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Research Article
Light and Scanning Electron Microscopy Studies of Male and Female Gubernaculum in Rats

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Keywords

Gubernaculum,
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sexual orientation.

Abstract

Background: The gubernaculum (helm / rudder) is a mesenchymal condensation intimately associated with the testis (and, to a lesser degree, with the ovary in females), and has been clearly identified as playing a key functional role in the mechanism of gonadal descent. **Objective:** To analyze the gubernacular tissues associated with reproductive tract development and providing the information towards elucidating the mechanisms of abnormal development in humans.**Methods:** The experimental animal model utilised in this study was the Sprague Dawley rats. Foetuses (n=6) at gestational days (Embryo, E16, E17, E18, and E20) and neonates at postnatal days (P0 [at birth], P2, and P4) were included.. Our present study was investigated by application of (LM, SEM study). Gubernaculum (Gb) at E16 consists of gubernacular cord (Gbc) and gubernacular cone (Gbn) at the region of future inguinal area. The primitive gonads appeared as thickening of coelomic epithelium from the mesonephros and positioned on the inferior pole of the developing kidneys. **Results:** At E17, the sexual orientation was determinate. At E20, male Gbn demonstrated maximum growth and exhibiting differential staining between Gbn core and periphery with short Gbc. In female, the Gbn demonstrated less growth and early degeneration. The Gbc appeared to be longer and thinner compared to counterpart in male. At postnatal P0, in male showed a remarkable inversion of Gbn and Gbn periphery were continuous with the abdominal musculature. In female, Gbn area showed fibro fatty structure remnants. **Conclusion:** The present study successfully determined some of the cellular factors required for a normal testis descent. Gb structure is unique to the male compared to female. In male, Gb showed a considerable contractility of Gbc and inversion of Gbn periphery as well as core to accommodate the testis.

Introduction

Cryptorchidism or failure of testis descent has been variously ascribed to *in utero* environmental agents, genetic predisposition or both[1]. It is observed that rising adverse trends in both male and female genital malformation are evident with resultant reproductive health problems[2].

During reproductive development, the testis descends from the posterior abdominal wall to its final anatomical location in the scrotum at / after birth which appears to be a complex process involving more than a simple physiological migration[3]. Although the ovary undergoes a distinct caudal shift in position, it is not to the same extent and manner as testicular descent[4].

The present study has focused on the detailed analysis of gubernacular tissues associated with reproductive tract development and providing the information towards elucidating the mechanisms of abnormal development in humans.

Materials and Methods

The experimental animal model utilized in this study was the Sprague Dawley rat. All animals were obtained from the

Animal Research centre, Faculty of Medicine, University of Malaya. The pregnant females were sacrificed and foetuses harvested at various gestational days (E16, E17, E18, and E20) and postnatal days (P0 [at birth], P2, and P4). A total number of 6 per experimental group of males were consistently compared against a similar group of females for each phase of the study. All foetuses were collected and assigned to containers filled either 4% formaldehyde fixative and 4% glutaraldehyde fixative for light (LM) and scanning electron microscopic (SEM) study respectively. Dissections of the anterior abdominal wall of the rats were carried out under a dissecting microscope to expose the developing gonads and associated gubernacular tissue. For LM analysis, routine H and E staining were carried out. For SEM, the specimens tissues were further cut into $1cm^3$ slices and fixed overnight with 2.5% buffered glutaraldehyde. After primary fixation, samples were then washed with 3 changes of 0.1 M sodium cacodylate buffer (at 10-minute intervals) and then stored at 4°C. Post–fixation was carried out in 1% osmium tetroxide (2 hours at 4°C) and then, specimens were again washed with 0.1 M sodium cacodylate buffer sodium (3 changes 10 minutes each). The specimens were dehydrated in a series of acetone. After the process of dehaydration, the specimens were transferred into critical point dryer (CPD) for 1 hour. After the critical point dryer, they were carefully mounted on mounting stubs. A coating of specimens was carried out using a gold sputter coater for 3 minutes. Specimens were viewed using a SEM and camera Lucida.

