International Journal of Anatomy and Research,
Int J Anat Res 2018, Vol 6(1.1):4777-83. ISSN 2321-4287
DOI: https://dx.doi.org/10.16965/ijar.2017.353

Original Research Article

HISTOMORPHOLOGICAL FEATURES OF HUMAN GUBERNACULUM IN THE MALE

Beulah Priyalatha Swamickan *[1], Aleyamma Fenn T.K [2].

[*1] Department of Anatomy, PSG Institute of Medical Sciences and Research, Coimbatore, Tamilnadu, India.
[2] Department of Anatomy, Govt. Medical College, East Yakkara Palakkad, Kerala State, India.

ABSTRACT

Background: Gubernaculum and the events related to the descent of testis have been studied since17th century, from the time of John Hunter onwards. Most of the studies were done in the animals and there is paucity of information regarding gubernaculum in the humans . Present study is an attempt to throw light on the morphological features and histological structure of gubernaculum in human male fetuses.

Aims: to study the appearance, extent and microscopic structure of gubernaculum testis and to explore the role of gubernaculums in causing cryptorchidism.

Materials and Methods: The study was conducted on 35 spontaneously aborted male fetuses and on 10 tissue samples of gubernaculum from children who have come for orchiopexy, obtained from PSG Hospital.

Results and Discussion: Gubernaculum was extended from the lower pole of testis and epididymis to pubic symphysis. Gubernaculum testis was made up of collagen fibres, elastic fibres, muscle fibres and loose mesenchyme with lymphocytes, macrophages and fibroblasts embedded within it. Collagen undergoes changes as the foetal age advances and this could contribute to the role of gubernaculumin testicular descent.

Conclusion: Gubernaculum testis possibly could play a significant role in the descent of the testis, by creating the channel for the passage of the testis and do not actively pull the testis down into the scrotum as thought earlier.

KEY WORDS: Gubernaculum, Mesenchymatous Tissue, Collagen Fibres, Fibroblast, Elastic Fibres, Skeletal and Smooth Muscle, Trimester of Pregnancy.

Address for Correspondence: Dr Beulah Priyalatha Swamickan, Department of Anatomy, PSG Institute of Medical Sciences and Research; Coimbatore – 641 004. Tamilnadu India.
Mobile No: +91 9363249025 **E-Mail:** beulahstephen@gmail.com

Access this Article online	Journal Information
Quick Response code	International Journal of Anatomy and Research
	ICV for 2016 90.30
	ISSN (E) 2321-4287 | ISSN (P) 2321-8967
	https://www.ijmhr.org/ijar.htm
	DOI-Prefix: https://dx.doi.org/10.16965/ijar
DOI: 10.16965/ijar.2017.353	

Article Information	
Received: 18 Jul 2017	Accepted: 16 Aug 2017
Peer Review: 19 Jul 2017	Published (O): 05 Jan 2018
Revised: None	Published (P): 05 Jan 2018

INTRODUCTION

During development, human testis migrates from the abdominal cavity through the inguinal canal into the scrotum during 15th to 28th weeks of development [1]. Even though there are many factors contributing towards this migration, gubernaculum has been shown to play an important role in this process, by means of its contracture [2], other factors being the steroid hormones and maternal gonadotrophins. The entire process of gonadal migration is conveniently divided into two stages: 1. Trans abdominal and 2. Extra abdominal. Gubernaculum, in the males is a gelatinous structure attached proximally to the testis and epididymis, extending variably to the scrotum or stopping short either in the inguinal canal or en route during the descent of the testis, obviously appearing

to guide the descent of the gonad [3].

Termination of gubernaculum as the 'mythical tails of Lockwood' proposed during the 18th century has now been refuted by the current biological evidence [4].

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Gubernaculum, continuous with the caudal extremity of the gonad as a mesenchymatous band extended downwards and merged with the urogenital ridge for a short distance before running around the abdominal wall, crossing the umbilical artery and ending in the inguinal region. Processus vaginalis, which is a diverticulum from the peritoneal cavity, grows into the gubernaculum and cremaster muscle developed therein without interference by body wall structures. Consequently, the scrotum and inguinal canal dilate to permit testicular descent. It has been shown that during the developmental process, ground substance within the gubernaculum increased resembling whartons jelly of the umbilical cord. Consequently, the scrotum and inguinal canal dilate to permit testicular descent. Gubernaculum consists of a proximal bulb, and a distal cord. Bulb of the gubernaculum has well demarcated mesenchyme, with densely arranged myocytes in bundles.

