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Original Research Article

Study of Histogenesis of Human Fetal Renal Medulla by Using Masson's Trichrome and PAS Stains

Rahul Kisan Ukey [1], Dharam Singh Rathia [*2], Tarkeshwar Devidas Golghate [3], Amit Purushottam Tirpude [4], Soumitra Trivedi [5].

[1] Assistant Professor, Department of Anatomy, AIIMS, Raipur, C.G. University – AIIMS, Raipur, Chhattisgarh, India. **ORCiD:** https://orcid.org/0000-0002-7775-5132

[*2] Assistant Professor, Department of Anatomy, AIIMS, Raipur, C.G. University – AIIMS, Raipur, Chhattisgarh, India. **ORCiD:** https://orcid.org/0000-0001-7141-1171

[3] Professor and Head of Department, Department of Anatomy, Government Medical College, Bhandara, M.S. University, Maharashtra University of Health Sciences, Nashik, Bhandara, Maharashtra, India. **ORCiD:** https://orcid.org/0009-0005-9867-2570

[4] Additional Professor, Department of Anatomy, AIIMS, Raipur, C.G. University – AIIMS, Raipur, Chhattisgarh, India. **ORCiD:** https://orcid.org/0000-0003-0223-2270

[5] Professor & Head, Department of Anatomy, AIIMS, Raipur, C.G. University – AIIMS, Raipur, Chhattisgarh, India. **ORCiD:** https://orcid.org/0000-0003-0379-2173

ABSTRACT

Introduction: Histogenesis of the human fetal kidney is the histological study of the maturity of the kidney at a given gestational age. Embryologically, the kidney is derived from the ureteric bud and the metanephric blastema. The ureteric bud forms the collecting part, and the metanephric blastema forms the secretory part. The details of the microscopic development of the kidney are not described in various textbooks of embryology.

Methodology: 50 aborted human fetuses between 13 to 36 weeks of gestational age without any gross congenital anomalies were included in the present study. Paraffin blocks of the cut sections of both kidneys were prepared. Slides of 5–7 micron-thick sections stained with Masson's trichrome (MT) and Periodic acid-schiff (PAS) stains.

Results and Conclusion: From 16 weeks onwards, the medulla showed well-differentiated collecting ducts, thick and thin segments of the loop of Henle. The amount of connective tissue decreased, and the vascularity of the medulla increased with an increase in gestational age. The transitional epithelium lining the renal pelvis was appreciated from 16 weeks onwards.

KEYWORDS: Histogenesis, Kidney, Renal medulla, Gestational age, Masson's Trichrome stain, PAS stain.

Corresponding Author: Dr. Dharam Singh Rathia, Assistant Professor, Department of Anatomy, AIIMS, Raipur, C.G. University – AIIMS, Raipur, Chhattisgarh, India.
E-Mail: dr.rathia@aiimsraipur.edu.in

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INTRODUCTION

Intra-uterine life is a very crucial period for human development. The intra-uterine life begins with the formation of the zygote, and ultimately, all the organ systems are developed from it. One should know the normal

developmental gross and microscopic anatomy of the kidney to understand the various congenital renal conditions.

Grossly, the interior of the kidney consists of an outer cortex and inner medulla. The renal medulla consists of triangular-shaped renal pyramids, their bases directed to the cortex and apices converging to the renal sinus. At the renal sinus, they project into calyces as papillae. One minor calyx receives 1 to 3 renal papillae [1].

The kidney develops from two embryologically distinct parts: the metanephric blastema forms the nephron, which produces urine, and the ureteric bud gives origin to the collecting duct, which completes the concentration of urine and through which urine passes out into the calyces of the kidney, the renal pelvis, the ureter, and the urinary bladder [1].

Metanephros, the primordia of permanent kidneys, appear in lumbosacral segments and start to develop early in the 5th week, and begin to function approximately after 4 weeks.

The description of the development of the kidney given in different textbooks [2-5] of embryology does not include the detailed microscopic appearance of various structures of the kidney and its maturation at different gestational ages. So, details of the microscopic development of the kidney are still unclear to us. For this purpose, it is essential to have knowledge regarding the histological maturity of the kidney and its functional status at the given gestational age. This study can also be used to determine the age of the fetus for medico-legal purposes.