Results

By E16, the gonads were positioned on the inferior poles of the developing kidneys but sexual orientation still remained indeterminate at this stage. The pair of genital ducts, primordia of the future mesonephric/Wolffian (WD) and paramesonephric / Müllerian duct (MD) was observed to be connected inferiorly from these developing gonads towards the primordial gubernaculum. The gubernaculum (Gb) consisted of a distinct gubernacular cone (Gbn) with proximally extending cord (Gbc). The gubernacular cord formed the caudal connection between the gonads and inguinal region. The cranial suspensory ligament (CSL) formed a cranial attachment for the mesonephros and primitive gonad to the posterior abdominal wall.

At E17, evidence of gonadal sexual differentiation was observed. The testis was ovoid in shape and was positioned just below the inferior pole of the kidney. The ovary was also ovoid in shape and was located lateral to the inferior pole of the kidney. The CSL was present in both sexes and remained attached to the cranial aspects of their respective gonads. At this stage, the first perceptible differences at the cellular level between male and female gubernacula were also demonstrable. In males, the Gbn exhibited increased cellularity, especially at its periphery compared to females.

By E18**,** the testis has descended halfway between the inferior pole of the kidney and the inguinal region. The male CSL was noted to be thinner and appeared to degenerate. Linear arrays of dense, spindle-shaped cells were present in the Gbn periphery while the Gbn core in the males, contained scattered eosinophilic and pyknotic cells, indicating possible apoptotic degeneration (Fig. 1). The ovary was more distinctly oval in shape and was positioned immediately lateral to the inferior pole of the kidney. The CSL, attached to the ovaries, was thicker and more vascular than in their male counterparts. The Gbn and Gbc in females still demonstrated reduced cellularity and increased fibrous tissue content compared to males; the Gbc remained connected to the broad Müllerian duct (Fig. 2).

In males, by E20, the ovoid testis and the curved epididymal tail descended towards the apex of the Gbn. The dilated dome-shaped Gbn exhibited differential staining between its core and periphery; the core exhibited reduced cellularity and comprised spindle-shaped mesenchymal cells while at the periphery, deep staining were present. In females at E20, the ovary was oval in shape, still positioned at the inferio-lateral pole of the kidney. The Gbn exhibited uniform cellularity lacking muscle fibres. The mesenchymal cells were spindly replaced by fibrous tissues. The Gbc was longer, slender and attached to the lateral aspect of the uterine horn. The ovary was surrounded by the fimbriae (Fig. 3).

At birth (P0) in the male, the oval testis and the epididymis were interposed between the testis and the internal inguinal ring, which was demarcated by the inferior margin of the anterior abdominal wall musculature. The peritoneum of the coelomic cavity formed an evagination, the primitive processus vaginalis, followed the course of the gubernaculum into the inguinal canal. The Gbn was involuted and consists of distinct deep staining at periphery and vacuolation within the core, the Gbc was short. SEM study also revealed early invagination of the Gbn and shortening of the Gbc. In the female, the elongated ovary containing primary follicles was still positioned at the inferio-lateral pole of the kidney. The region of the internal inguinal ring showed the presence of Gbn with reduced cellularity and vascularity. The Gbc in female was elongated and slender compared to the male counterparts (Fig. 4).

At P2, the epididymis appeared comma-shaped and the testis was seen to contain distinct seminiferous tubules and a capsule. The Gbn had partially evaginated through the inguinal canal (like the inversion of glove fingers). The Gbc had shortened, but still connecting the apex of the Gbn to the caudal epididymis and this part of epididymis preceded the testis in descending through the inguinal canal. In the female, the shaped and positioned of ovary, there was no change from P0. Gbn was reduced and consisted of fibro-fatty tissue.

