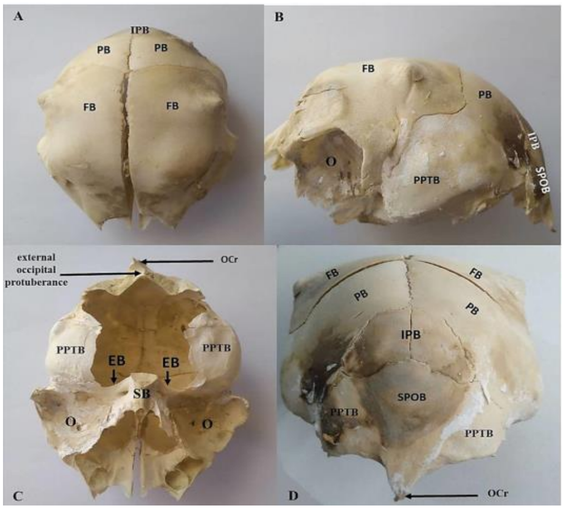


**Figure 15.** Showed the cranium and skull base bones of an indigenous sheep fetus at twenty-two weeks of pregnancy, prepared by maceration with fly larvae method. (A) (dorsal view): FB: Frontal bone, PB: Parietal bone, IPB: Inter-parietal bone, (B) (lateral view): SPOB: Squamous part of the occipital bone, PPTB: Petrous part of the temporal bone, O:Orbit, (C) (ventral view): OCr: Occipital crest, EB: Ethmoidal bone, PSB: Pre-sphenoid bone, (D) (caudal view).

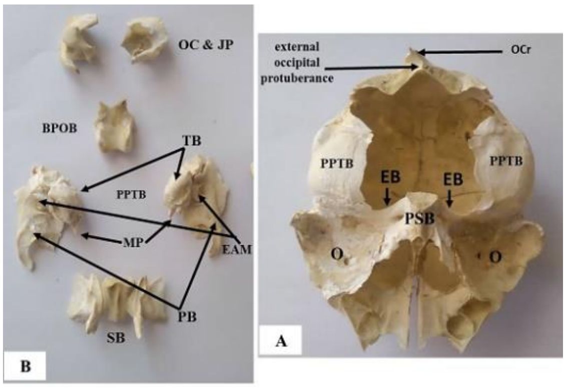


**Figure 16**. Showed the cranium and skull base bones of an indigenous sheep fetus at twenty-two weeks of pregnancy, prepared by maceration with fly larvae

method. (A) (ventral view): OCr: Occipital crest, EB: Ethmoidal bone, PPTB: Petrous part of the temporal bone, SB: Sphenoid bone, O:Orbit, OC&JP: Occipital condyles & jugular process, BPOB: Basilar part of the occipital bone, TB: Tympanic bulla, PPTB: Petrous part of the temporal bone, MP: Muscular process, EAM: External acoustic meatus, PB: Pterygoid bone.

## Discussion

The results of the current study are similar to what [21] reported about the development of the skull before and early after birth in Nigerian sheep breeds, they indicated that in 2nd trimester, ossification of the frontal, parietal, occipital, and basal sphenoid bones was observed. The results of the current study contradict what was mentioned in their study is that the interparietal bone was absent in 2nd trimester and appeared in 3rd trimester of pregnancy. The results of the current study found that the interparietal bone appeared ossified in the middle and end of 2nd trimester of pregnancy in local Iraqi sheep breeds, and the results of the current study differed also from [22] who mentioned that the interparietal bone was absent during the fetal stage and was formed at birth and merged on the 20th day after birth in Spanish sheep breeds. This difference could be due to the basic genetic factor that the breed of sheep affects the development of the skeleton, in addition to the difference in environment between the two countries [23, 24]. The results of the current study agreed with [19] in their study of radiography of the skull and calvaria histogenesis before birth in the Uda and Yankasa sheep breeds, where they mentioned that the ossification of the parietal and frontal bones was not completely complete after the neuron skull has an X-ray-permeable area in the rostral fontanelle, and the size of the X-ray-permeable area varies depending on the size of the fetus. The X-ray-permeable rostral fontanel increases in size as the fetus grows toward the end of 2nd trimester of pregnancy [25]. The results of the current study are also similar to those of the researchers mentioned above, in the late 3rd trimester, it was detected that the zygomatic process of the frontal bone and the frontal process of the zygomatic bone ossify and converge to form the orbital rim. The results of the current study differed from [21] in terms of the growth and development of the jugular process, the squamous part of the occipital bone, and the external occipital protuberance began in 3rd trimester of pregnancy in the studied Nigerian sheep breeds, while these bones appeared in the local Iraqi sheep in the middle and end of 2nd trimester of pregnancy, this may be due to poor maternal nutrition for Nigerian sheep, which affects the health status of the fetus's growth and the main genetic factor that affects the development of the skeleton, which is the breed of sheep, in addition to the difference in environment between the two countries [23, 24].