MATERIALS AND METHODS

The present study was conducted in the department of Anatomy, Government Medical College, Aurangabad, Maharashtra, India. Spontaneous abortuses, stillborn and terminated fetuses under the Medical Termination of Pregnancy Act of India (total 50 fetuses, 29 females and 21 males) were included in this study. These foetuses, between 13-36 weeks of gestational age with no obvious congenital anomalies, were obtained from the Department of Obstetrics and Gynaecology of the same institute with the consent of respective parents and prior permission of the Head of Department. The institutional Ethical Committee approved the study.

10% formalin was injected into the thoracic and abdominal cavity of the fetuses to fix them, and they were kept in a 10% formalin jar and then dissected within 2 hours of collection. The anterior abdominal wall was opened by giving a midline vertical incision, and both kidneys were removed. The longitudinal sections (LS) of both the kidneys were taken to include cortex, medulla, and hilum, each section being 3-4 mm thick. Specimens were kept in Bouin's fluid for 4-5 days. The blocks were labelled and cut with the help of a rotary microtome, 5-7 microns in thickness, in the form of a ribbon. Slides included in this study, stained by Masson's trichrome (MT) and Periodic acid-schiff (PAS) stains, were observed under low and high power of the light microscope.

OBSERVATIONS AND RESULTS

Masson's trichrome stain:

At 13 weeks: Mesenchymal connective tissue between the tubules stained blue and was found to be abundant. Collecting ducts, thick and thin segments of the loop of Henle, and capillaries were clearly differentiated. RBCs in capillaries stained red (Figure 1). The developing renal pelvis lined by multi-layered epithelium was seen.

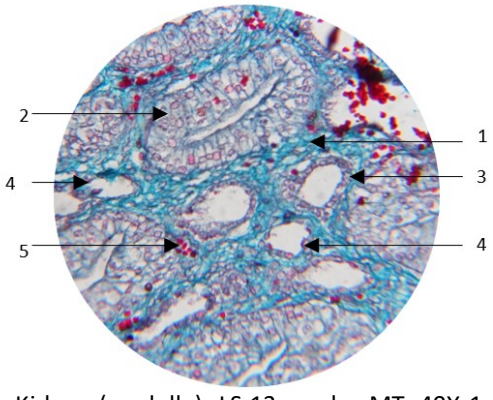

Fig 1: Kidney (medulla); LS;13 weeks; MT; 40X 1. Mesenchymal tissue (blue), 2. Collecting duct, 3. Thick segment of loop of Henle, 4. Thin segment of loop of Henle,5. RBCs in capillaries (red).

At 16 weeks: The Mesenchymal connective tissue between the tubules stained blue and

was reduced compared to the previous stage. Therefore, the collecting ducts, thick and thin segments of the loop of Henle, were seen to be densely arranged, and capillaries were better differentiated than in the previous stage. Capillaries increased in number, indicating increased vascularity of the medulla (Figure 2). Minor calyces were lined by transitional epithelium and opened into the extended renal pelvis. Mesenchymal connective tissue was found to be abundant below the transitional epithelium (Figure 3).

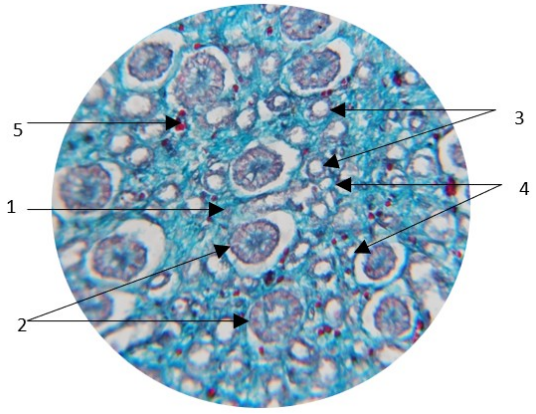

Fig 2: Kidney (medulla); LS; 16 weeks; MT; 40X. 1. Mesenchymal tissue (blue), 2. Collecting duct, 3. Thick segment of loop of Henle, 4. Thin segment of loop of Henle, 5. RBCs in capillaries (red).

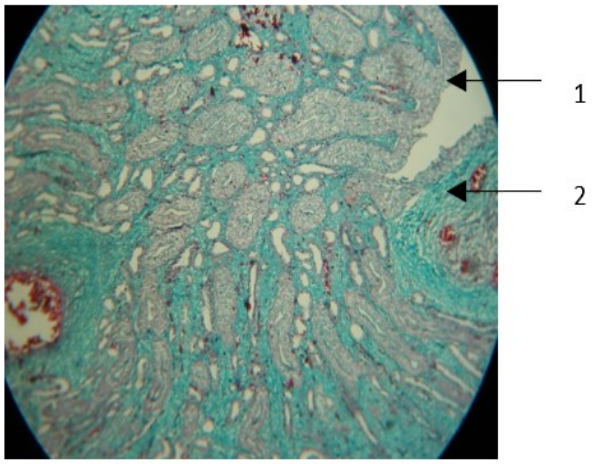

Fig 3: Kidney (medulla); LS; 16 weeks; MT; 40X. (1. Transitional epithelium, 2. Mesenchymal tissue (green).