By P4, the oval shaped testis containing a distinct elongated differentiated seminiferous tubule distributed throughout the testis. The epididymis continued their passage through the inguinal canal into the scrotum. On either side of the invaginating Gbn, a marked inferio-medial invagination of the mesothelium lining was also observed into the inguinal region. In the female, the shaped and positioned of ovary, there was no change from P0. The gubernacular tissues, including cone appeared much shrunken and increasingly fibrous and slender. Gbc was contributing to the formation of the round ligament precursor. This round ligament clearly possessed a distal attachment which could be traced from the internal inguinal ring continuing lateral to the urinary bladder and along with the posterior abdominal wall, with its proximal attachment to the uterine horn (Fig. 5).

At P8, in the male, the testis and caudal part of the epididymis was located well inside the scrotal sac. The Gbc had become a short stump attaching the epididymis to the base of the future scrotum (Fig. 6).

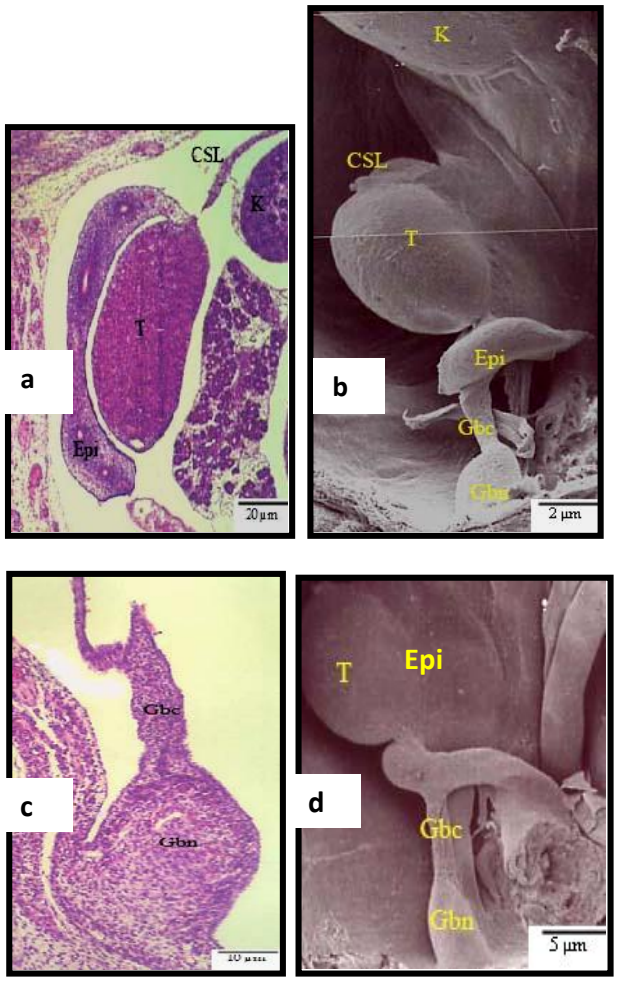

Fig.1. Male gubernaculum at E18. **(a)**Photomicrograph **(b)**scanning electron micrograph.**(c)** Photomicrograph showing linear orientation of cells in gubernacular cone (Gbn) periphery and in Gbn centre showing scattered pyknotic cells. **(d)** scanning electron micrograph. Gubernacular cone(Gbn); gubernacular cord (Gbc); epididymis (Epi); testis(T); cranial suspensory ligaments (CSL); kidney (K). Scale bar. (a) 20 µm (b) 2 µm. (c) 10µm (d) 5 µm.

