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# HISTOGENESIS OF MUSCLE LAYERS OF HUMAN URINARY BLADDER

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## ABSTRACT

**Background:** Urinary bladder is an organ of considerable importance in mammals, being a site where urine is collected before micturition and without undergoing any significant exchange of water or ions with surrounding. Urinary epithelium (urothelium) is unique in being non-reabsorptive and non-secretory in nature. The earlier studies on the development of urinary bladder were mainly on its gross anatomical features.

**Objectives:** 1) To note structural differentiation and maturity of muscularis externa layer which it attains at different stages of development to show the adult picture. 2) To compare and contrast differences between different age groups and with previous studies and available literature.

**Material and Method:** 50 aborted human fetuses (29 females and 21 males) of different gestational age from 9th week onwards were collected, urinary bladder were taken out and fixed in a fixative. Blocks of tissues were made from bladder wall proper, trigone & bladder neck and processed to get sections which were stained with 1) Haematoxylin and Eosin 2) Masson's trichrome stain.

**Results:** Beginning with 12th week, muscularis externa showed well developed circular muscle layer and a thin inner longitudinal layer in the form of thin, scattered bundles. Outer longitudinal layer appeared as discrete, nonuniform bundles of muscle fibres by 13th week. All three layers were seen well developed and thick by 16th week and found thickest by 32nd week with slight variations in their arrangement. Muscle layer thickness at bladder neck and trigone increased progressively from 18th wk onwards.

**Conclusion:** Muscular layer in bladder wall showed all three layers progressively increased in thickness and indistinctly well developed from 16th week onwards and found thickest at 32nd week. Thus Histogenesis of urinary bladder in this instance may represent a powerful tool to delineate structure – function relationship.

**Key Words:** Histogenesis, Fetal urinary bladder, Muscularis externa, Trigone, Bladder neck

## INTRODUCTION

Understanding of the processes involved in the formation of various organs and systems of body has disclosed most cryptic secrets of nature. The earlier studies on the development of urinary bladder were mainly on its gross anatomical features. Not much work has been done on the development and differentiation of muscular layers of human fetal urinary bladder especially of Indian origin which differ largely from their western counterparts in the rate of growth, differentiation and maturity. Similar studies have been done in western countries by many researchers<sup>(1,2,3,4,5,6,7,8,9)</sup>.

## Material and method:-

**Study design:** Observational (Qualitative) study

**Statistical Analysis:** No measurements have been taken as it is an Observational study, so statistical analysis is not applicable.

## Material and method

**Collection of materials:** After approval from the institutional ethical committee, during period of 2 years, 50 aborted human fetuses (29 females and 21 males) of different gestational age from 9<sup>th</sup> week onwards were collected from the

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department of Obstetrics and Gynaecology, Aurangabad (Fig-1). Written consent from parents of aborted fetuses was taken.

**Inclusion criteria:** Spontaneously aborted fetuses from 9<sup>th</sup> week onwards, stillborn fetuses, terminated fetuses under the Medical termination of Pregnancy Act of India 1971.

**Exclusion criteria:** Fetuses less than 9 weeks, twins, presence of any congenital anomalies, post mortem decomposition.

Fetuses were obtained within 1-2 hrs of abortion to avoid post-mortem decomposition changes and preserved immediately in 10% formalin. Gestational age was calculated from Body weight and Crown-rump length (CRL). They were dissected within 2 hrs of collection by taking a midline vertical incision extending from umbilicus to pubic symphysis (Fig 2 & 3). Bladder was then carefully removed along with its neck (Fig 4). Subsequently bladders were passed through following procedures: <sup>(10)</sup>

