

Prenatal Development of Pinna in Indian Sheep

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Received: 28 July 2025; Accepted: 16 September 2025

ABSTRACT

Gross and microanatomical studies of pinna were conducted in embryos and fetuses collected from Nellore sheep aged between 22 to 145 days. External ear comprised of pinna (Auricle) and external acoustic meatus. Morphogenesis revealed that the otic placode and four pairs of pharyngeal pouches were visible externally in the ventro-lateral aspect of neck region by 22 days. Five aural hillocks were appeared at 23 days. Auricle shifted its position from neck to temporal region by 63 days. The pinna presented 5-6 prominent ridges by 74 days. Histogenesis revealed that the first and second pharyngeal arches developed with an intervening first pharyngeal cleft by 22 days. Small cone shaped pinna at 24 days modified into irregular sigmoid shaped structure with mesenchymal condensation at the centre of the auricle in 31 days embryos. Differentiation of hair follicles was first initiated on the auricular epidermis by 69 days and presence of elastic cartilage in the center of pinna by 80 days. Biometric analysis of pinna revealed that the average length, width increased significantly between different age groups. It may be concluded that the development of pinna continues postnatally also in sheep.

Keywords: Auricle, Pinna, Morphogenesis, Histogenesis, Morphometry, Sheep Fetus

INTRODUCTION

The external ear comprises the pinna (Auricle) and the external acoustic meatus. The pinna is visible on either side of the head; its function is to direct the vibrations of sound waves into ear canal towards the tympanic membrane (eardrum) and serves to protect the middle and inner ear. Unlike in man, the auricle can be moved in different directions without turning the head by means of ear muscles in the domestic mammals. Much of our understanding of external ear has been derived from the adult mammals. Indian sheep have a gestation period of 142-154 days with individual and breed variations. Many well documented studies are available in the field of production, reproduction and other aspects of Sheep biology but the developmental anatomy of sheep is still a growing branch of veterinary medicine in India. However, the anatomical and morphometrical study of middle ear ossicles was conducted in 2 to 3-month-old Makouei sheep fetuses (Simaei *et al.*,

2017) and some literature was also present on the sequential changes in the ossification pattern and morphogenesis of middle ear ossicles in Nellore sheep fetuses (Karunasri *et al.*, 2018 & Karunasri *et al.*, 2023) but there is a paucity of literature on the morphological and histological features on the prenatal development of the pinna in Indian sheep and also worldwide. The details mentioned in the present study may help the veterinarians and researchers in understanding the teratology of pinna in external ear of sheep in a systematic manner.

MATERIALS AND METHODS

The present study was conducted on 60 local Nellore sheep embryos and fetuses. The embryos and fetuses irrespective of the sex were collected immediately after slaughter from uterus of adult sheep at the abattoirs in and around Vijayawada, Andhra Pradesh. These were collected irrespective of age and nutritional status of the mother. Therefore the approval of ethical committee was not required. Some of the term fetuses were collected from sheep (undergone dystocia) immediately after caesarean section from department of Veterinary Gynecology and Obstetrics, N.T.R. College of Veterinary Science, Gannavaram with the willingness of owner.

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The fetuses were randomly collected from 22 days (0.9 cm Crown Rump Length (CRL)) to 145 days (42 cm CRL). Among these 60 fetuses, 42 were male, 10 were female and 8 were undifferentiated grossly. The CRL was measured from the most anterior part of crown to the base of tail by using a measuring scale and thread. The embryos and fetuses were categorized into twenty three numbers in group I (< 7.0 cm CRL), twenty nine in group II (< 25.0 cm CRL) and eight in group III (>25.0 cm CRL) on the basis of crown rump length (CRL). The approximate age of embryos and fetuses was determined on the basis of their CRL up to 3 cm CRL length (Bryden *et al.*, 1972) and for later ages by using the following formula (Hejazi *et al.*, 2011).

$$X = 2.74Y + 30.15$$

Where 'X' was the age of fetus and 'Y' was the crown rump length of fetus in cm. The earliest age embryos and fetuses were collected using a magnifying lens and flushing of the uterus. After measuring the CRL, fetuses were dissected; gross anatomical positions of both the pinnae were noted. Based on the position of pinna, the external acoustic meatus was identified and dissected along with tympanic bulla and inner ear in age groups II and III.