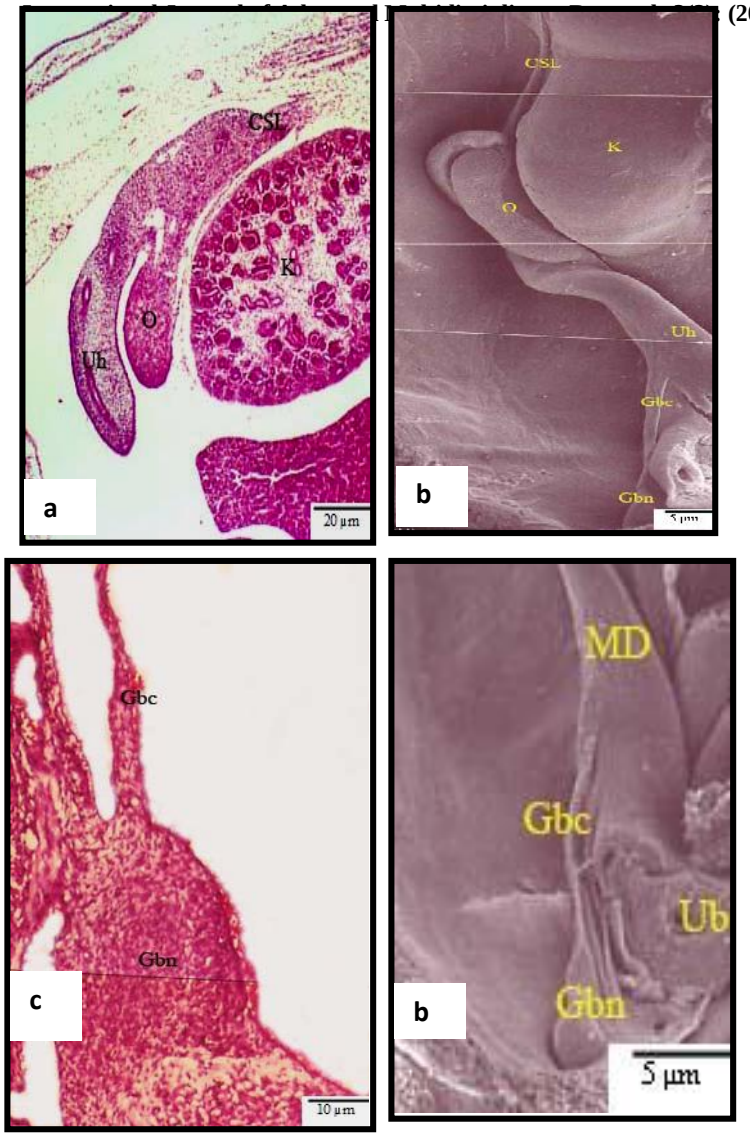

Fig. 2. Female gubernaculum at E18. **(a)** Photomicrograph **(b)** scanning electron micrograph showing the developing cranial suspensory ligament (CSL); kidney (K); ovary (O); elongated uterine horn (Uh) curving superiorly around the upper aspect of the ovary; gubernacular cord (Gbc); gubernacular cone (Gbn). **(c)** Photomicrograph showing uniform cellularity / non-differentiation of gubernacular cone (Gbn) with slender gubernacular cord (Gbc). **(d)** scanning electron micrograph showing the relationship of the Müllerian duct (MD) and the urinary bladder (Ub) to the gubernacular structures. Scale bar. (a) 20 µm (b) 5 µm. (c) 10 µm (d) 5 µm.