- 1) Fixation of Bladder: in Bouin's fluid for 4-5 days. Longitudinal and transverse sections of specimen were taken from bladder wall proper, trigone region and bladder neck region, each section being 3-4 mm thick.
- 2) Dehydration: The tissue was processed in ascending grades of 50%, 70% and 90% alcohol.
- 3) Clearing: done to remove alcohol from tissue. Tissue was placed in xylene for about 30 minutes. It also increases the refractive index of tissues.
- 4) Paraffin bath: It involves soaking of tissue in molten soft paraffin wax (melting point 45-50°C). Tissue was subjected to two changes of paraffin wax each for three hours.
- 5) Casting (block making): The blocks were prepared by pouring molten paraffin wax (melting point 55-60°C) into a mould. Using two 'L' moulds, suitable size blocks were prepared and wax impregnated tissue was placed eccentrically and oriented so that it could be sectioned in the right angle plane.
- 6) Microtomy (section cutting): The block was cut with the section thickness of 5-7 microns in the form of ribbon with the help of rotary microtome.
- 7) Fixing sections on the slide: The ribbon of sections was placed on the surface of warm water in the flotation bath. This removes all wrinkles from the tissue and wax (flattening). The glass slide was smeared with egg albumin and sections were mounted on it and slides were placed on the hot plate at 45°C - 50°C for 2 hours or more as per the requirement for drying.

The sections were stained with the following stains:

#### A) Haematoxylin and Eosin staining: <sup>(10)</sup>

##### Technique:

- 1) Removal of paraffin wax from the sections was done by dipping the slide into two changes of xylene for one to two minutes each.
- 2) Removal of xylene done by dipping the slide into two changes of absolute alcohol for one to two minutes each and then treated with descending grades of alcohol- 90%, 80%, 70% for one minute each.
- 3) The slide was kept under running tap water for 2-3 minutes.
- 4) The slide was stained with Haematoxylin for about five to seven minutes followed by washing under running tap water for 2-3 minutes. This leads to bluing of the section.
- 5) Excess stain is removed (Differentiation) by dipping the slide in acid alcohol for few seconds. This changes blue color to red because of the acid.
- 6) The blue color was regained by washing in running tap water for 5 minutes and it was checked under the microscope, for nuclear staining.
- 7) The section was counterstained with 5% aqueous solution of eosin for about 5 minutes and dehydrated by dipping in ascending grades of alcohol as 70%, 90%, and absolute alcohol (100%) for one minute each.
- 8) Clearing was done in two changes of xylene for one minute each.
- 9) The slide was mounted in DPX (Distrene Plastsizer and Xylene) and coverslip was applied and the slide was kept at room temperature for some hours for firm adhesion of the coverslip to the section.

**Result:** Nuclei-blue, cytoplasm- pink, muscle cells- pink, collagen fibres- light pink.

#### B) Masson's Trichome staining: <sup>(10)</sup>

##### Technique:

- 1) Wax was removed and section was brought to water.
- 2) Nuclei were stained with Weigert's Iron Haematoxylin and then slide was washed well in water.
- 3) It was stained with diluted Ponceau Acid Fuschin for five minutes.
- 4) The slide was rinsed in distilled water.
- 5) Section was differentiated in 1% Phosphomolybdic acid until collagen was decolorized and again rinsed in distilled water.
- 6) Section was counterstained with light green or aniline blue for two minutes.
- 7) Light green was differentiated in water.
- 8) Slide was dehydrated and cleared.
- 9) Lastly the slide was mounted.

**Result:** Nuclei- blue to black.

Muscle, red blood cells, fibrin and some cytoplasmic granules- red.

Collagen, some reticulin, basement membrane, amyloid and mucin- green or blue according to counterstain used.

**Observations and Results:-** The slides were stained with Haematoxylin and Eosin and Masson's Trichome stain, observed by unioocular light microscope under low (10X) and high (40X) magnifications for three indistinct anastomosing layers- inner and outer longitudinal muscle layers and intermediate circular muscle layer.