At 18 weeks: Mesenchymal connective tissue between the tubules stained blue and appeared reduced in amount as compared to the previous stage. Collecting ducts, thick and thin segments of the loop of Henle, and capillaries were better appreciated than in the previous stage. An increased number of capillaries was identified compared to the previous stage (Figure 4).

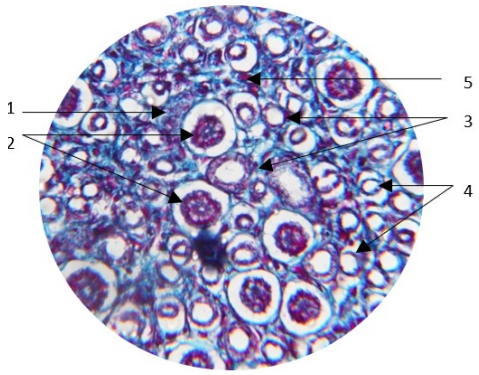

Fig 4: Kidney (medulla); LS; 18 weeks; MT; 40X. 1. Mesenchymal tissue (blue), 2. Collecting duct, 3. Thick segment of loop of Henle, 4. Thin segment of loop of Henle, 5. RBCs in capillaries (red).

At 20 weeks: Mesenchymal connective tissue between the tubules stained blue and was found to be negligible than in the previous stage. Collecting ducts, thick and thin segments of the loop of Henle, and capillaries were more clearly identified than in the previous stage. Numerous capillaries were identified compared to the previous stage (Figure 5). The pelvis was lined by distinct transitional epithelium. Mesenchymal connective tissue below the transitional epithelium was reduced in amount (Figure 6).

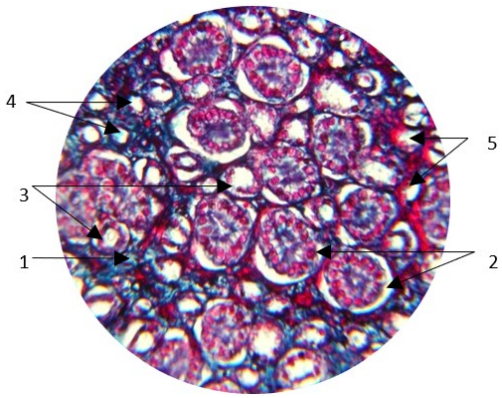

Fig 5: Kidney (medulla); LS; 20 weeks; MT; 40X. (1. Mesenchymal tissue (blue), 2. Collecting duct, 3. Thick segment of loop of Henle, 4. Thin segment of loop of Henle, 5. RBCs in capillaries (red)]

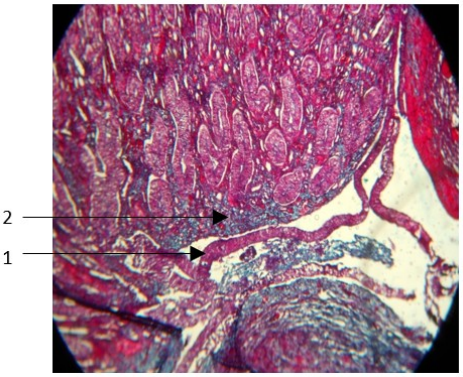

Fig 6: Kidney (medulla); LS; 20 weeks; MT; 40X. (1. Transitional epithelium, 2. Mesenchymal tissue (green)

At 28 weeks: The findings were similar to those of the previous stage (Figure 7).

At 36 weeks: Collecting ducts, thick and thin segments of the loop of Henle were distinctly seen (Figure 8).