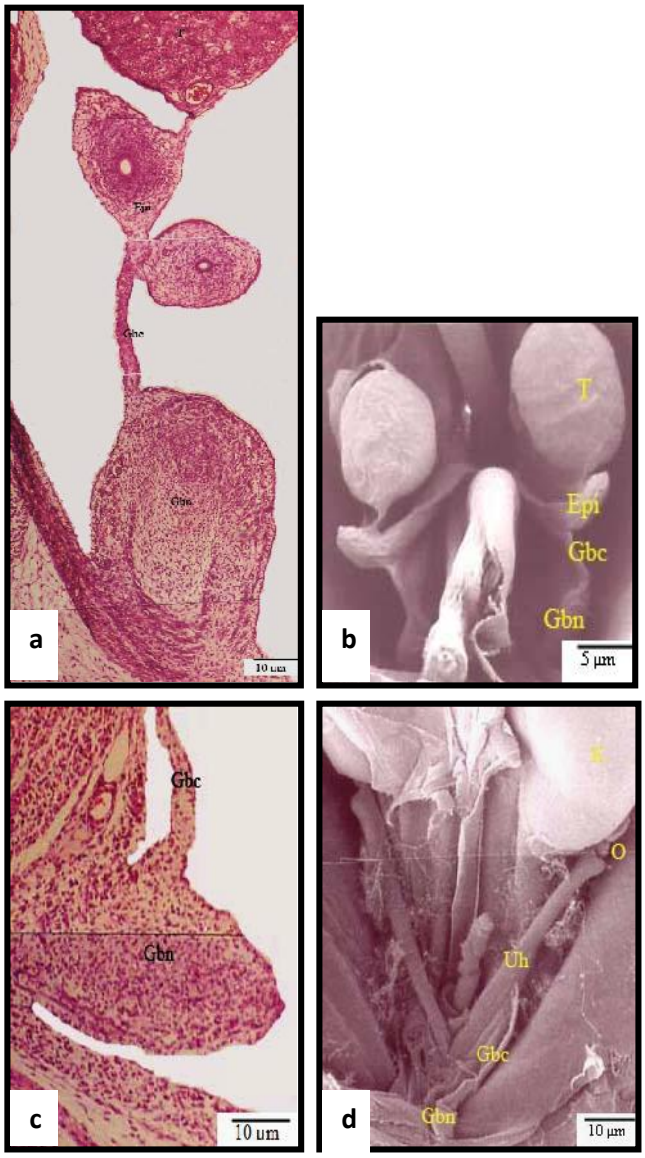

Fig. 3. Male and female gubernaculum at E20. **(a)** Photomicrograph showing a distinct orderly arrangement of muscle-like fibres at Gbn periphery and mesenchymal cells at the Gbn core. **(b)** scanning electron micrograph. Gubernacular cone (Gbn); short gubernacular cord (Gbc); epididymis (Epi); testis (T). Scale bar. (a) 10 µm (b) 5 µm. (**c**) gubernacular cone (Gbn) exhibited uniform cellularity largely lacking muscle like-fibres and mesenchymal cells appearing spindly. **(d)** scanning electron micrograph showing reduced gubernacular cone (Gbn), and elongated gubernacular cord (Gbc) attached to uterine horn (Uh); ovary (O); kidney (K). Scale bar. (a) 10 µm (b) 10 µm.