The results of the radiographs of the head of an indigenous sheep fetus at age 90 days, approximately at the end of 2nd trimester of pregnancy, showed that the parietal bone, the inter-parietal bone, the squamous part of the temporal bone, and the caudal rim of the orbit had not yet fully ossified, as they appeared transparent to radiolucent, while the frontal bone, the sphenoid bone, and the basilar part of the occipital bone appeared ossified. In 3rd trimester of pregnancy, all the cranium bones appeared radiopaque, meaning they were ossified, and the joints connecting the skull bones (the sutures) were not completely clear in the radiographs that had been made.

These results agreed with [19] in their research on prenatal radiography of the skull and calvaria histogenesis in the Uda and Yankasa breeds of sheep, in that the frontal and parietal bones have not yet ossified in 2nd trimester of pregnancy, with the neurocranium containing a radiolucent area known as the rostral fontanel, where the size of the radiolucent area varied according to the size of the fetus, as the size of the fontanelle increases with the increasing of the growth of the fetus towards the end of 2nd trimester. The rims of the orbit and teeth in the upper and lower jaws have not yet appeared. The results of the current study also agreed that the zygomatic process of the frontal bone and the frontal process of the zygomatic bone were ossified and articulated to complete the caudal rim of the orbit in the late 3rd trimester of pregnancy.

The results of the current study did not agree with the results of [26] who studied the evaluation of skeletal ossification of Iranian sheep fetuses in the stages of pregnancy from the age of 20 to 95 days using radiography and complete staining of the skeleton, as they found that in the radiography of a fetus aged 67 days of pregnancy, the skeleton can be seen ossified. All parts of the skull, forelimbs and hind limbs, spinal vertebrae, and ribs appeared ossified and clearly defined in the fetus aged 78 days, and by comparing the radiographs of ages 65 to 95 days of pregnancy, the process of ossification of the fetus's skeleton had been completed, the results of the current study showed that at the age 90 days of pregnancy, the parietal bone, the inter-parietal bone, the squamous part of the temporal bone, and the caudal rim of the orbit had not ossified completely.

The results of [7] in their study of the heads of fetal camels with one hump also showed that the beginning of calcification can be identified even in the first three months by the opaque appearance of the skull (which appears whiter in radiograph). This appearance increases with increasing gestational age, as fetuses in 3rd trimester of pregnancy are more radiopaque because the skull is completely ossified. The results of the current study agreed with these results in that in 3rd trimester of pregnancy the skull bones were completely ossified.

[27] who studied bone formation in the skull of cow fetuses, they found that at the age of 97 days of pregnancy, that is, at the beginning of 2nd trimester of pregnancy, five osteogenetic loci that form the occipital bone, and at this age, only three among them can be observed, which are the squamous part, the occipital condyles, and the basal part of the occipital bone. The results of the current study showed that at the end of 2nd trimester of pregnancy, there were parts that appeared to ossify in the squamous part of the occipital bone, the occipital condyles, and the basal part of the occipital bone, but these parts did not ossify completely. They also mentioned that in the region of the temporal bone, which arises from multiple primary and secondary ossification centers, the tympanic part is a specific area that forms the tympanic ring is also incomplete dorsally at the beginning of 2nd trimester of pregnancy in cows, and differs from our results in terms of the date of appearance, as it appeared in sheep at the end of 2nd trimester, this difference is due to the difference in the duration of pregnancy between cows and sheep.

The results of the current study using the fly larvae showed that the bones of the skull (the cranium and the skull base) appeared clean and separated from each other at young ages, while at the ages close to birth they also appeared clean and not separated from each other after the end of the maceration process using larvae, and these results were consistent with [2] who compared different maceration methods using Dermestid larvae, potassium hydroxide, and sodium hypochlorite in the skulls of Wistar rat cranium, the results of their study showed that maceration using insect larvae is an ideal method for removing soft tissue with preserving all bone components, while maceration with potassium hydroxide was an effective method, but because it’s a highly corrosive chemical agent, was aggressive on bone tissue, while successful maceration could not be achieved with sodium hypochlorite. The results of the current study were also consistent with other studies conducted using fly larvae on other parts of the skull, such as the jaw bone, [16] studied the development of the jaw bone in local sheep embryos using fly larvae method where the jaws were clean and separated from each other and were suitable for anatomical and measurement study; the researchers stated that using fly larvae method is an effective method to preserve samples from the effects of chemicals and also saves on the use of chemicals and allows studying each one individually; and were consistent with the study carried out by [20], who also used maceration by fly larvae, for the heads of goat fetuses where he got the skull bones clean and separated from each other.

## Conclusion

Maceration by NaOH solution macerates fetuses' skulls in a speedy and odorless method, nonetheless, quick consideration should be given to the fetuses' skulls when using this compound technique so that the concentration used is in increasing concentration demand to escape softening, fracturing, and melting the fetuses skulls bones. Only bones of the fetus skulls of the indigenous sheep were well ossified can be seen in the radiograph. Fly larvae are valuable as a method to clean fetuses' skulls since they do a brilliant debridement of the tiniest holes nonetheless numerous thousands are necessary to create fast cleaning and if left with fetuses' skulls for an extended time can consume and terminate them.

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## Conflict of interests

The authors declare no potential conflict of interest in the publication of this research paper.

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