Testicular descent, marked by the growth of gubernaculum has been shown to be under the influence of factors involved in limb bud growth ensuring the movement of the testis from below the external inguinal ring down to the bottom of the scrotum [5].

During early gestation, in rats, the bulb of gubernaculum consisted of loose mesenchymal cells that develop into fibrillar cells [6,7] which later thicken to form rhabdomyoblasts and differentiate into spiral striated muscle towards the end of gestation. Striated musculature, found at the periphery of gubernaculum, formed the strip-like cremaster muscle. Orientation of the muscle fibres varied from being transverse to longitudinal in direction.

Transabdominal migration of the testis has been attributed to the active mitotic activity and an increase in glycosaminoglycans and hyaluronic acid, causing the caudal enlargement of the gubernaculum [8]. Removal of the extracellular matrix of the gubernaculum left a fibrous remnant that attached the testis and caudal epididymis to the scrotum after testicular descent. The hydrophilic nature of the hyaluronic acid makes the end of the gubernaculum bulky and gelatinous. Gene association studies in canine and procine models have indicated subtypes of quantity of collagen to influence testicular descent [9,10].

In rats, during the early postnatal period the gubernaculum was not in close proximity to the developing scrotum at any stage. Gubernacular eversion was more apparent than real and some degree of migration was needed for complete extension of the cremasteric sac to the bottom of the scrotum [11]. Gubernaculum has been shown to be innervated by genitofemoral nerve. Immunohistochemical studies using androgen receptor expression and estrogen receptor knock-out (ERKO) have thrown light on the details of differentiation of the mesenchyme and the time of appearance of skeletal and smooth muscle within gubernaculum

In goats, gubernaculum appeared as solid mass of highly eosinophilic mesenchymal cells, with spherical or ovoid nuclei at 56th day of gestation then became vascular and further differentiated histologically by 108th day of gestation [12]. Histological analysis of the gubernaculum of pigs ranging from 53 to 116 days of gestation revealed that they consisted of primitive mesenchymal cells with an abundant intercellular material containing glycosaminoglycans [13,14]. In 16 mm human fetuses, gubernaculum was seen as a mesenchymatous tissue covered by coelomic epithelium extending as a ridge from the differentiating gonad over the mesonephros up to inguinal canal. Sexual dimorphism did not exhibit until the foetus was 120 mm in length. Processus vaginalis and cremaster grew into the gubernaculum before

the testis descended. The swelling of this part by absorption of water might have produced a channel for the passage of testis. It is highly unlikely that cremaster muscle is differentiated from the gubernaculum [15]. Gubernaculum was seen to be made up of varying degrees of elastic fibres, skeletal muscle cells and collagen fibres [16].

They reported absence of smooth muscle cells in gubernaculum, in contrast to the reports [17] confirming the presence of both skeletal and smooth muscle cells, generating the propulsive force for the descent of testis through the processus vaginalis. Failure of descent of testis was associated with a decrease in smooth muscle content and a decrease in sympathetic tonus which in turn is depended on androgens. There seem to be controversial reports regarding the appearance of skeletal and smooth muscles and their roles within gubernaculum.

Striated muscle was seen by around 12 weeks and at 22 weeks of gestation myofibroblasts were detected followed by appearance of smooth muscle in the 27thweek in the gubernaculum of the male foetus [18]. It was suggested that cremasteric muscle might have trans-differentiated from the vascular smooth muscle.

Although laboratory animals rarely were cryptorchid, faulty mechanism of testicular descent results in cryptorchidism which was seen more common in pigs or humans (2- 12%) than in cattle or sheep [19].

Similarly, remodeling of the histological components of the gubernaculum was noticed in normal human fetuses of the age group 15 to 29 weeks and in children who had undergone orchiplexy.