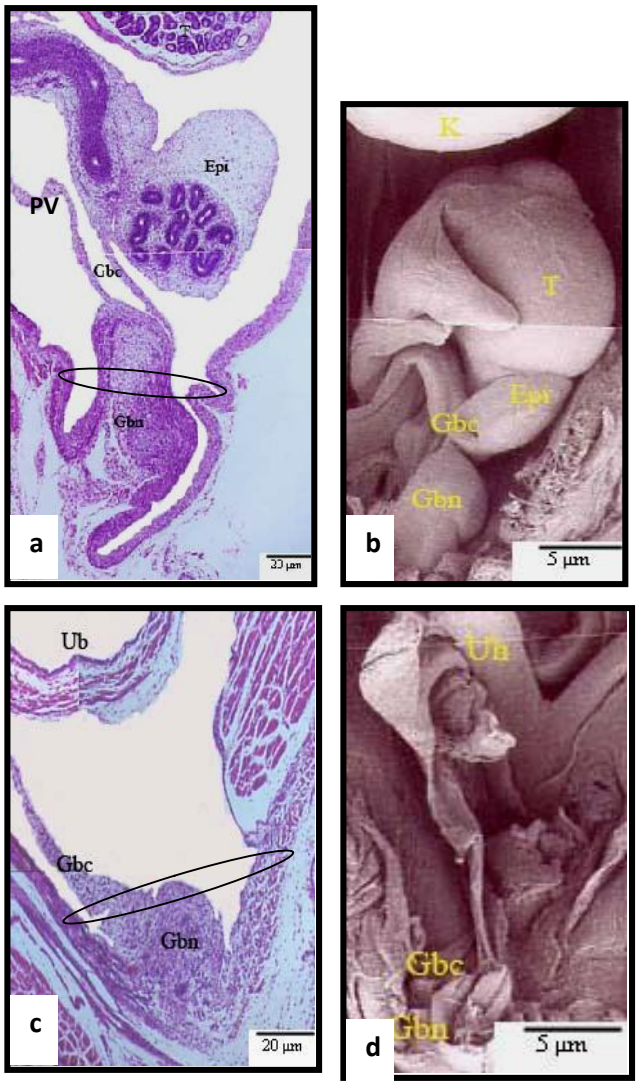

Fig. 4. Male and female gubernaculum at P0. **(a)** Photomicrograph showing the invagination of the gubernacular cone (Gbn) and the internal inguinal ring (oval ring). **(b)** scanning electron micrograph showing invagination of the gubernacular cone (Gbn); short gubernacular cord (Gbc); epididymis (Epi); processus vaginalis (PV); testis (T); kidney (K). **(c)** Photomicrograph showing no sign of invagination of the gubernacular cone (Gbn), the gubernacular cord (Gbc) is slender in appearance and internal inguinal ring (oval ring). **(d)** scanning electron micrograph showing gubernacular cone (Gbn) with slender gubernacular cord (Gbc) attached to the uterine horn (Uh). Scale bar. Scale bar. (a) 20 µm (b) 5 µm.(c)20 µm (d) 5 µm.

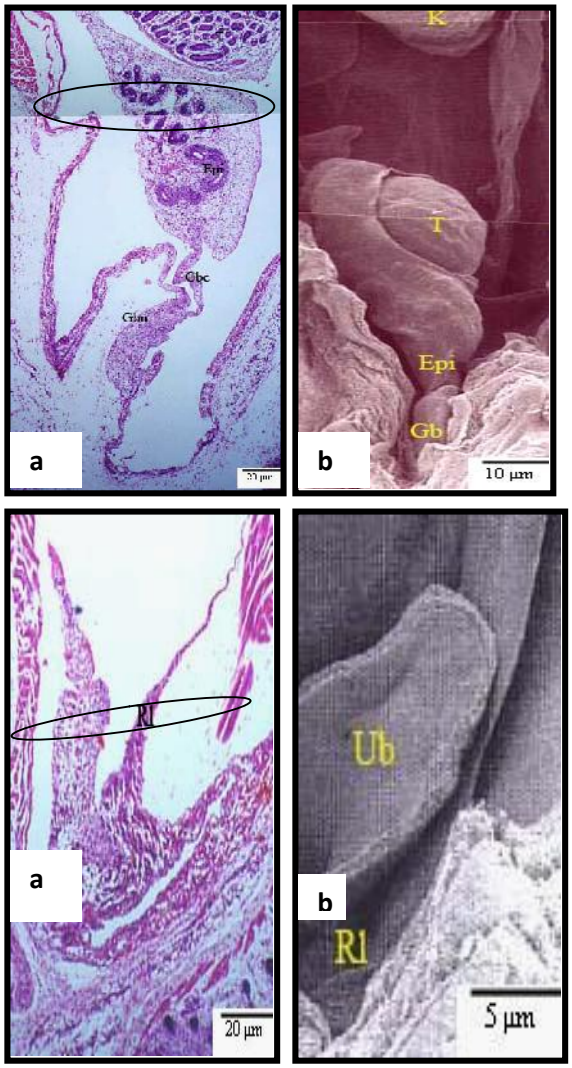

Fig. 5. Male and Female gubernaculum at P4. **(a)** Photomicrograph showing entry of the testis through internal inguinal ring (oval ring). **(b)** scanning electron micrograph showing entry of the caudal part of the epididymis (Epi) into inguinal canal accompanied by the testis (T). Scale bar.(a) 20 µm (b) 10 µm.

