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Histogenesis of the Gubernaculum During Testis Descent in the Goat Fetus (*Capra marghoz*)

Histogénesis del Gubernaculum Durante el Descenso Testicular en el Feto de Cabra (Capra *marghoz*)

Sajjad Hejazi[1,2]; Morteza Rasekh[3]; Ibrahim Al Hawz[1]; Arman Sabet[2] & Mohammad Matin Maroufi[2]

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SUMMARY: Testicular descent is a complex process that only occurs in mammals. The role of the gubernaculum during testicular descent has been explained mainly by its capacity for dilatation and contraction. This study tried to investigate the changes in the structure of the fibers and cells of the gubernaculum in different age levels of testicular descent in goat fetuses. Embryo samples were collected and grouped in such a way that 60 male goat fetuses were obtained from 100 pregnant does (*Capra marghoz*). The samples were classified based on the average length (CRL) of the used embryos into 6 age groups. Tissues of the gubernaculum were stained using Masson's Trichrome method to observe collagen fibers under light microscopy. In the present study, growth and orientation of collagen fibers of gubernaculum were observed from the age of 51 days in a manner that the arrangement and order of fibroblasts and collagens to be associated with the onset of testicular migration order and collagen fibers until the end of the third month. Further, changes in the cell arrays and strings were observed after the age of 111 days in such a way that near the birth date, the gubernaculum converted into atrophy tissue. It can be said that from the beginning of the period of testicular descent until its completion, the tissue of the gubernaculum undergoes cellular changes, such as deformation and increase and secretion in connective fibers.

KEY WORDS: Histogenesis; Gubernaculum; Goat fetus; Testis descent.

INTRODUCTION

Testicular descent is a complex process that only occurs in mammals. The location of the scrotum varies between species, as does the scrotal development (Hutson *et al*., 2009). Internal organs of reproduction, including sexual glands or gonads, and excretory structures are all derived from the intermediate mesoderm. The somatic mesoderm condenses in the inguinal cord which is called the gubernaculum, extending from the scrotal ridge to the gonad (Noden & De Lahunta, 1985). The gubernaculum seems to be the most important anatomical structure in the testicular descent process. The gubernaculum is an elongated, cylindrical structure that connects the inferior pole of the testis and the tail of the epididymis to the inguinal canal and scrotum (Heyns & Hutson, 1995; Favorito *et al*., 2000). These changes are caused by the swelling of the gubernaculum in front of the inguinal canal. The swelling results from the proliferation of mesenchymal cells and increases the high secretion of mineralized extracellular matrix that mainly includes hyaluronic acid. The accumulation of this extracellular secretion dilates the inguinal canal and causes the gubernaculum to become soft and have a jelly-like stature, decreasing its resistance to drop down the gonad (Bloom & Fawcett, 1975). There is no contractible tissue to take up the gonad actively to the canal. The role of the gubernaculum during testicular descent has been explained mainly by its capacity for dilatation and contraction (Costa *et al*., 2002). During the descent phase, the integration of the intra-abdominal mesenchymal gubernaculum with the extra-abdominal component causes a certain shortening of the gubernaculum. Simultaneously, the extra-abdominal mesenchymal gubernaculum is still swollen in the swelling of the scrotum. After the descent of the testis, the mesenchymal gubernaculum is assimilated from the inguinal canal and causes a definite shortening in its length (Noden & De Lahunta, 1985). The descent of testis from the inguinal canal usually occurs for a bull at 106 days,

[1] Department of Anatomy, Faculty of Veterinary Medicine, Near East University, Nicosia, Cyprus.
[2] Department of Anatomy, Tabriz medical sciences branch, Islamic Azad University, Tabriz, Iran.
[3] Department of Basic Science and Hygiene, Science and Research Branch, Islamic Azad University, Tehran, Iran.

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in male pigs at 70 days after gestation, in stallions at or near the time of birth, and in dogs 3-4 days after birth (Noden & De Lahunta, 1985). The gubernaculum is essential for normal testicular descent and its swelling, elongation, and atrophy are controlled by the secretion of the testis. Therefore, testis runs its descent by itself. This external growth of the gubernaculum is simulated with a non-androgenic testicular factor increased by the testosterone of the testis. The assimilation of gubernaculum is caused by testosterone (McGeady *et al*., 2006). In this study, due to insufficient research, we tried to investigate the changes in the structure of the fibers and cells of the gubernaculum in different age levels of testicular descent in goat fetuses.