In another interesting study, components of the gubernaculum testis, were altered significantly when treated with hCG in cryptorchid patients [20].

There seem to be controversial reports regarding the appearance of skeletal and smooth muscles and their roles within gubernaculum. The foregoing background establishes that there is paucity of information and controversies regarding the embryonic structures related to the gonads and their fate in the humans, and the present work aims to throw light on the structural features of gubernaculum in human males.

MATERIALS AND METHODS

Structure of gubernaculum was studied histologically using the material obtained from aborted fetuses made available from the Department of Obstetrics and Gynaecology, PSGIMS&R; Coimbatore. Graded series of human foetuses from the age group of 13 weeks to full term (II and III trimesters) and surgical specimen from children aged 6 months to 10 years were examined.

Consent for the study was obtained from the parents of the diseased infants. This study was approved by the Institutional Human Ethics Committee of PSGIMS&R. No:11/198.

Aborted fetuses collected from the labour room of PSG Hospitals and fixed in 10% formalin were carefully dissected within 24 – 48 hours.

They were classified into 1st trimester : <12 weeks, 2nd trimester : 12-24 weeks , 3rd trimester : 24-37weeks. Weight, head circumference and crown rump length were noted.

A midline incision extending from the xiphoid process down to the pubic symphysis and a transverse incision above the umbilical stump were made, thus dividing the anterior abdominal wall into four flaps. The peritoneum was opened likewise, thus exposing the peritoneal and pelvic cavities. The small intestines were pushed aside to identify the testis. Subsequently, gubernaculum was identified and its attachment to the testis and epididymis was established. Its course through the deep inguinal ring, inguinal canal, superficial ring into the perineum was traced and the gubernaculum was removed. Gubernaculum was cut into bits measuring 1.5cm X 1.5cms and these bits were refixed in 10% formalin overnight. The tissue bits, processed conventionally were sectioned at 4 microns and were stained with haematoxylin and eosin. Histochemical constituents of the tissue were studied using Masson's trichrome and Periodic acid – Schiff for mucins. Various connective tissue components were studied using Verhoff's stain, as well. Slides were examined under 10X, 40X & oil immersion.

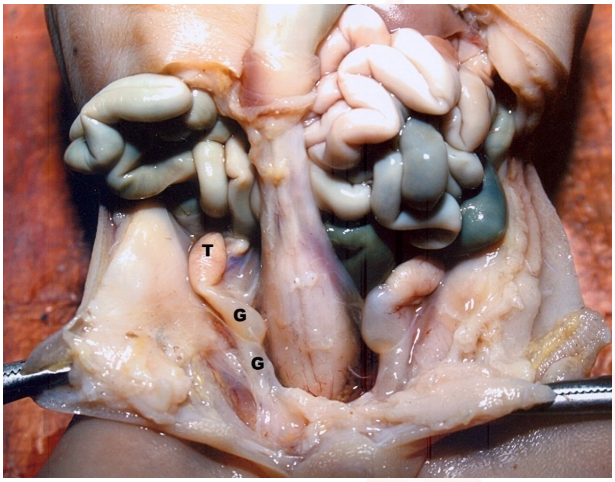
Exclusion criteria: Fetuses with severe macera-

tion. Fetuses with congenital malformations of testis & gubernaculum.

RESULTS

Gubernaculum was traced from the lower pole of the testis till the level of pubic crest. Thereafter it became less discernable and could not be traced further. Blood vessels supplying gubernaculum when traced were seen to be branches of inferior vesical artery, a branch of the umbilical artery.

Fig. 1: 24 Weeks Male Fetus dissected showing G-Gubernaculum T-Testis.



Histologically, gubernaculum during all the stages of development contained differing amounts of collagen and elastic fibres. Muscular tissue was apparent only from 19th week of gestation and showed a steady increase from then onwards and maximum amount of muscular tissue was seen during full term.

Collagen fibres formed the main content of the gubernaculum testis and maximum amount was observed in the foetus of 13 weeks. These fibres were diffusely dispersed within the gubernaculum (fig.2) of earlier fetuses. Their arrangement changed from 16 weeks onwards, when they started to be seen in bundles, running parallel to the direction of gubernaculum. These bundles appeared to be thicker at the periphery (fig.3). By the age of 26 week old foetus, these bundles became wavy (fig.4).

