Original Research Article

Morphologic and histological differentiation of gubernaculum in female fetus: a cadaveric study

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ABSTRACT

Background: In both male and female fetuses inguinal canal development entails a complex sequence of anatomic events involving the gubernaculum and processus vaginalis. Much has been written about the embryological development of the genital system, particularly the male genital system and the descent of the testes from the abdominal cavity into the scrotum. In this process, the gubernaculum plays a relevant although still unclear role. Despite all the studies that have been performed, controversy still exists in this anatomical region.
Methods: Twenty round ligaments of uterus were dissected from female fetuses and microscopic structure was studied under light microscope using haematoxylin and eosin stain. The specimens were collected from female fetuses (8wks-26wks). One male fetus was also dissected.
Results: Gubernaculum plays a crucial role in the development of the inguinal region. The gubernaculum is directly associated with the migration of the testis through the inguinal canal and probably to the scrotum; but the inguinal canal is present before testicular descent and females have both an inguinal canal and gubernaculum, although the ovaries do not migrate through the abdominal wall.
Conclusions: In this anatomical region, and despite all the studies that have been performed, controversy still exists. This article attempts to study the morphology and histology and the differentiation of the gubernaculum with age.

Keywords: Gubernaculum, Round ligament, Uterus

INTRODUCTION

The round ligament of uterus represents a mammalian developmental novelty. The unusual structure of the round ligament is related to one or more of the many unusual features of human uterine development. The uterus develops as a single organ with a position deep in the abdominal cavity below the pelvic brim and far away from the posterior abdominal wall. The unusual anatomical position may require an unusual construction of the uterine suspensory apparatus of which the round ligament is one component.[1] Its exact role in females has many claims.

The round ligament is continuous with the ligament of ovary and the two represent in continuity, the gubernaculum, the counterpart of the gubernaculum testis of the male. Arey et al claims that the ligament appears as early as 7th week of IUL.[2] By the beginning of 3rd month a continuous cord of dense mesenchyme extends from the uterus to the labium majus. The ligament passes through the abdominal muscles which organize about it to form the tubular inguinal canal with the round ligament in females or spermatic cord in males. Shallow peritoneal pockets persist as diverticula of Nùck correspond to the primary vaginal processus of the male. There is no external descent of ovary, one reason being the

mechanical block presented by the uterus which is interposed between ovarian and round ligaments.

The aim of the study was the morphological and histological differentiation of gubernaculum in female fetus of 8 wks to 26wks (Cadaveric study).
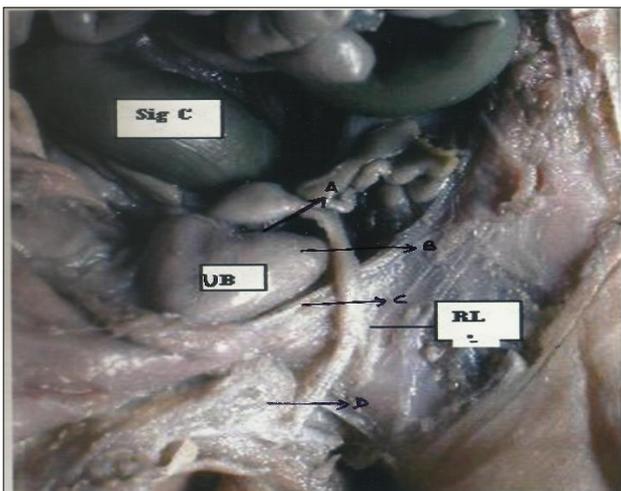
METHODS

Twenty round ligaments of uterus were dissected from female fetuses and microscopic structure was studied under light microscope using haematoxylin and eosin stain. The specimens were collected from female fetuses (8wks-26wks). One male fetus was also dissected.

The round ligament was divided into four regions- at uterine cornu, in the pelvis, in inguinal canal and at its termination i.e., just beyond the superficial inguinal ring. These were named A, B, C and D respectively. The proximal part of each of these segments was taken for the microscopic study (Figure 1).

To study the microscopic structure of the round ligament, each round ligament was cut transversely and cut parts were subjected to tissue processing for histological study. The specimens were dehydrated in ascending grades of alcohol, cleared in chloroform and embedded in paraffin wax. 5µ thick sections were cut and were examined with H and E stain.

RESULTS

The gross anatomy and microscopic structure of the round ligament of uterus has been studied in each of 20 round ligaments from fetuses ranging from 8-26wks. The microscopic structure of the gubernaculum testis was studied for comparison.