At 12<sup>th</sup> week, all four layers of urinary bladder were clearly visible. Muscularis externa showed well developed circular muscle layer and inner longitudinal layer in the form of discrete, scattered bundles with absence of outer longitudinal layer (photomicrograph-1). At 13<sup>th</sup> week, all three muscular layers were seen with inner and outer longitudinal layers appeared as discrete, nonuniform bundles of muscle fibres (photomicrograph-2). All three layers were well developed and thick from 16<sup>th</sup> week onwards (photomicrograph-3&4) and distinct and thickest by 32<sup>nd</sup> week (photomicrograph-5&6). At this stage muscle thickness was found comparative to adult picture in relation to the bladder size. With Masson's Trichome, muscularis externa stained bright red 16<sup>th</sup> week onwards. Pattern of muscle layers in muscularis externa were different within same and different gestational age groups (Table 1).

In the trigone region at 18<sup>th</sup> week, muscle layer showed intermingling of inner ureteral longitudinal and outer circular detrusor muscle fibres which became more pronounced from 20<sup>th</sup> week onwards (photomicrograph-7).

In the bladder neck at 16<sup>th</sup> week, muscular layer showed indistinct internal longitudinal and outer circular layers (photomicrograph-8). Male fetus of 32<sup>nd</sup> week showed circular muscle layer more thicker than that in 34<sup>th</sup> week female fetus.

## DISCUSSION

In the present study, at 12<sup>th</sup> week muscularis externa showed well developed circular muscle layer and a thin inner longitudinal layer in the form of discrete, scattered bundles. Outer longitudinal layer appeared as discrete, nonuniform bundles of muscle fibres by 13<sup>th</sup> week. All three layers were seen well developed and thick by 16<sup>th</sup> week and found thickest by 32<sup>nd</sup> week with slight variations in their arrangement. These findings were corroborative with the findings of others according to whom, musculature in the bladder begins to develop at 13<sup>th</sup> week and proceeded from apex of bladder towards the urethral orifice<sup>(1,2,3,4)</sup>. It is said that muscle coat in bladder develops well when kidney starts producing urine, by the end of 2<sup>nd</sup> month (9 week) to 11 weeks of gestation<sup>(2)</sup>. Mechanical distention of bladder stimulates myogenesis in the wall<sup>(2)</sup>. By the 5<sup>th</sup> month (24 weeks) the cells had acquired most of adult picture in their arrangement and relationship with each other and co-ordinated activity<sup>(4)</sup>. In the present study, considering trimester wise, at 12<sup>th</sup> week circular and inner longitudinal

muscle layer were present while outer longitudinal layer of muscularis externa was absent. In the second trimester, out of total 44 cases 14 cases (32%) showed presence of circular and outer longitudinal muscle layer and absence of inner longitudinal layer, while 7 cases (16%) showed presence of circular and inner longitudinal muscle layer and absence of outer longitudinal muscle layer. 1 case (2%) showed presence of only circular muscle layer with absence of both inner and outer longitudinal muscle layer. Remaining 22 cases (50%) of second trimester showed presence of all three layers of muscularis externa. In the third trimester 1 out of total 5 cases showed absence of outer longitudinal layer. Other study also shows similar findings<sup>(5)</sup>.

In trigone region muscle layer showed intermingling of inner ureteral longitudinal and outer circular detrusor muscle fibres at 18<sup>th</sup> week and was more pronounced from 20<sup>th</sup> week onwards. Musculature of trigone developed between 11-20 cm CRL stage (13-18 weeks) and appeared continuous with the urethral smooth muscle<sup>(1,3)</sup>.

At bladder neck at 16<sup>th</sup> week muscle layer showed indistinct inner longitudinal and outer circular layer and this found similar with others<sup>(3)</sup>.

The male fetus of 32<sup>nd</sup> week showed circular muscle layer more thicker than that in 34<sup>th</sup> week female fetus. In male bladder at vesical orifice, circular muscle layer thickens and encircles proximal urethra completely to form 'Trigonal ring'<sup>(1,6)</sup>.