For histological study, entire heads were collected from 22 days (0.9 cm CRL) to 46 days of gestation (5.5 cm CRL). Serial sections were made for histogenesis since the ears could not be separated from heads at this age. Rest of the gestational fetuses, after recording morphological details, the petrous temporal part of head along with pinna was cut for proper fixation by adopting cross, frontal and sagittal planes. The ear samples were fixed in 10% Neutral buffered formalin and Bouin's fluid. The fixed samples were subjected to standard processing techniques for histological studies (Luna, 1968). The tissues were processed and paraffin sections of 5-8 μ m thickness were stained by the Haematoxylin and Eosin (H&E) stain, Masson's Trichrome staining method for the development of collagen fibres (Luna, 1968) for histological study. Paraffin sections of 5-8 μ m thickness were stained with Periodic Acid Schiff (PAS) technique for mucopolysaccharides (Bancroft and Gamble, 2003).

RESULTS AND DISCUSSION

Morphogenesis

In group I, the otic placode and four pairs of pharyngeal pouches were visible externally in the

ventro-lateral aspect of neck region by 22 days (0.9 cm CRL) of gestation in sheep. Five aural hillocks were appeared at 23 days (1 cm CRL) which fused to form the pinna later (Fig.1a). Similarly, Sinowatz (2013) described that the nodular masses of mesenchyme take shape along each side of first pharyngeal cleft early in development ultimately fuse to form the auricle as age advances in domestic animals. However, Bryden *et al.* (1972) mentioned that aural hillocks were visible on 25th day indicated the early development of auricle in ovine fetus. Whereas, Wareing *et al.* (2014) and Wright (1997) stated that development of the auricle began at five weeks of gestation with development of the auricular hillocks numbered from one to six derived from the first (mandibular) and second (hyoid) branchial arches in humans.

Pinna was visible externally little above the neck region in 24 days (1.3 cm CRL) sheep embryos (Fig.1b). Medial to the auricle a depression was present; the future external acoustic meatus (EAM) appeared first at this age. In later days, shape of the pinna was changed from round hillocks to small spatula. The auricle appeared as a short triangular flap covering the orifice of the external auditory meatus during 31 to 46 days of gestation. By 49 days, the shape of auricle was modified into an elongated triangular flap. Similar findings were made by Bruzewicz (2000) in swine fetuses.

In group II, pinna/auricle was soft and flap like in 52 days (8 cm CRL) fetuses, but it was first erected and appeared as small cone shaped structure by 63 days (12 cm CRL) (Fig.2). Pinna was gradually shifted its position from the neck region to temporal region by this age according to Kagurasho *et al.* (2012) and Sinowatz (2013) in humans and in domestic animals but the changes of auricular location were the result of intensive growth of the mandible in domestic animals as per Noden and de Lahunta (1985). However, during the initial stages of its development, the auricle was located in the general area of the neck, behind the lower jaw, but by the 20th week of gestation it moved upward to attain its adult location and overall configuration according to Wright (1997) in humans. In the present study, the ridges appeared on the internal surface of pinna at 63 days. At 74 days (16 cm CRL) the pinna presented 5-6 prominent ridges (Fig.3) which are corroborated with the findings of Bruzewicz (2000) that the ridges were noted as tragus, anti tragus, helix, incisura intertragica, plica craniale, plica mediale, caudal

plica longitudinalis, plica antitragra during 58 to 115 days in swine fetuses.