The round ligament was divided into four regions- at uterine cornu, in the pelvis, in inguinal canal and at its termination i.e., just beyond the superficial inguinal ring. These were named A, B, C, and D respectively.

The proximal part of each of these segments was taken for the microscopic study (Figure 1).

Gross anatomy

The length of the round ligament is found to vary from a minimum of 0.8 cms to a maximum of 2.6 cms in the fetuses.

The round ligament in fetuses from 8-17wks is seen attached to the proximal part of the uterine tube (Figure 2, 3). In older fetuses it is seen attached separately to the lateral border of the uterus as in adult females.
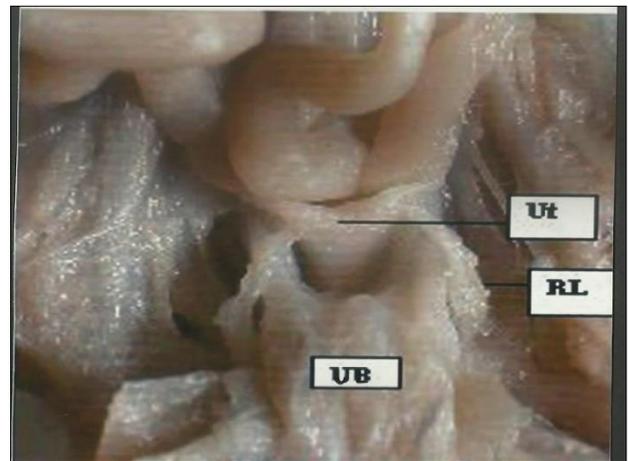

Figure 2: Fetus 10wks. The round ligament (RL) is seen arising from the uterine tube UB: Urinary bladder, Ut: Uterus.

Figure 1: Fetus 26wks. The four regions from where the specimen is taken for histological study are shown A: at uterine cornu B: in the pelvis C: in inguinal canal D: At Termination Sig C: Sigmoid colon, UB: Urinary Bladder, RL: Round ligament.
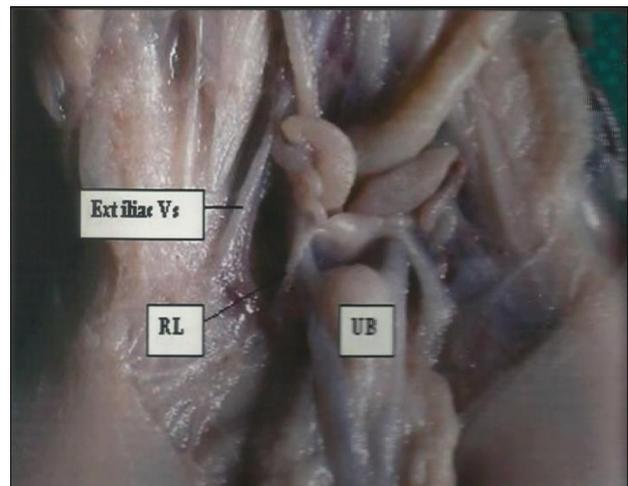

Figure 3: Fetus 17wks. The Round ligament(RL) is seen passing medial to the external iliac vessels (Ext iliac Vs).

It passes forwards and laterally in the anterior leaf of broad ligament. In the fetus, the external iliac vessels are lateral to the ligament (Figure 3) while in the adult it reaches the side wall of the pelvis and crosses the vescical vessels, obturator vessels and nerve, obliterated umblical artery and also the external iliac vessels.

At the deep inguinal ring, it hooks round the inferior epigastric vessels and then traverses the inguinal canal. In its course in the inguinal canal it receives coverings corresponding to those of the spermatic cord in male.

Further it is accompanied by a branch from the ovarian artery and in the caudal part by a branch from the inferior epigastric artery. Ilioinguinal nerve and the genital branch of genitofemoral nerve also accompany it in the inguinal canal. In the fetus, a tubular process of the peritoneum is carried with it for a short distance into the inguinal canal (Canal of Nück). The ligament loses its identity among the deep tissue of the labium majus after coming out of the superficial inguinal ring in all the specimens although the mode of termination of the ligament is different in the fetuses and the adult.

Figure 4: Fetus 24wks. Depicting termination of the Round ligment in one specimen.

