



*Original Research*

## Pronephros in Sheep (*Ovis aries*)

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### Abstract

The present research work carried out in 11 embryos and fetuses of local Nellore sheep from 20 days (0.7 cm CRL) to 31 days (2 cm CRL) of gestation. The tissue samples were fixed in 10% NBF and Bouin's fluid and processed for routine histological studies. The pronephros was located on either side of the dorsal aorta which was surrounded by undifferentiated mesenchymal cells in 20 day (0.7 cm CRL) old embryo. It showed one giant glomerulus of  $225 \pm 1.3 \mu\text{m}$  in size and many tubules were present in the pronephros, some tubules were fully formed and some other was incomplete and open type. At 27 days of gestation (1.7 cm CRL) the degenerative changes were noticed in glomerular network due to which vacuolation was noticed between the tuft of glomerulus. The complete degeneration of pronephros with proliferating mesenchymal tissue replaced with degenerated structures by 31 days (2 cm CRL) of gestation.

**Key words:** Glomerulus, Histogenesis, Pronephros, Tubules

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### Introduction

Sheep plays a significant role in the agricultural economy of several tropical countries and occupies unique position among livestock. Down through the centuries, rearing of sheep has remained a primary occupation for a vast section of the rural population in India. Sheep rearing is a major source of income for landless labor and small and marginal farmers of semi-arid regions in Andhra Pradesh (Ekambaram *et al.*, 2013). Development of the pronephros and mesonephros, prior to the origin of metanephros, has been exemplified in man and animals as an expression of retained ancestral characters of urinary system during early embryonic life. The present study was therefore, planned in sheep embryos of different gestation periods to elucidate histogenesis and degeneration of the pronephros.

### Review of Literature



### **Pronephros**

Fraser (1920) studied the inter-relationship between pronephros and mesonephros in cat and concluded that the two organs developed in a strictly cranio-caudal sequence with no sharp dividing line between them. Davies (1951) reported that the intermediate cell mass at the 7<sup>th</sup> segment and for some distance caudally, presented a well-marked bulge dorsally in sheep foetus. It was referred to as a 'nephric ridge' since the pronephric structures were doubtful. At 11- 12 somite stage, the nephric ridge showed differentiation and separation from its dorsal margin of a rod of cells and the appearance of vesicles was initiated. The duct primordium was closely applied to the ectoderm at about the level of the future 14<sup>th</sup> somite. Pronephros persisted until late stage of embryonic life. According to Gopinath and Singh (1998), there was aggregation of loose mesenchymal tissue at the cranial end of the mesonephros, around pre-existing pronephric ducts thus representing its site of occurrence in 6 mm CRL (15 day) goat embryos. The area of pronephros changed into an organized mass of fibrous tissue by 10 mm CRL (20 day) stage with the progressive condensation of mesenchymal cells.

### **Pronephric Glomeruli**

As per Davies (1951), at 14 somite stage in sheep embryo, the nephrogenic cord showed intense angioblastic activity and development of the glomerular complex. About fourteen vesicles were observed in the nephric ridge dorsal to nephrogenic cord. In 23 somite stage, the glomerular complex was located approximately from 7<sup>th</sup> to 14<sup>th</sup> or 15<sup>th</sup> somite, and was almost completely developed. At 29 somites stage, the glomerular complex was almost fully developed and probably functional as judged by the histological and anatomical appearances.

### **Pronephric Tubules**

At 23 somites stage of sheep embryo, the associated tubules (about twenty-five) were short and entirely dwarfed by the massive glomerular pronephric complex that developed ventrally. The most rudimentary tubule was present at the caudal most end (Davies, 1951).

### **Materials and Methods**

The present study was conducted on 11 local Nellore sheep embryos and foetuses. The embryos and foetuses were collected immediately after slaughter from the abattoirs located in and around Vijayawada, Andhra Pradesh. The foetuses were randomly collected from 20 days (0.7 cm CRL) to 31 days (2 cm CRL). The approximate age of embryos and foetuses was determined on the basis of their CRL upto 3 cm CRL size (Bryden *et al.*, 1972). The number of specimens collected based on their age (Table1).