In group III, the ridges were very prominent on pinna in addition to these, hairs were apparent on external acoustic meatus (EAM) and pinna by 126 days (35 cm CRL). As age advanced pinna was folded at edges and modified to form pendulous pinna at 145 days (42 cm CRL). The morphometrical analysis revealed that the average length, width of pinna at middle point and width of pinna at base gradually increased as age advanced from 36 to 140 days of gestation (Table 1). However, Bruzewicz (2000) stated that the increase of the auricular height seemed to be more intensive than the growth in width of the auricle in swine fetuses.

Table 1: Morphometric analysis of pinna at different ages in sheep fetuses (Mean±SE).

S.No.	Age of fetus (days)	length of pinna (cm)	Width of pinna at middle point (cm)	Width of pinna at base (cm)
1	36	0.413±1.05	0.2±0.051	0.183±1.083
2	46	0.63±1.052	0.281±1.013	0.28±1.0376
3	52	0.91±2.031	0.37±1.051	0.286±1.418
4	58	0.993±1.067	0.49±0.071	0.49±2.071
5	63	1.116±1.213	0.59±2.052	0.486±1.081
6	66	1.205±1.15	0.683±3.051	0.59±0.883
7	71	1.513±0.087	0.79±1.052	0.783±1.912
8	85	2.79±1.03	1.786±3.033	1.49±2.103
9	93	3.866±2.057	2.283±2.075	1.496±0.508
10	134	4.693±2.013	2.19±1.805	2.156±1.028
11	140	6.79±3.705	2.683±1.056	2.65±2.062

Histogenesis

In group I, at 22 days (0.9 cm CRL) of embryonic life, four pairs of pharyngeal pouches were identified at the ventro-lateral aspect of developing head and neck in the present study. First three pairs were pushed towards ectoderm in between branchial arches and 4th pair did not reach the ectoderm. The first and second pharyngeal arches developed with an intervening first pharyngeal cleft which was closely opposing otic capsule in 24 days embryos (Fig.4). These findings were in accordance with Wright (1997) in humans and Sinowatz (2013) in domestic animals.

During 31-49 days of gestation, pinna modified into irregular sigmoid shaped structure with mesenchymal cell condensation at the centre as a concentric / regular line throughout the auricular

length in 31 days embryos. The mesenchymal cell population was increased and modified as precartilaginous mass and occupied approximately half of its width. The shape of the auricle changed to elongated comma by 39 days (3.3 cm CRL) (Fig.5). Auricle was highly vascularised with single layer of cuboidal cells covering externally and many layers of cells towards the inner surface.

In group II, pinna was in precartilaginous form and cells were oriented as a nodule for future conchal cartilage during 55 to 60 days. Developing hair follicles were first appeared on the auricular epidermis by 69 days (Fig.6). Lining epithelium of pinna was modified to stratified squamous cells with developing hair follicles on either surface; presence of elastic cartilage in the center of pinna by 80 days. The superficial layers of stratified squamous epithelium were keratinized with increased size, number of hair follicles and sebaceous glands on both the surfaces of auricle. Conchal cartilage increased in width and placed at the center of auricle by 99 days (Fig.7). These findings were in accordance with Wright (1997) in humans at 22 weeks of gestation.

In group III, the stratified squamous epithelium of auricle and EAM was progressed in keratinisation with well developed hair follicles and sebaceous glands during 104 (27 cm CRL) to 140 days (40 cm CRL) gestation as in adult (Fig.8). Similarly, Wright (1997) stated that the auricle was well formed by the time of birth but did not attain their full size and adult configuration until about 9 years of age.

CONCLUSION

The timeline in the development of pinna in sheep can be compared with other mammalian species including humans. This may be useful in understanding the developmental evolution. The information about the prenatal development of auricle can be useful as an adequate option for training and research in otologic surgery in humans. The biometric and histo-morphogenesis data of auricle may be introduced as an accessory method of evaluating the fetal age of sheep. The development of auricle continued postnatally also in Sheep.

ACKNOWLEDGEMENT

Authors are thankful to Sri Venkateswara Veterinary University for providing the necessary infrastructure, laboratory facilities and chemicals to complete the research work.