It remains as a fibrous band in the fetuses (Figure 4) while it breaks up into 4-5 fibrous strands in the adult females. The distance from termination of the round ligament to caudal labium is found to increase with gestational age, 0.6cms in 8wks fetus to 1.5cms in 26wks fetus. This is due to increase in the size of body wall.

Microscopic structure (Table 1)

Uterine cornu

In the smallest fetus (8-10wks), the round ligament is seen as a collection of mesenchymal cells with fibroblasts (Figure 5). Very few blood vessels and myoblasts are seen. Mesenchymal cells are stellate to fusiform, nucleus is large and pale with scanty cytoplasm and they form a syncytium.
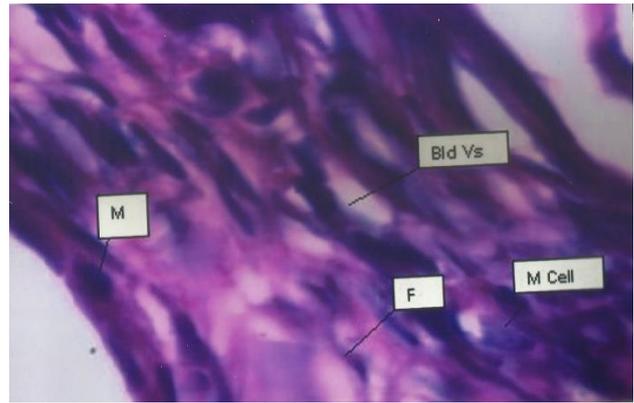

Figure 5: (X40) TS through RL at uterine cornu in 10wks fetus showing fibroblasts, mesenchymal cells(M), very few blood vessels (Bld Vs) and myoblasts (M).

Fibroblasts are irregular to fusiform, branching basophilic cells with ovoid euchromatic nucleus. In 10-12wks, the section shows plenty of fibroblasts in the mesenchyme. Mesenchymal cells differentiate into myoblasts which give rise to the muscle. Myoblasts are seen as darkly staining elongated basophilic structures with elongated nucleus as compared to the surrounding mesenchymal cells.
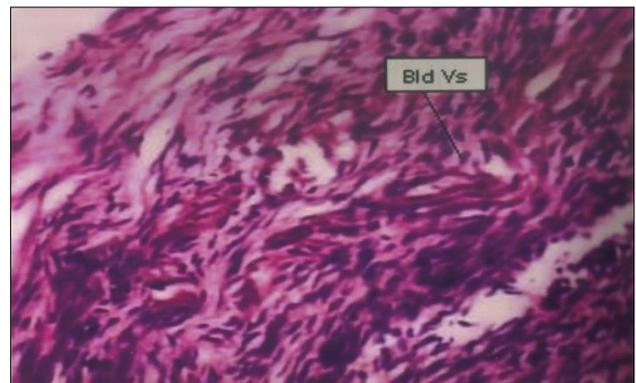

Figure 6: (X20) TS through RL at uterine cornu in 17 wk old fetus showing increase in vascularity.

Plenty of myoblasts and smooth muscle are observed in 17wks fetus. Mesenchymal cells are few in number. The myoblasts start arranging as a central longitudinal core and oblique peripheral region and smooth muscle is also seen in the peripheral region. Vascularity is increased (Figure 6). In 22-26wks fetuses, large congested blood vessels with mesenchymal cells, fibroblasts and myoblasts are seen. In one 22wk old fetus, central zone of blood vessels (vascular core) is seen surrounded by fibroblasts, mesenchymal cells and peripheral myoblasts. In a 26wk old fetus, central zone of blood vessels, mesenchymal cells, myoblasts, smooth muscle and peripheral fibroblasts are seen.

Table 1: Microscopic structure of fetal round ligament at 10, 17, 22 and 26wks.