Study of the tissues collected from children undergoing orchiplexy was restricted to the distal end of the gubernaculum and the specimens were from the age group from 6 months up to 10 years. Collagen fibres were seen in all but no specific arrangements had been observed.

The amount of collagen in the cryptorchid gubernaculum was less compared to those of foetal tissue.

There was a steady increase in the amount of elastic fibres as the age advanced and maximum amount was noted at 19 weeks of age (fig.5). There after there was reduction in the amount of elastic fibres. Gubernaculum of cryptorchid children, the elastic fibres were few compared to collagen.

Although a few myoctes were visible in gubernaculum by 19 weeks of age, (fig.6,7) well differentiated skeletal muscle characterized by striations was detected only by, 23 weeks old foetus (fig.8).Gubernaculum of full term fetuses and that of cryptorchid testis exhibited longitudinal and cross striations (fig.9). Smooth muscle could not be detected in the specimens studied.

Blood vessels were observed from 13 weeks age onwards and were present in all the specimens studied. Gubernaculum of older foetuses appeared more vascular. Vascularity was seen in gubernaculum associated with undescended testis as well.

Fibroblasts were seen from the specimens of 13 to around 22 weeks of gestation. A few lymphocytes and sparse macrophages also could be identified in the specimens till 20 weeks of gestation. Scanty amount of fat was seen in the gubernaculum of 24 weeks onwards. Orchiplexy specimen also exhibited a small amount of adipose tissue.

Grossly, the proximal attachment of the gubernaculum was seen at the lower end of the testis, in concurrence with the observations of earlier workers [1,2,3,15,26].

However, the distal extent of the gubernaculum in our series was seen to terminate diffusely at the level of pubic symphysis, opposed to the observations of very early Anatomist like Curling (1840). Obviously 'tails of Lockwood' (Lockwood, 1888) also could not be demonstrated, agreeing with the more recent work [2]. Varying morphology wherein there was difference in the consistency between proximal, middle and distal parts of gubernaculum has not been documented earlier. Our observation is contrary to the findings of Favoirto [21] of 326

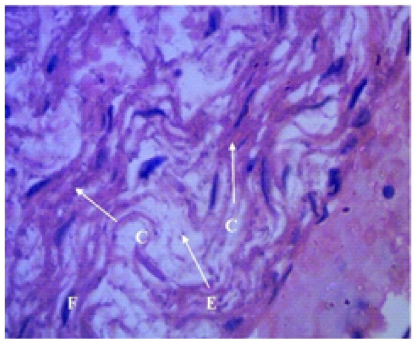


Fig. 2: Histological section of gubernaculum of male foetus of 13weeks, showing collagen bundles(C) elastic fibres (E) and fibroblasts (F).(H and E X 400).

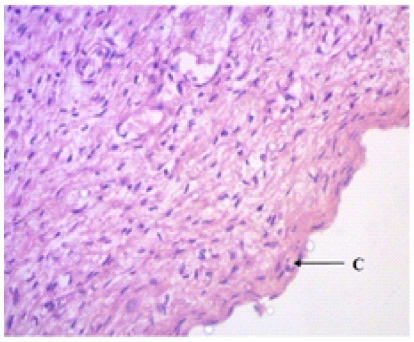


Fig. 3: Middle part of Gubernaculum of male foetus of 13 weeks. Note the collagen bundles (C) in the periphery (H and E stain X 100).

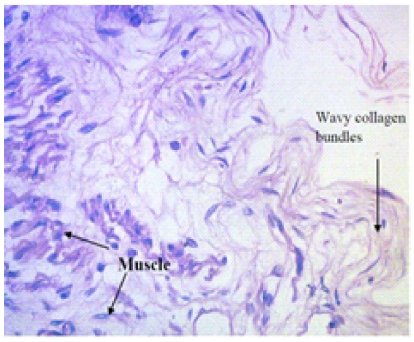


Fig. 4: Gubernaculum of male foetus of 26 weeks. Collagen seen in wavy bundles. (H and E stain X 400).