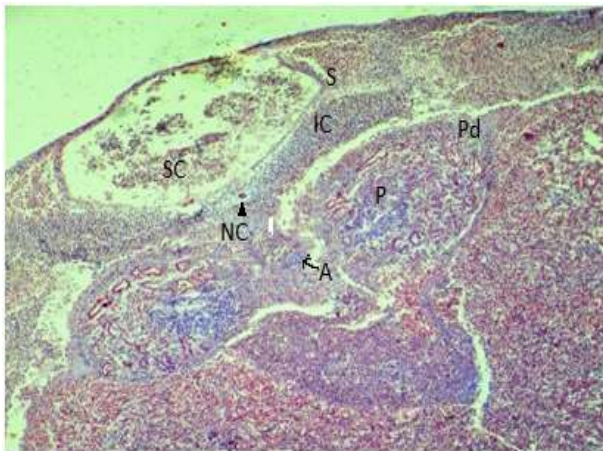
**Table1:** Showing age of embryos based on their crown rump length (CRL)

| No. of Animals | CRL (cm) | Gestational Age (days) |
|----------------|----------|------------------------|
| 2              | 0.7      | 20                     |
| 1              | 1.1      | 24                     |
| 2              | 1.5      | 25                     |
| 1              | 1.7      | 27                     |
| 2              | 1.9      | 29                     |
| 3              | 2        | 31                     |

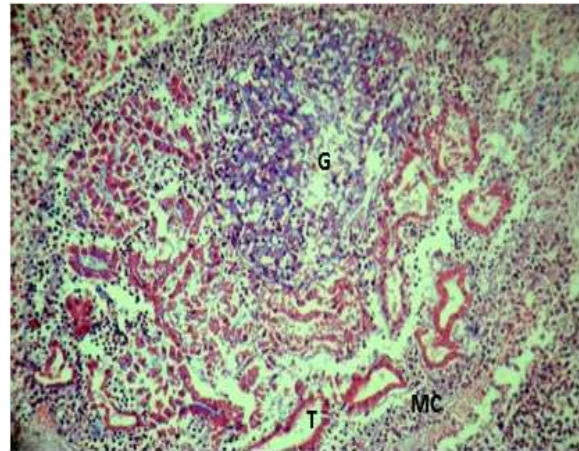
## Result and Discussion

### Pronephros

The pronephros in 20 days (0.7 cm CRL) old sheep embryo was located on either side of the dorsal aorta at the level of body somites and ventral to the intermediate cell mass towards the dorsal median line. It was surrounded by undifferentiated mesenchymal cell mass of nephric ridge (Fig.1). Davies (1951) reported that the intermediate cell mass was present at 7<sup>th</sup> segment from its anterior to caudal end and appeared as a wellmarked dorsal bulge referred to as nephric ridge. Pronephric vesicles were surrounded by a layer of mesenchymal cells and fibers (Fig. 2).



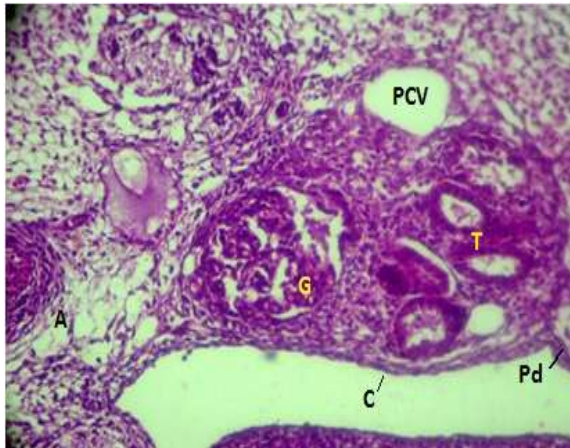
**Fig.1:** Photomicrograph of 20 day old sheep embryo showing pronephros (Masson's Trichrome X100). (S-Somite, SC-Spinal cord, IC-Intermediate cell mass, NC-Notocord, A-Dorsal aorta, P-Pronephros, Pd-Pronephric duct)