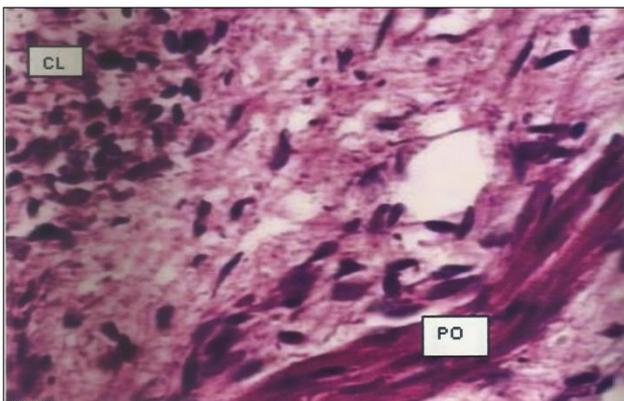| Uterine Cornu (A)	10wks	17wks	22wks	26wks
Blood vessels	+	++	+	+ in central zone
Mesenchymal cells	+	+few	+ few	+
Fibroblast	+	+	+	+
Myoblasts	+	+ Smooth muscle seen (Central longitudinal & peripheral oblique)	+ more at periphery	+
Pelvis (B)	10wks	17wks	22wks	26wks
Blood vessels	+	+	+	+
Mesenchymal cells	+	+	+	+
Fibroblast	+	+	+ at periphery	+
Myoblasts	+	+ at periphery	+ (Central longitudinal & peripheral oblique)	+in centre
Inguinal Canal (C)	10wks	17wks	22wks	26wks
Blood vessels	+	+	++	++
Mesenchymal cells	+	+	+few	+few
Fibroblast	+at periphery	+	+few	+few
Myoblasts	+ very few	+few	+ Smooth muscle (Central longitudinal & peripheral oblique) Skeletal muscle outermost	+ Smooth muscle (Central longitudinal & peripheral oblique) Skeletal muscle outermost
Termination (D)	10wks	17wks	22wks	26wks
Blood vessels	+	+	+	+
Mesenchymal cells	+	+	_	+
Fibroblast	+	+	+	+
Myoblasts	+few	+few skeletal muscle scattered	+	+ longitudinal in centre

Figure 7: (X20) TS through the RL in pelvis in 22wk old fetus showing central longitudinal core (CL) & peripheral oblique (PO) arrangement.

Pelvis

In 8-10wks fetus, only mesenchymal cells and fibroblasts are seen with few blood vessels. Myoblasts are seen to appear first in 10wks old fetus. The ligament resembles loose areolar tissue in 17wks old fetus and a covering of peritoneum can be seen accompanying it. In 22-26 wks old fetuses the number of fibroblasts is reduced, myoblasts show a central longitudinal core and peripheral oblique arrangement (Figure 7).

Figure 8: (X20) TS through the RL in the inguinal canal in 22wk old fetus showing increased vascularity. Central longitudinal core (CL) & peripheral oblique (PO) arrangement of myoblasts (M) seen along with mesenchymal cells (M cells).

Inguinal canal

Mesenchymal cells and fibroblasts are seen in 8-10wks. Myoblasts are very few. Undifferentiated mesenchyme is seen in between. In 10-17 wks fetus the picture remains the same and the specific arrangement of myoblasts has not appeared as yet. 22-26 wks old fetuses show a marked increase in vascularity. Fibroblasts and mesenchymal cells are very few. Myoblasts arrange themselves in a central longitudinal core with obliquely arranged myoblasts at the periphery (Figure 8). Skeletal muscle appears first in 22wks old fetus. Vascular core is seen in one 26wks old fetus.

Termination

In 8-10 wks fetus mesenchymal cells and fibroblasts are seen. Myoblasts are also seen in 10-12wks fetus in addition to mesenchymal cells and fibroblasts. In 17 wks fetus blood vessels, mesenchymal cells, fibroblasts, myoblasts and a few scattered skeletal muscle are seen. Skeletal muscle fibre shows peripheral nucleus in transverse section while prominent cross banding or striations and multiple peripheral nuclei can be seen in longitudinal section. In 22-26wks fetus fibroblasts, myoblasts, mesenchymal cells are seen with plenty of blood vessels. In one 24wks old fetus smooth muscle and peripheral skeletal muscle are also seen (Figure 9).
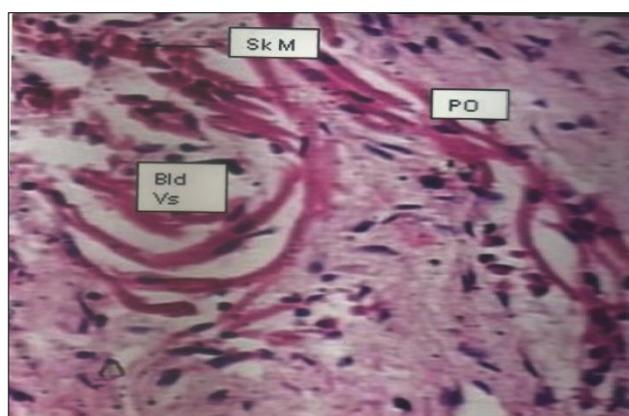

Figure 9: (X20) TS through the RL at its termination in 24 wk old fetus showing smooth muscle (PO) and peripheral skeletal muscle (Sk M) as well.