MATERIAL AND METHOD

Collection of fetal samples and grouping: Embryo samples were collected and grouped in such a way that 60 male goat fetuses were obtained from 100 pregnant does (*Capra marghoz*) killed in an industrial slaughterhouse. Each fetus was measured for its crown rump length (CRL) in centimeters. The approximate age of collected embryos was estimated using a formula presented by Gall *et al*. (1994); the formula is as follows: Y= 2.47 X + 30.15. Then, the age and length of the fetuses were recorded, and they were divided into five groups (Table I).

Fetal sex differentiation: To determine the fetal sex at an early age, there is a need to observe external genital differentiation by a stereomicroscope system. Therefore, the sex of the fetus was determined by the appearance of the genital tubercle in the form of genital swelling in the first group. In other groups, the sex was identified by the development of the penis and scrotal sac. The fetuses were dissected by giving ventral abdominal incision to observe the development of the gubernaculum adjacent to the testis (Fig. 1). For microscopic examination of gubernaculum histological changes, the gubernaculum was collected in 10 % formaldehyde solution and then processed by the usual paraffin embedding method and cut into 5-6 μ dimensions. The sections were stained with the Specific Masson's Trichrome method to observe collagen fibers under light microscopy (Nikon, Eclipse E200, Japan).

Table I. Division of groups based on the average length, weight, and age of the fetuses used in the study (n=10)

Fetus	Fetal CRL (cm)	Fetal weight (g)	Approximate age of fetus (day)
Group 1	5.2-6.4	5.5-8	41-46
Group 2	8.5-10.5	25-35	51-56
Group 3	16.5-18.5	170-200	71-76
Group 4	24.5-26.0	420-500	91-96
Group 5	32.5-34.5	800-900	111-116
Group 6	36.5-40.0	1300-1500	121-130

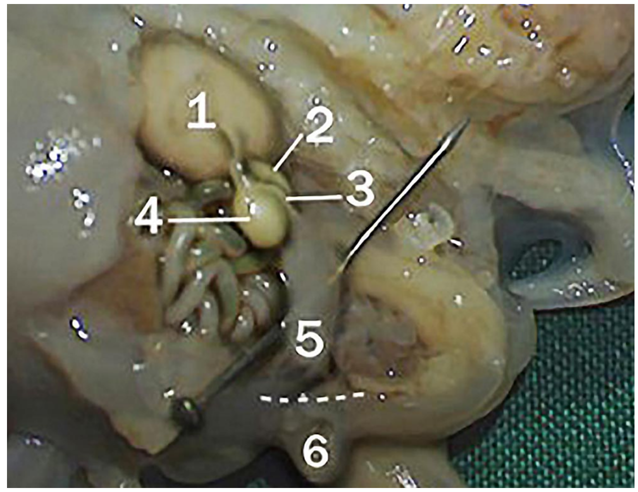

Fig. 1. Macroscopic view of the fetal abdomen with CRL= 8.5cm, the left side view.1.Metanephrosis; 2. Mesonephrosis; 3. Mesonephric duct; 4. testis; 5. Gubernaculum; 6. scrotum.

RESULTS

In the microscopic examination of the gubernaculum in group I (CRL= 52-64 mm), Mesenchymal cells with oval and clear nuclei were found to be strongly eosinophilic. Fibroblast cells were also seen in an irregular arrangement. In Masson's Trichrome Staining, we saw the secretion of delicate type III collagen fibers by fibroblasts (Fig. 2.A). In group II (CRL= 85-105 mm), fibroblasts formed the spindle shape and were observed in a messy and tangled arrangement. Further, the secretion of collagen fibers was increased (Fig. 2.B). In group III (CRL= 165-185 mm), fibroblast cells were seen in parallel rows, and collagen fibers were seen with higher density and parallel and regular form (Fig. 2.C). In group IV (CRL= 245-260 mm), aggregation and density in the order of fibroblasts and collagen fibers increased; fibroblasts kept their spindle form. Collagen fibers were thicker compared to those in previous days. The maximum thickness and density of collagen fibers were observed at this age (Fig. 2.D). In group V (CRL=325-345 mm), changes in the placement pattern of cells and fibers of the gubernaculum were observed. The spindle shape of fibroblasts with elongated cytoplasmic extras was not observed at this age, and the nuclei of the cells were ovserved as spherical and dark. Collagen fibers were observed with high density but an irregular and overlapping arrangement (Fig. 2.E). In group VI (CRL = 365-400 mm), a noticeable change in the order of the gubernaculum tissue structure was observed, so that the disorder of collagen fibers and fibroblasts was evident. Fibroblast with dark spherical and hyperchromatic nuclei was observed as well. In general, the tissue of the gubernaculum at this age from the descent of the testis was in the state of tissue regression and atrophy (Fig. 2.F).