Urinary bladder is studied for Histogenesis upto Preprostatic part of urethra.

## CONCLUSION

In Urinary bladder wall, muscularis externa shows all three layers indistinctly well developed and thick from 16<sup>th</sup> week onwards and found thickest at 32<sup>nd</sup> week.

Trigone region shows intermingling of inner ureteral longitudinal and outer circular detrusor muscle fibres at 18<sup>th</sup> week with increase in thickness and intermingling from 20<sup>th</sup> week onwards.

In the bladder neck, at 16<sup>th</sup> week the muscular layer shows indistinct internal longitudinal and outer circular muscle layer. Male fetus from 32<sup>nd</sup> week onwards shows circular muscle layer more thick than that in female fetus of comparative gestational age.

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### REFERENCES

1. Drees JT. Observations on the musculature of the urinary bladder and the urethra in the human fetus. . Br J Urol. 1974 Apr; 46(2):179-85.
2. Newman J, Antonakopoulos GN. The fine structure of the human fetal urinary bladder. Development and maturation. A light, transmission and scanning electron microscopic study. J Anat. 1989 Oct; 166:135-50.
3. Gilpin SA, Gosling JA. Smooth muscle in the wall of the developing human urinary bladder and urethra. J Anat. 1983 Oct; 137 (Pt 3):503-12.
4. Hoyes AD, Ramus NI, Martin BG. Differentiation of the muscle of the human fetal bladder: an ultrastructural study. Micron; 1972; 3 (4); 414–28.
5. Cankara N, Ozguner G, Koyuncu E, Kadir D, Sulak O. The development of muscular layers in the wall of fetal urinary bladder during the fetal period. S.D.U. Typ Fak. Derg. 2009;16(1); 16-20.
6. Healy JC. Urogenital system in Standring S, editor. Gray’s anatomy: The Anatomical basis of Clinical Practice. 40<sup>th</sup> ed. London: Churchill Livingstone Elsevier; 2008. p. 1245-50.
7. Hicks RM. The mammalian urinary bladder: an accommodating organ. Biol Rev Camb Philos Soc. 1975 May; 50(2):215-246
8. Matsuno T, Tokunaka S, Koyanagi T. Muscular development in the urinary tract. J Urol. 1984 Jul; 132(1):148-52.
9. Oswald J, Schwentner C, Lunacek A, Fritsch H, Longato S, Serigi C et al. Reevaluation of the fetal muscle development of the vesical trigone. J Urol. 2006 Sep; 176(3):1166-70.
10. Drury RAB, Wallington EA. **Carleton’s histological technique**. 5<sup>th</sup> ed. Oxford, New York, Toronto: Oxford University Press; 1980.

**Table 1: Observed Differentiation of three muscle layers of muscularis externa**

Fertilization age (weeks)	Muscle layers differentiation
12 <sup>th</sup> week	A circular and inner longitudinal layer was present with absence of outer longitudinal layer in muscularis externa.
13-14 weeks	All three layers of muscularis externa present.
15-16 weeks	Circular layer of muscularis externa present in all cases. Inner longitudinal layer absent in 2 and outer longitudinal in 2 of total 9 cases.
17-18 weeks	Circular layer of muscularis externa present in all cases. Inner longitudinal layer absent in 4 and outer longitudinal in 2 of total 9 cases.
19-23 weeks	Circular layer of muscularis externa present in all cases. Inner longitudinal layer not seen in 6 and outer longitudinal in 2 and both layers in 1 of total 16 cases.
24-30 weeks	Circular layer of muscularis externa present in all cases. Inner longitudinal layer not seen in 1 and outer longitudinal in 2 of total 10 cases.
31-38 weeks	Circular layer of muscularis externa present in all cases. Outer longitudinal layer not seen in 1 of total 3 cases.