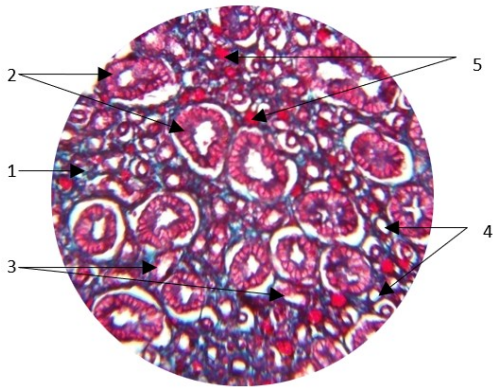

Fig 7: Kidney (medulla); LS; 28 weeks; MT; 40X**.** (1. Mesenchymal tissue (blue), 2. Collecting duct, 3.Thick segment of loop of Henle, 4. Thin segment of loop of Henle, 5. RBCs in capillaries (red)

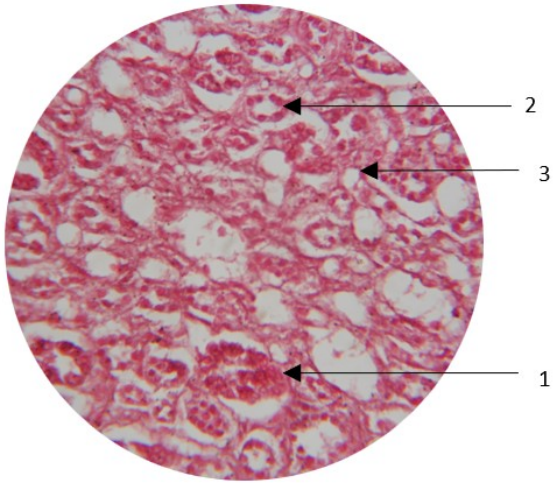

Fig 8: Kidney (medulla); LS; 36 weeks; MT; 40X. 1. Collecting duct, 2. Thick segment of loop of Henle, 3. Thin segment of loop of Henle

PAS stain:

At 13 weeks: The basement membrane of the lining epithelium of the collecting tubule, thick and thin segments of the loop of Henle, stained faint magenta in colour (Figure 9).

At 16 weeks: The basement membrane of the lining epithelium of the collecting tubule, thick and thin segments of the loop of Henle, stained more deeply with magenta colour than in the previous stage (Figure 10).

At 18 weeks: The basement membrane of the lining epithelium of the collecting tubule, thick and thin segments of the loop of Henle, stained more deeply magenta than in the previous stage, indicating their better differentiation than in the previous stage (Figure 11).

At 20 weeks: Deep magenta colour of the basement membrane of the lining epithelium of the collecting tubule, thick and thin segments of the loop of Henle indicated their better differentiation than the previous stage (Figure 12).

At 32 weeks: The basement membrane of the lining epithelium of the collecting tubule and the thick and thin segments of the loop of Henle stained more deeply with magenta colour than the previous stage, indicating that these tubules were better differentiated than the previous stage (Figure 13).

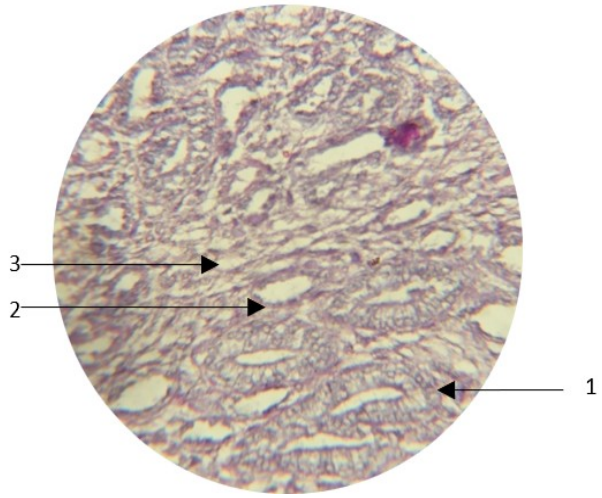

Fig 9: Kidney (medulla); LS; 13 weeks; PAS; 40X. 1. Basement membrane of collecting tubule, 2. Basement membrane of thick segment of loop of Henle, 3. Basement membrane of thin segment of loop of Henle)

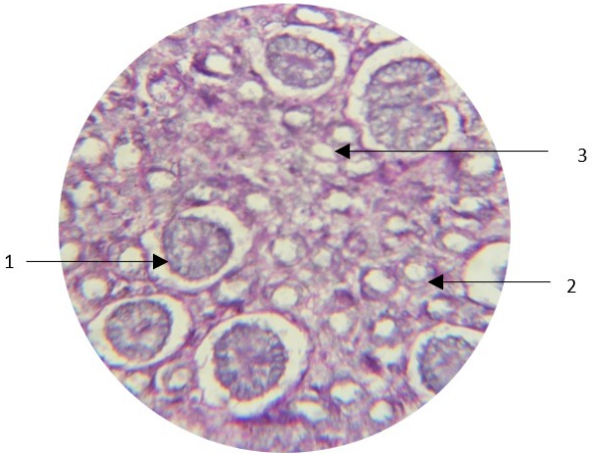

Fig 10: Kidney (medulla); LS; 16 weeks; PAS; 40X. 1. Basement membrane of collecting tubule, 2. Basement membrane of thick segment of loop of Henle, 3. Basement membrane of thin segment of loop of Henle)