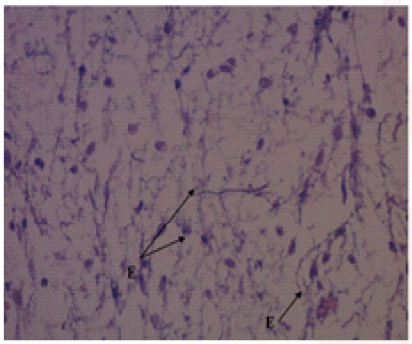


Fig. 5: Gubernaculum of male foetus of 19 weeks, showing elastic fibres. (H and E stain X 400).

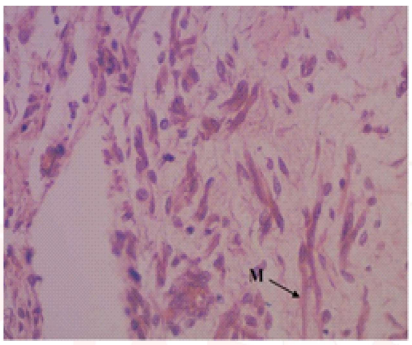


Fig. 6: Gubernaculum of male foetus of 19 weeks, showing solitary myocytes. (H and E stain X 400).

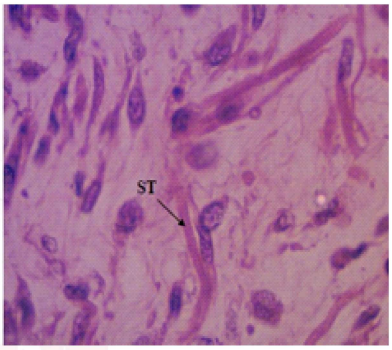


Fig. 7: Cross striations (ST) within individual myoctes. (H and E stain X 400).

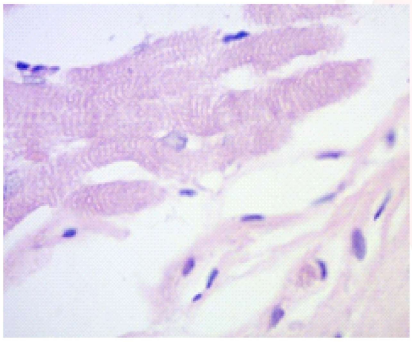


Fig. 8: Muscle fibres within the gubernaculums of 23 weeks old male foetus. Note the cross striations. (H and E. x 1000).

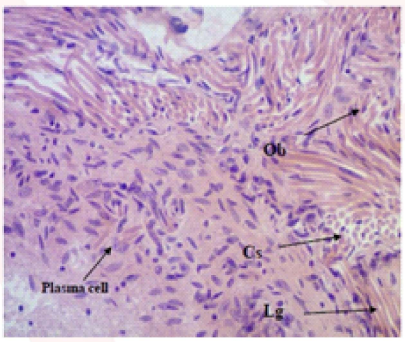


Fig. 9: Muscle fibres traversing in all directions within the gubernaculums of 36-weeks-old male foetus. (H and E x 400).

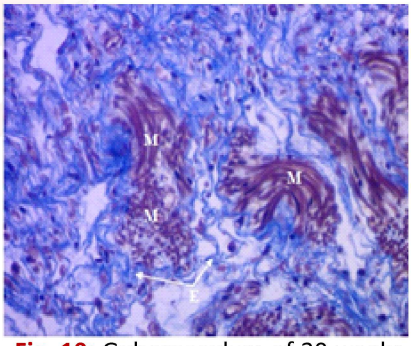


Fig. 10: Gubernaculum of 30 weeks-old male foetus showing muscle (M) and elastic (E) fibres. (Masson's trichrome x 400).

testis and gubernaculum wherein the authors have documented five of its extensions. Since the role of gubernaculum in 'pulling down' the testis has long been debated, the differing consistency of gubernaculum across its length does not seem to be of much significance.