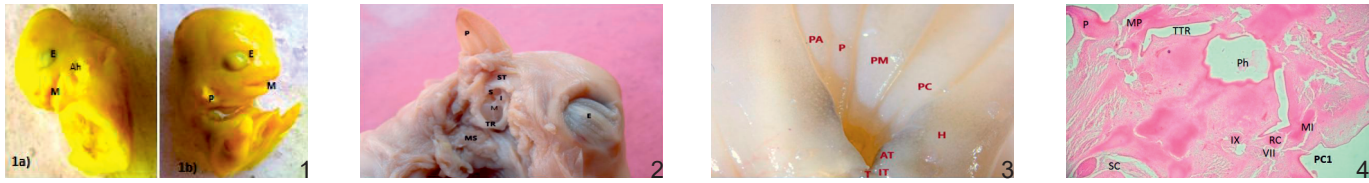


Fig 1: Stereozoom photograph showing 1a) Head of embryo at 23 days 1b) Head of embryo at 24 d Ah- Aural hillocks,P-Pinna, E- Eye; **Fig 2:** Stereozoom photograph showing dissected external and middle ear at 63 days M-Malleus,I- Incus,S- Stapes, P-Pinna,ST-Squamous Temporal bone,TR- Tympanic Ring,MS-Mandibular Salivary gland; **Fig. 3:** Stereozoom photograph showing the folds on pinna at 74 days, T-Tragus,AT-Anti Tragus,H- Helix, IT-Incisura interTragica,PC- Plica Craniale, PM- Plica mediale, P- caudal plica longitudinalis, PA- Plica Antitragica; **Fig 4:** Photomicrograph showing external and middle ear at 24 days P-Pinna,Ph-Pharynx, TTR-Tubo Tympanic Recess, Sc-Spinal cord, VII- 7th cranial nerve, IX- 9th cranial nerve, RC- Reichert's Cartilage, PC1-Pharyngeal Cleft1, MI-Malleus Inus complex, MP-Meatal Plate. H&EX40;

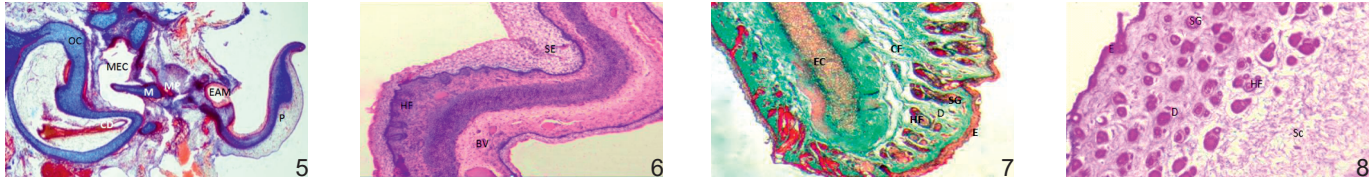


Fig 5: Photomicrograph showing external, middle and inner ear at 39 days. P- Pinna, M- Malleus, EAM-External Auditory meatus, MP- Meatal Plate, OC- Otic Capsule, CD- Cochlear Duct, MEC- Middle Ear Cavity. Masson's TrichromeX100; **Fig. 6:** Photomicrograph showing the development of pinna at 69 days. HF- Hair Follicle, SE- Stratified squamous epithelium, BV- Blood vessel. H&E 100X; **Fig 7:** Photomicrograph showing the development of pinna at 99 days. HF- Hair Follicle, E-Epidermis, D-Dermis, EC-Elastic Cartilage, SG- Sebaceous Glands, CF- Collagen Fibres. Masson's Trichrome Staining X 100; **Fig 8:** Photomicrograph showing the pinna at 140 days of gestation. HF- Hair Follicle, E- Epidermis, D-Dermis, SG- Sebaceous Glands, Sc-Subcutis. PAS stainingX100

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