The microscopic structure of the gubernaculum testis in 10 wk old fetus resembles that of round ligament of 10wk old female fetus of corresponding age.

It has predominantly fibroblasts and mesenchymal cells (Figure 10). The spermatic cord of adult male shows thick walled muscular arteries, vas deferens together with abundant connective tissue fibres.

The skeletal muscle (cremaster) is present in the periphery.

Age changes

Due to limitation of sample size available for the study it is difficult to comment on the age changes in the fetus however it can be said without doubt that with increasing age of the fetus there is marked increase in vascularity. Mesenchymal cells and fibroblasts are reduced in number with increasing age. The number of myoblasts increase with increasing gestational age. Differentiation of myoblasts into smooth muscle is first seen in 17 wk old fetus while presence of skeletal muscle is seen earliest in the 22wk old fetus. The distance from termination of the round ligament to caudal labium is found to increase with gestational age, 0.6cms in 8wks fetus to 1.5cms in 26wks fetus. This is due to increase in the size of body wall.
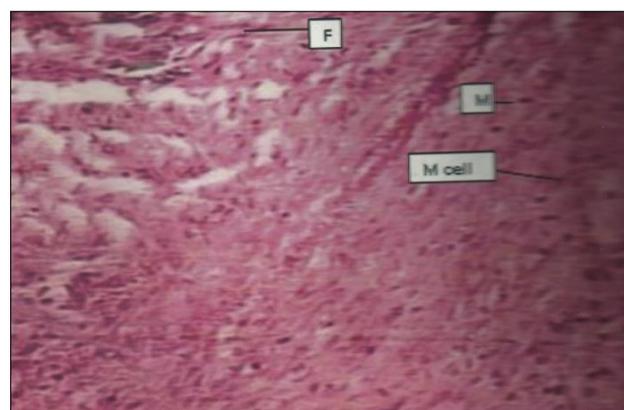

Figure 10: (X10) TS of Gubernaculum testis in a 10 wk old male fetus showing mesenchymal cells(M cells), fibroblasts (F) & myoblasts (M).

Knowledge of the detailed histology would be of value in the understanding of its physiological role.

DISCUSSION

According to Mustafi the gubernaculum affects the change in position of the ovary. About the 3rd month, ovary descends to the level of pelvic brim and remains there till birth.[3] At a later period it descends into pelvis minor and assumes its normal position. The canal of Nück accompanies the gubernaculum along the inguinal canal into labium majus, thus in a rare abnormality the ovary may pass through the inguinal canal into labium majus.

Until sexual differentiation begins, the gubernaculum is similar in both sexes. The gross structure is more or less the same as in the males until the 4th month of intra uterine life.

By the 4th month, testis is producing significant amount of androgenic steroids and Mullerian Inhibitory Substance (MIS) and from then on, growth and development of the gubernaculum ceases in the females.[4,5] Up to about the 10cm CR length stage there is

virtually no difference between the gubernacular apparatus in the male and female, but thereafter development continues in the male but ceases in the female, the mesenchyme gradually changing into connective tissue and smooth muscle of the round ligament, at the same time as the body wall fascia are laid down.

According to Emmen et al gubernaculum develops rapidly in the male fetus whereas development in the female fetus is lacking.[6] Possible factors responsible are androgens, Anti mullerian hormone (AMH) and insulin like factor (Insl3). Mitotic activity of cells in the gubernaculum bulbs from male and female fetus is correlated to the sexual dimorphism in gubernaculum development. When the gubernaculum bulges from the external ring at 25-30 weeks, a migration phase is believed to occur and then it moves into the scrotum at 35 weeks. Dissolution of its bulk forms the fibrous attachment of the testis to the scrotum, once descent is complete.

The gubernaculum in females does not shorten, deform or regress. In the absence of male hormones, the female gubernaculum remains intact and grows in step with the rest of the body.[7] In the females, the gubernaculum remains small and thin , after the fourth month of uterine life it differentiates to form the suspensory ligament of the ovary and the round ligament of the uterus. No migration of the round ligament occurs leaving it ending just outside the external ring.[8]

The processus vaginalis forms as a small evagination of the peritoneum on the gubernacular mesenchyme at the future superficial inguinal ring.[9]

Hollinshead described the round ligament as a flattened band arising from the lateral border of the uterus, immediately below the attachment of the ovarian ligament.[10] It runs in the broad ligament beneath its anterior peritoneal surface raising a distinct ridge as it curves forward. It gradually becomes more rounded crosses the pectineal line of pelvis and then the external iliac vessels to enter the inguinal canal at the internal inguinal ring. It represents the lower part of the homologue of the gubernaculum testis and should therefore extend to the labium majus, the homologue of the scrotum.