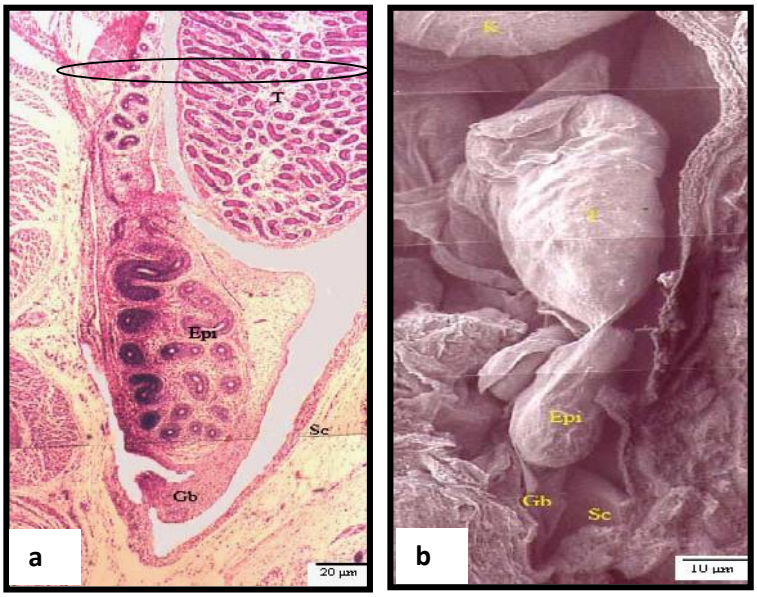

Fig. 6. Male gubernaculum at P8. **(a)** Photomicrograph showing the testis in the inguinal canal indicated by internal inguinal ring (oval ring); epididymis (Epi) into the scrotal sac (Sc) and attachment of the gubernaculum (Gb). **(b)** scanning electron micrograph. Gubernaculum (Gb); scrotal sac (Sc); epididymis (Epi); inguinal canal has been dissected to demonstrate these structures. Scale bar: (a) 20 µm (b) 10 µm.

Discussion

At E16, the sexual orientation in the present study remained indeterminate and the Gb was morphologically similar in both sexes. This is in agreement with Van der Schoot[5] who reported that the gubernacular primordia of the females develop initially in a similar fashion to the males and are indistinguishable.

At E17, males and females were clearly distinguishable by LM, with the testis, located just below the inferior pole of the kidney and the ovary located lateral to the kidney. Similar findings were reported by Van der Schoot and Elger[6] in rats, the ovary remained in close proximity to the caudo-lateral pole of the developing kidney. Morphologically, the Gb in males exhibited increased cellularity especially at the Gbn periphery demonstrating undifferentiated mesenchymal cells in the Gb core, while in the female, no clear distinction was observed. Similar findings in the male were also reported by George[7] but these researchers did not report any microscopic differences between male and female Gb.

Light and scanning electron microscopy analysis of the testis of E18 males highlighted its oval shape and its descent halfway down between the inferior pole of the kidney and bladder neck in this study. The ovary in females appeared only slightly enlarged and positioned lateral to the inferior pole of the kidney. Morphologically, the Gbn at this stage exhibited linear arrays of spindle shaped cells seen at the Gbn periphery. The Gbn core however, was composed of loosely arranged mesenchymal cells. In contrast, the female Gbn demonstrated reduced cellularity at the periphery compared to the Gbn of the male. The difference between the cellularity of the male and female Gbn suggests that the male Gbn had higher growth activity compared to the female Gbn.

In the present study, by E20, the size of the Gbn in males was maximal. Morphologically, the Gbn at this stage exhibited differential staining density between the Gbn core and periphery, whereas, the female Gbn persisted in demonstrating uniform staining. This indicates increased cellularity activity in male Gbn compared to female Gbn, a comparable finding to that by Heyns[8] who concluded that growth of the Gbn was caused by cell proliferation, increased in glycosaminoglycans and hyaluronic acid. In fact, it has been postulated that the removal of such hydrophilic extracellular matrix might cause involution of the Gbn. Heyns and Hutson[9] stated that the dynamic development of the Gbn in rats before testicular descent has an important role in that activity.