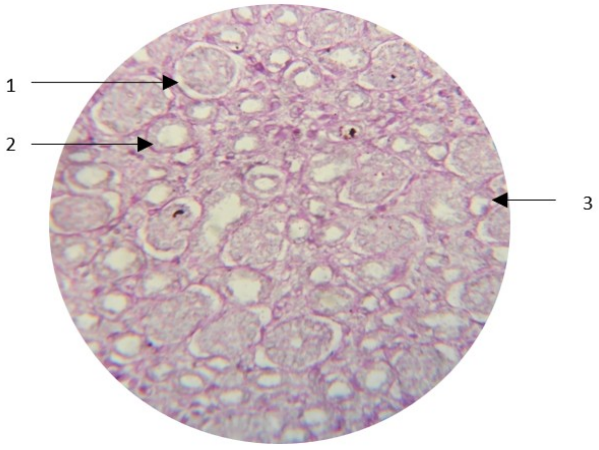

Fig 11: Kidney (medulla); LS; 18 weeks; PAS; 40X. 1. Basement membrane of collecting tubule, 2. Basement membrane of thick segment of loop of Henle, 3. Basement membrane of thin segment of loop of Henle)

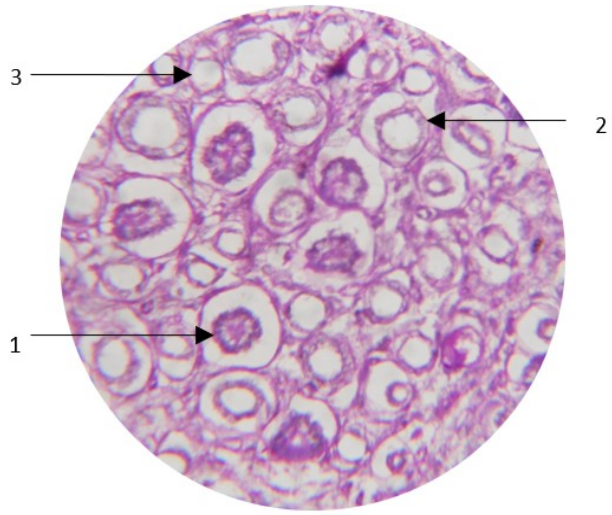

Fig 12: Kidney (medulla); LS; 20 weeks; PAS; 40X. 1. Basement membrane of collecting tubule, 2. Basement membrane of the thick segment of the loop of Henle, 3. Basement membrane of the thin segment of the loop of Henle)

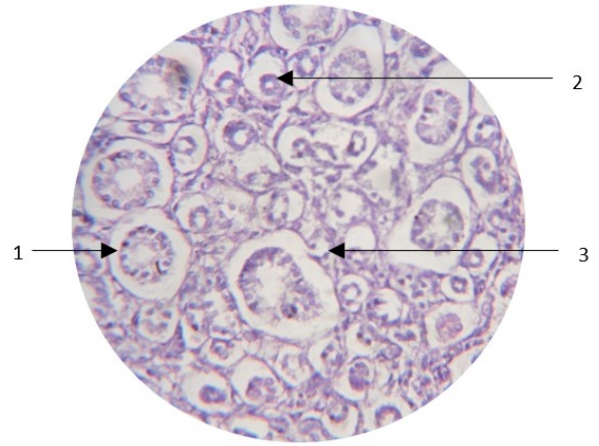

Fig 13: Kidney (medulla); LS; 32 weeks; PAS; 40X**.** 1. Basement membrane of collecting tubule, 2. Basement membrane of the thick segment of the loop of Henle, 3. Basement membrane of thin segment of loop of Henle)

DISCUSSION

Masson's Trichrome staining: In the present study, we found that, at 13 weeks, in the medulla, mesenchymal connective tissue between the tubules stained blue and was found to be abundant. Collecting ducts, thick and thin segments of the loop of Henle, and capillaries were clearly differentiated. RBCs in capillaries were stained red.