The course of the round ligament, as mentioned in the standard text books and as described by the recent research does not show any variation but termination of the ligament is controversial. According to Schafer, Symington and Bryce, like the spermatic cord in the male, the round ligament also passes through the inguinal canal.[11] It then reaches the fore part of the pubic symphysis where its fibres expand and become united with the substance of the mons pubis.

Last mentions that the round ligament after passing through the inguinal canal is attached at its distal extremity to the fibrofatty tissue of the labium majus of the vulva.[12]

Williams et al claim that the ligament after coming out of the external ring finally breaks up into strands which merge with the areolar tissue in the labium majus.[13] Hamilton describes that the ligament loses its definition and eventually loses its identity among the deep tissue of the labium after coming out of the superficial inguinal ring.[14]

According to Hollinshead tissue of the round ligament within the inguinal canal fuses with that of the canal itself and the ligament may end through such fusions within the inguinal canal; however a reduced part of it may continue through the inguinal canal and the external ring to blend with the connective tissue of the labium majus.[10]

Attah and Hutson observed the gubernaculum in the 30wk old male fetus and found that it ended outside the external ring, later migrating into the scrotum under androgenic control.[8] Anatomy of the round ligament was defined by dissection and photography on post mortem examination of 10 females (0-11yrs) dying of non genital disorders. The round ligament ended just outside the external ring in all females with neither attachment nor extension to the caudal labium. The round ligament entered the internal ring as a flat, cord like bundle of fibres. Outside the external ring, the flattened band attached inferiorly to abdominal wall muscles or got embedded in the substance of the fatty tissues of the mons pubis. The average distance from these attachments to the caudal end of labium majus was 67mm which increased with the age of the patient.

Histology

It was claimed that the round ligament is mainly formed of smooth muscle tissue and so it should be called as the round muscle.[15-17]

Schäfer, Symington and Bryce, Bunim, Hendricks and Moawad, McVay, Pernoll et al and Van der Schoot described that the proximal portion of round ligament had smooth muscle which were anatomically continuous with smooth muscle of uterus.[11,18-21,1] Besides this, it also contained certain amount of connective tissue, small blood vessels, nerves and lymphatics.

Musgrove et al studied the microscopic structure of the round ligament in rat and guinea pig and found it to be made up of longitudinally oriented smooth muscle bundles surrounding a connective tissue core.[22] The smooth muscles were separated from myometrium by collagen except at tubo-uterine junction where it extended to become continuous with outer layer of myometrium.

The studies of Anson mention that it is a fibrous cord containing smooth muscle tissue.[23] According to Gardner, Gray and O' Rahilly it contains some smooth muscle near its attachment.[24] Hamilton states that the round ligament is a fibromuscular cord.[14] It contains some striated muscle fibres from the abdominal musculature around the inguinal canal and these may be regarded as the counterpart of the male cremaster muscle. Mcgregor observed that the ligaments of the uterus including the round ligament contains muscular and elastic tissue.[25] The proportion of fibrous tissue, muscle and elastic tissue varies somewhat with the individual but more according to age.

Last's anatomy describes that the extraperitoneal areolar tissue is condensed to form the ligament which is composed histologically of a high proportion of smooth muscle fibres.[12]

Gray's Anatomy mentions that the round ligament contains much non striated muscle which becomes progressively less in amount and the terminal part is purely fibrous. It is accompanied by blood vessels, nerves and lymphatics which drain from the parts of the uterus near the entry of the uterine tube and pass to the superficial inguinal lymph nodes.[13]

According to the study by Van der Schoot round ligament of uterus consists of fibrous cords containing smooth muscle (longitudinal) from the outer layer of the corpus. The ligament consists mainly of smooth musculature.[1]

According to Ozbey et al round ligament consists of striated and smooth muscle fibres, abundant nerves and vessels.[26]

CONCLUSIONS

In this anatomical region, and despite all the studies that have been performed, controversy still exists. This article attempts to study the morphology and histology and the differentiation of the gubernaculum with age.

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