Presence of collagen, elastic fibres and skeletal muscle is in confirmation [22,23].There has been a decrease in the collagen content from 13th week to 22nd week, while elastic fibres were seen to increase in quantity during this period. Then onwards both collagen and elastic fibres decreased. Role of collagen in gubernacular development has been documented earlier [24]. It has been suggested that contracture of collagen within gubernaculum is the effective mechanism responsible for descent of fetal testis, which occurs during 80 to 90 days of gestation. It can be assumed that collagen forms the framework for gubernaculum. Content of collagen decreased when traced from 13 weeks on wards. This may be to make sure that the size of gubernaculum decreased as gestational age advanced. Contracture of collagen fibres in the gubernaculum has been suggested as contributing to the mechanism of testicular

descent [24].Relative concentration of collagen and elastic fibres has also been suggested as very important in gubernaculum of normal foetuses, since it has been observed that in conditions of Cryptorchidism, in certain syndromes like Prune Belly, the relative concentrations of collagen and elastic fibres are altered. Analysis of the gubernacula across the second and third trimester of human gestation clearly illustrates that connective tissue within the gubernaculum undergoes extensive remodeling before they become highly fibrosed. Such changes within the gubernaculum have been accepted as a mechanism contributing to the natural movement of the testis towards the scrotum.

The present study has brought out the pattern of distribution of collagen within gubernaculum, which showed pattern change as age advanced. This observation is in agreement with that of Chaber [20] and Soito [21]. Based on our observations, it is tempting to suggest that the changes in the distribution of collagen reflects extensive remodeling of gubernaculum during testicular descent, a hypothesis put forward by Soito [21]. Furthermore, the same workers have shown rather fibrous nature and a reduction in collagen in cryptorchid testis and have suggested this morphology as a causative factor for undescended testis.

Gene association of collagen content with testicular descent has been documented in recent years [10]. Our observations on the possible roles of these factors is limited by lack of A decrease in the number of fibroblasts, as the foetal age advanced seems to be in line with the amount of fibres exhibited in the gubernaculum across the ages. This observation may be justified by the fact that fewer number of fibroblasts are enough to produce lesser amount of fibres and this seems to be self limiting.

Lots of lymphocytes and macrophages were seen in the younger fetuses, while in the older foetus above 26 weeks lymphocytes were few or absent. Early appearance of them could be for general immunological protection. Absence of these cells in the gubernaculum of children with undescended testis cannot be correlated with function. There seems to be paucity of information on these cells within gubernaculum.

Role of muscular tissue within gubernaculum has long been debated much. Our present observations have revealed appearance of muscular tissue later than in earlier reports [23]. Since the present study did not reveal physical continuity of musculature to anterior abdominal wall or to other regions, it was not possible to contribute to the theory that these muscle fibres have derived from either transversus abdominis or internal oblique abdominis [25]. Presence of smooth muscles along with skeletal muscle was recorded by Tanyel [18], who attributed the presence of both skeletal and smooth muscles to the propulsive forces seen within the gubernaculum, helping in accelerating the descent of testis. However, the present study did not reveal the presence of smooth muscles, in conformation with the findings of Costa [16].

Presence of myxomatous tissue increasing as age advances is a feature shared with the observations of Blackhouse [15] and Tanyel [23], although the Wharton's jelly like consistency as noted by the latter was not apparent in our series. Significance of scanty lymphocytes and macrophages, seen till around the same time period could not be verified.

CONCLUSION

The above findings concerning the histological configuration of gubernaculum testis seems to support the existing evidence in of its function contributing to guide the testis towards the scrotum and also to suggest the possible factors which may result in Cryptorchidism. This essentially involves time bound remodeling of the histological components of gubernaculum [19]. The present study, however could be extended further to evaluate the genetic factors involved during the normal development and differentiation of the gubernaculum.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the guidance and help of Dr. Pananghat A Kumar. Professor of Anatomy and Coordinator, Clinical Simulation Laboratory at PSGIMSR, Coimbatore, in preparing this manuscript.

Conflicts of Interests: **None**

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How to cite this article:

Beulah Priyalatha Swamickan, Aleyamma Fenn T.K. HISTOMORPHOLOGICAL FEATURES OF HUMAN GUBERNACULUM IN THE MALE. Int J Anat Res 2018;6(1.1):4777-4783. **DOI:** 10.16965/ijar.2017.353