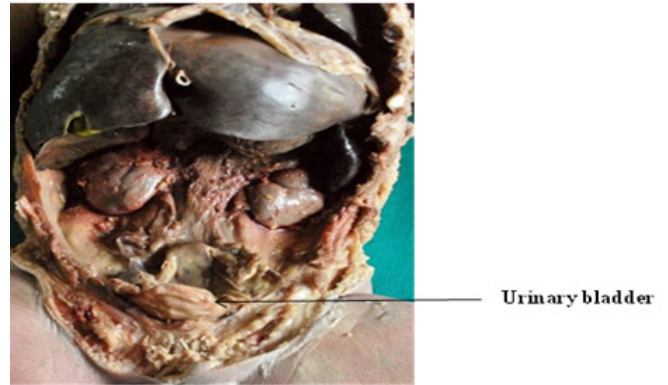
**Figure 1:** Fetuses of different age groups.



**Figure 2:** Instruments used for dissection.

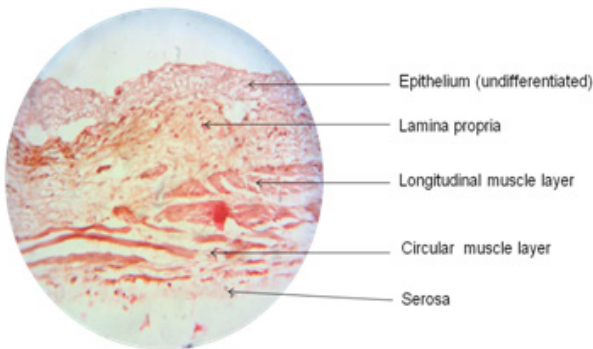


**Figure 3:** Dissection of 34 weeks old fetus and urinary bladder in situ.

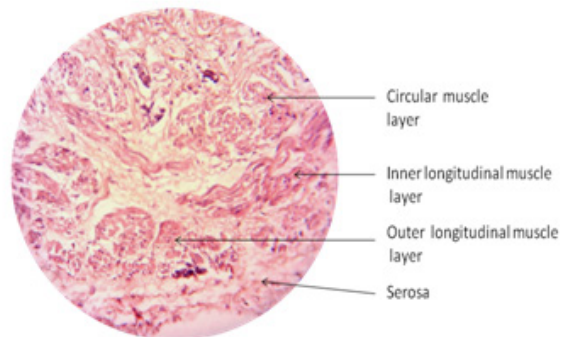


**Figure 4:** Urinary bladders of fetuses of different age groups.

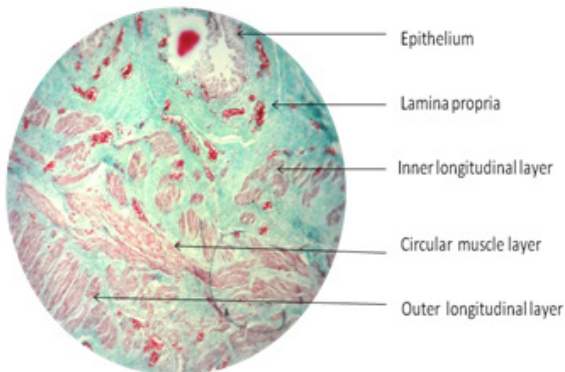
**Photomicrographs:**



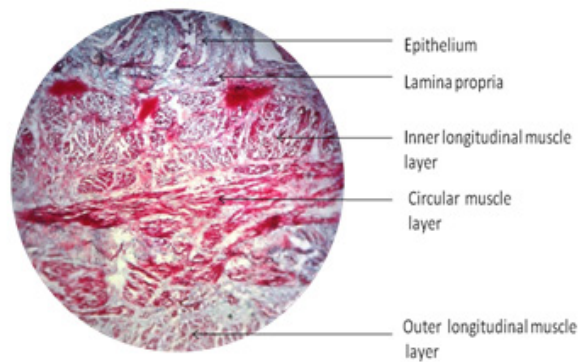
**Photomicrograph 1:** 12 weeks: 40 X; Haematoxylin and Eosin