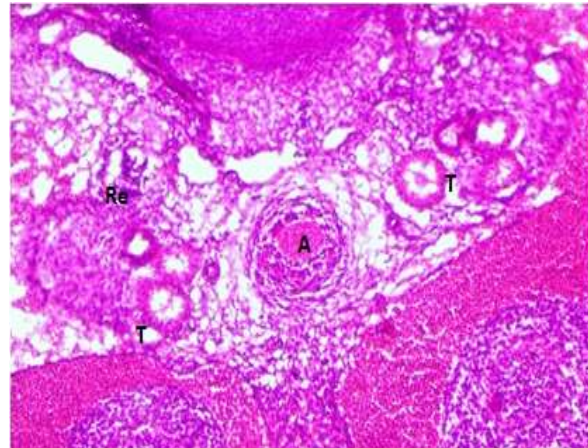
**Fig.2:** Photomicrograph of 20 day old sheep embryo showing pronephric glomerulus (Masson's Trichrome X400). (G-Glomerulus, T-Tubules, MC- Mesenchymal)

At 24 days (1.1 cm CRL) of gestation, the pronephros was surrounded by a thin layer of connective tissue capsule which was lined by squamous to cuboidal cells. The posterior pole of pronephros that represented the future opening of pronephric duct was lined by stratified cuboidal cells (Fig. 3) as mentioned by Rao and Jagapathi (2013). The degeneration of pronephros was first observed at cranial pole in 27 days (1.7 cm CRL) embryo. The mesenchymal covering around the pronephros disappeared by leaving few tubules (Fig.

5). The rest of the pronephros was filled with differentiating mesenchymal cells and completed the process of degeneration by 31 days (2 cm CRL) of gestation. In contrary, Davies (1951) recorded that the pronephros persisted until late stages of embryonic life in sheep foetus.



**Fig.3:** Photomicrograph of 24 day old sheep embryo showing pronephric glomeruli (H&E X400) (A-Aorta, G-Giant Glomerulus, T-Tubules, PCV- Posterior cardinal vein, C-Capsule, Pd- Pronephric duct



**Fig.5:** Photomicrograph of 27 day old sheep embryo showing pronephric tubules (H&E X400) (Rg- Remnants of glomerulus, A- Aorta, T- Tubule)

### Pronephric Glomeruli

Intense angioblastic activity was observed on either side of the dorsal aorta in 20 day old embryos (7 mm CRL). The pronephros consisted of only one giant glomerulus of  $225 \pm 1.3 \mu\text{m}$  in size (Table 2) which was placed medially and varied from middle to caudal in its location.

**Table 2:** Showing micrometrical details of Pronephros

| S. No. | Gestational Age (days) | Capsular Space( $\mu\text{m}$ ) | Glomerular Diameter ( $\mu\text{m}$ ) | Tubules                 |                          |
|--------|------------------------|---------------------------------|---------------------------------------|-------------------------|--------------------------|
|        |                        |                                 |                                       | Width ( $\mu\text{m}$ ) | Length ( $\mu\text{m}$ ) |
| 1      | 20                     | $2.5 \pm 0.5$                   | $225 \pm 1.3$                         | $25 \pm 1.2$            | $105 \pm 1.6$            |
| 2      | 24                     | $7.5 \pm 0.8$                   | $193.7 \pm 2$                         | $55 \pm 1.2$            | $95.6 \pm 1.7$           |
| 3      | 25                     | $10 \pm 1.2$                    | $150 \pm 1.6$                         | $31.2 \pm 1.5$          | $62.5 \pm 1.5$           |
| 4      | 27                     | $15 \pm 1.5$                    | $75 \pm 1.8$                          | $29.3 \pm 1.6$          | $48.1 \pm 1.9$           |