At 16 weeks, in the medulla, mesenchymal connective tissue between the tubules stained blue and was reduced than the previous stage. Collecting ducts, thick and thin segments of the loop of Henle, and capillaries were better differentiated than in the previous stage.

In subsequent weeks, the medulla showed better differentiated collecting ducts, thick and thin segments of the loop of Henle and the transitional epithelium lining the minor calyces and the renal pelvis.

PAS staining: In the present study, we observed that, at 13 weeks, in the medulla, the basement membrane of the lining epithelium of the collecting tubule and the thick segments of the loop of Henle stained faint magenta in colour.

At 18 weeks, in the medulla, the basement membrane of the lining epithelium of the collecting tubule, thick and thin segments of the loop of Henle, stained deep magenta in colour, indicating that these were better differentiated than in the previous stage.

In subsequent weeks, in the medulla, the basement membrane of the lining epithelium of the collecting tubule, thick and thin segments of the loop of Henle, stained more deeply with magenta colour, indicating their better differentiation.

These findings were compared with those of the other workers. Most of these workers had used only the haematoxylin and eosin (H&E) stains in their studies, and similar findings were reported in our earlier study [6]. In the available literature search, there are limited studies conducted using the Masson's Trichrome and PAS staining techniques for the histological sections of the medulla of the developing kidney, except for the work of

Shalika Sharma et al (2014) [7] used the Masson's Trichrome stain alone. After comparison, it is found that the differentiation of collecting tubules, thick and thin segments of the loop of Henle, took place earlier than that of the other workers reported. Other findings of this study were broadly comparable.

Mishra S et al (2006) [8] mentioned that at 16 weeks of gestation, mesenchymatous tissue with some developing tubules was seen in the medulla.

Tank KC et al (2012) [9] stated that, at 12 weeks, the medulla showed undifferentiated mesenchymal tissue. At 22 and 24 weeks, an increase in the number of collecting tubules, thick and thin segments of the loop of Henle, with a decrease in the amount of connective tissue was seen in the medulla. At 36 weeks, the medulla showed well-differentiated collecting tubules and thick and thin segments of the loop of Henle.

Patil et al. (2012) [10] found that at 16 weeks, the medulla showed connective tissue, primitive blood vessels, and a few clusters of cells. At 19-26 weeks, connective tissue was decreased, and vascularity was increased, accompanied by an increase in the number of tubules. At 29 weeks, the number of collecting tubules, thick and thin segments of the loop of Henle, was increased.

Syed SA et al (2012) [11] described that at 14 weeks, undifferentiated mesenchymal tissue was observed in the medulla. Medulla showed scattered primitive blood vessels with RBCs, lined by simple squamous epithelium. At 37-40 weeks, collecting tubules, thick and thin segments of the loop of Henle, and blood vessels were better differentiated.

Shalika Sharma et al (2014) [7] stated that, in Masson's trichrome stain, in a 12-week fetus, mesenchymal cells were found to be loosely arranged in the periphery. By 16 weeks, many developing glomeruli were observed to be densely arranged due to a decrease in the amount of interstitial connective tissue. The pelvis was seen extended and was receiving the openings of many minor calyces. A multilayered epithelium lined the pelvis at this stage. At 22 weeks, the pelvis was lined by well-developed transitional epithelium. By 23 weeks in H & E stain, the amount of interstitial connective tissue was negligible in the cortex.

SUMMARY AND CONCLUSION

In the medulla of the kidney at 13 weeks of gestation, collecting tubules, thick and thin segments of the loop of Henle were identified with abundant mesenchymal connective tissue and few blood vessels. At 32 weeks, the medulla of the kidney appeared more mature. Connective tissue was significantly less in amount. The number of collecting tubules, the thick and thin segments of the loop of Henle, was increased. At 36 weeks, the medulla showed well-differentiated collecting tubules, thick and thin segments of the loop of Henle.

Conclusion

· The medulla became well differentiated from 16 weeks onwards.

· Vascularity of the medulla increased with an increase in gestational age.

· Connective tissue of the medulla decreased with an increase in gestational age.

· The renal pelvis was lined by transitional epithelium.

Most of the findings of the present study were in corroboration with the studies performed by the previous workers.

Author Contributions

Rahul Kisan Ukey: Study conception, methodology and data collection.
Dharam Singh Rathia: Mauscript writing and correspondance.
Tarkeshwar Devidas Golghate: Methodology rivew and literature review.
Amit Purushottam T irpude: Analysis of data and drafting mauscript.
Soumitra Trivedi: correction and edditing.

Conflicts of Interests: None

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