In this study, at birth (P0) in the male, the oval testis was demonstrated to possess the epididymis interposed between it and the internal inguinal ring. In the female, the elongated ovary containing distinct primary follicles remained positioned at the inferio-lateral pole of the kidney. The region of the internal inguinal ring was a useful landmark to locate the presence of Gbn. In the present study, it was observed that the processus vaginalis followed the course of the gubernaculum into the inguinal canal. Van der Schoot and Elger[6] similarly reported in rats that at P0, the Gb starts inversion as the first steps towards the formation of the muscular cremaster sac. This finding was confirmed by our SEM study which revealed invagination of the Gbn and shortening of the Gbc. Female Gbn cellularity, however, was reduced and Gbc appeared slender and much elongated compared to male counterparts. The importance of the course of processus vaginalis in this study supported the findings by Fallat et al[10], where the rat gubernaculum and processus vaginalis migrate to the scrotum before testicular descent. In our study, the female Gbn had reduced cellularity, and no sign of Gbn inversion compared to male Gbn. This finding also explained the presence of vacuolation in the Gbn core which ultimately also created a space for the testis to be pushed into the invaginated Gb.

The findings of this study clearly show that Gb in males plays an important role in testicular descent. Heyns and Hutson[9] who postulated that descent occurred when the upper part of the hollow Gb inserted itself into the lower part through the contraction of muscle fibers surrounding the upper part of the fluid-filled Gb, which pushed the abdominal muscles apart. The route for descent was then opened through re-absorption of this fluid. In this study, the presence of processus vaginalis was noted. Tanyel et al[11] suggested that testis descent through the processus vaginalis was by coordinated propulsive activity of surrounding smooth muscles and cremaster muscle in a way that resembles the transport of a bolus through the esophagus.

Morphologically at P2, the position of the testis was slightly below the bladder neck, and the caudal part of the epididymis was first delivered into the inguinal canal in this study. In the present study, the Gbn structure at P2 was altered, exhibiting reduction in size and regression at this stage. Whereas, in females, the Gb also appeared to contain reduced cellularity and comprised fibro-fatty tissue. This finding was similar to Husmann and Hasman[12], where the male Gbn core decreased dramatically in volume and almost completely disappeared by postnatal day P3.

In this study, at P4, the epididymis began to enter the inguinal canal followed by the testis. The invaginating Gbn was accompanied by a marked invagination of the mesothelium lining of the Gbn along a medial and inferior direction within the inguinal region. In female gubernacular tissue, the Gbn appeared much reduced / shrunken with increasingly fibrous and slender Gbc forming the round ligament precursor. The distal attachment of this primitive round ligament could be traced from the internal inguinal ring, continued alongside the urinary bladder and up the posterior abdominal wall with its proximal attachment to the uterine horn in the present study. Ando et al[13] reported in humans that the ligament which runs along an inguinal hernia sac in females was believed to be the round ligament of the uterus.

Attah and Hutson[14] reported that in the human female, the Gb remained small and thin after the fourth month of uterine life. The Gb finally differentiated to form the suspensory ligament of the ovary and the round ligament of the uterus. The Gb was attached to the uterus near the origin of the uterine tube. The cranial part of the Gb became the ovarian ligament; the caudal part formed the round ligament of the uterus.

In this study, morphologically, at P8 in males, the caudal part of the epididymis entered the scrotal sac and the Gbc remnant appeared as a short stump attached to the base of this sac. This finding was similar to Heyns et al[15] that testicular descent into the scrotum was influenced primarily by the need for migration of the caudal part of the epididymis to this cooler scrotal region. Whereas, Shono et al[16] reported in mice and rodents that inguinoscrotal descent began at birth as a real migration of the testis towards the scrotum. Clarnette and Hutson[17] claimed that in humans, the inguinoscrotal descent began between 26 – 28 weeks of gestation during which the testis and the Gb descended rapidly through the inguinal canal, and then moved slowly to the scrotum.

Conclusion

The present study successfully determined some of the cellular factors required for a normal testis descent. Gb structure is unique to the male compared to female. In male, Gb showed a considerable contractility of Gbc and inversion of Gbn periphery as well as core to accommodate the testis.

Acknowledgments

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