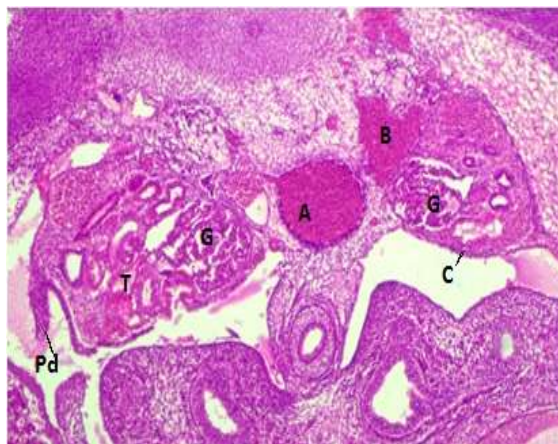
The whole of the glomerulus was surrounded by very thin layer of cuboidal cells with connective tissue fibers (Fig.1) which is in accordance with Davies (1951) at 14 somite stage in sheep embryos. However, Rao and Jagapathi (2013) denoted them as external glomeruli in mammals. At 24 days of gestation (1.1 cm CRL), the giant glomerulus was surrounded by a distinct capsular space only at certain places. The pronephric glomerulus was surrounded by a single layer of squamous to cuboidal cells at posterior pole. These cells appeared as tall cuboidal cells indicating the tubular continuation (Fig.3). However, Davies (1951) identified that the glomerular complex was almost fully developed and probably functional at 29

somite stage in sheep. In the present study, degenerative changes were noticed in glomerular network due to which vacuolation was noticed between the tuft of glomerulus at this age. Glomerulus was not developed in association with the cranially situated nephric tubules. It ultimately disappeared and complete degeneration of glomerulus was noticed as indistinct glomerulus in the pronephros by about 27 days (1.7 cm CRL) of gestation (Fig.5). No reviews are available in this regard.

### **Pronephric Tubules**

In 20 days old sheep embryo (0.7 cm CRL), many tubules were present in the pronephros. Some of them were fully formed while others were incomplete open type. Periphery of the pronephros consisted of irregular tubules that varied in size. The tubules present at the dorsal end of the vesicles were elongated and irregular in shape and traversed towards the caudal part of the vesicle. Tubules present close to the glomerulus appeared as incomplete and oriented with clusters of angioblast cells. The caudal ends were connected to coelomic epithelium by cellular strands like stalks that probably represented the rudimentary peritoneal funnels on either side (Fig.1). Davies (1951) observed similar changes at 23 somite stage in sheep. About forty-five tubules in various ages of development were reported with the most rudimentary tubule lying caudally. At 29 somite stage, increase in length and an early differentiation of the cranial tubules into secretory and a collecting segment was elucidated.

At 24 days of gestation (1.1 cm CRL), the mesenchymal differentiation progressed. The number of tubules were 6 to 7 and restricted to caudal end of pronephros. They were lined by cuboidal cells with discontinuous brush borders. The lumen of the tubules was occupied by eosinophilic material. The tubules increased in size upto this foetal age and were located postero-dorsal to the glomerulus (Fig. 3). The degeneration of the tubules was observed from cranial end to the periphery of the pronephros at 25 to 27 days of gestation (1.5 cm CRL to 1.7 cm CRL). The number of tubules were restricted to 3 to 6 and were lined by large/tall cuboidal cells with prominent centrally placed nucleus surrounded by eosinophilic cytoplasm. Some tubules had brush borders with little narrow lumen whereas the other tubules were without brush border. The size of the tubules started decreasing at 25 days than the earlier gestation periods. There was increase in the concentration of cellular strands at the caudal end and length of the stalk increased thus indicating the union of pronephros with primary excretory duct, which appeared as solid rod of cells in the coelomic cavity (Fig.4).



**Fig.4:** Photomicrograph of 25 day old sheep embryo showing pronephros (H &E X100) (B-Blood islands, G- Giant glomeruli, T-Tubules, A-Aorta, C-Capsule, Pd-Pronephric duct)

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