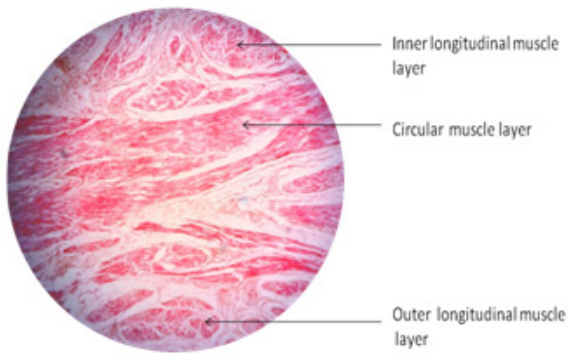
**Photomicrograph 2:** 13 weeks: 40 X; Haematoxylin and Eosin



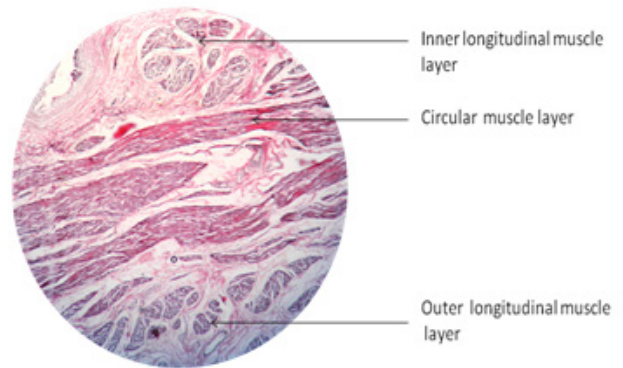
**Photomicrograph 3:** 16 weeks: 10 X; Masson's Trichome



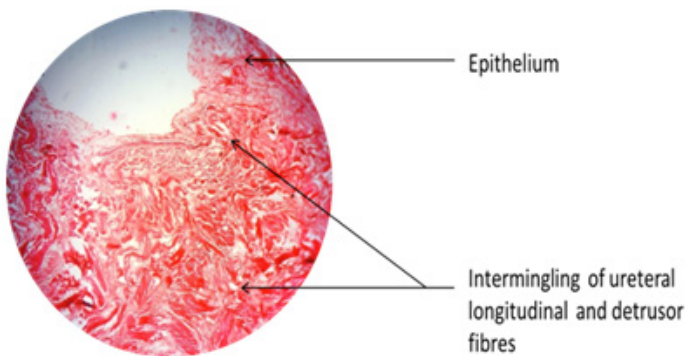
**Photomicrograph 4:** 22 weeks: 10 X; Masson's Trichome



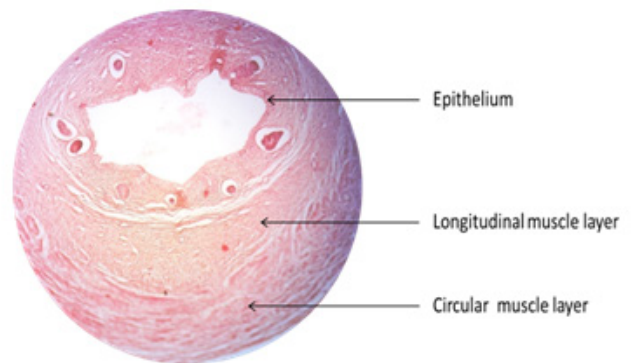
Photomicrograph 5: 32 weeks: 10 X; Haematoxylin and Eosin



Photomicrograph 6: 32 week: 10 X; Masson's Trichrome



Photomicrograph 7: Trigone: 18 weeks: 10 X; Haematoxylin and Eosin



Photomicrograph 8: Bladder neck: 16 weeks: 10X; Haematoxylin and Eosin