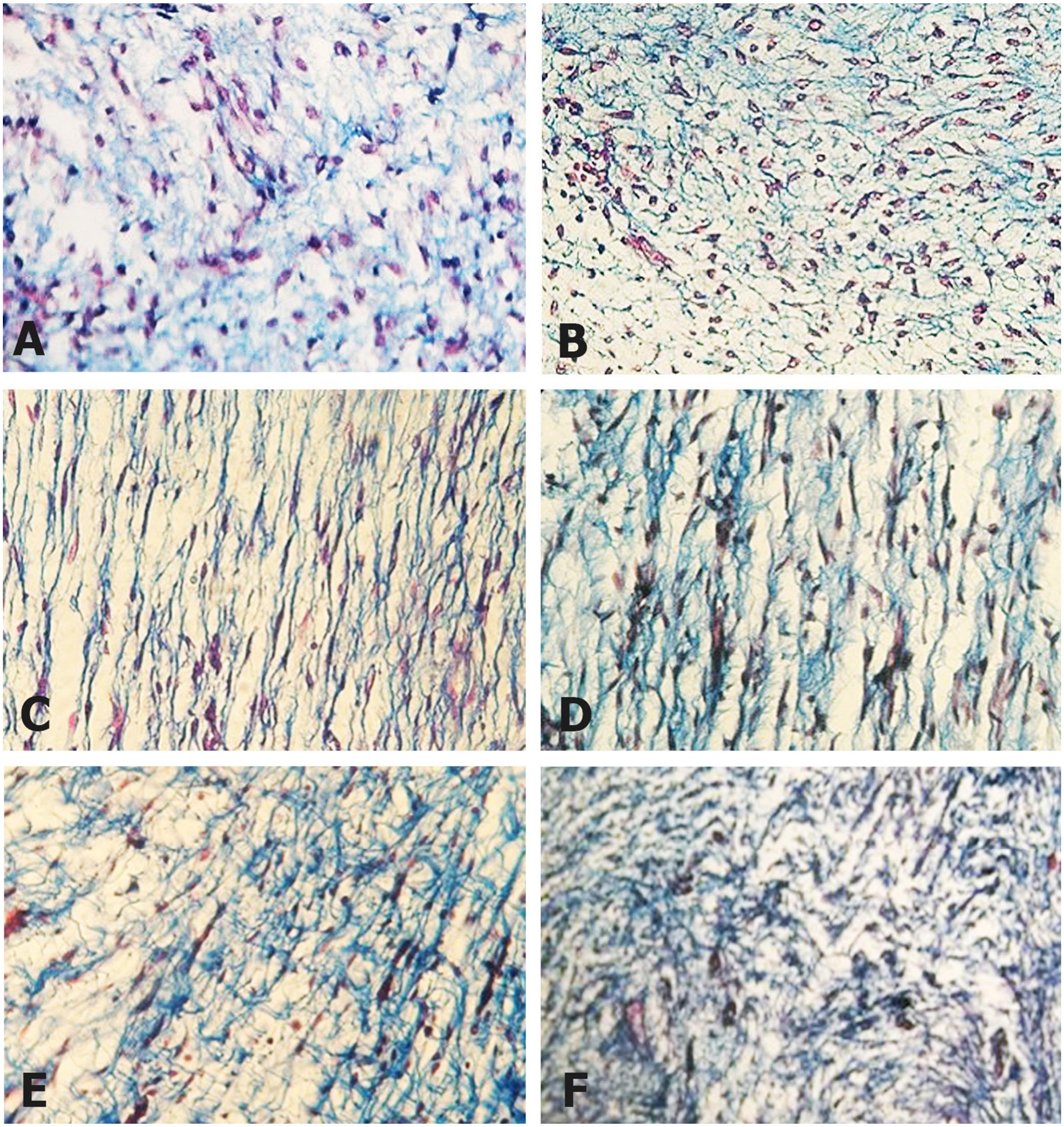

Fig. 2. Microscopic view of fetal gubernaculum tissue. (Masson's Trichrome Staining , magnification ¥ 40). A: Group I, B: Group II, C: CRL= Group III, D: Group IV, E: Group V, F: Group VI

DISCUSSION

The role of the gubernaculum during testicular descent has been explained mainly by its capacity for dilatation and contraction (Costa *et al*., 2002). The gubernaculum plays an important role in testicular migration because of its ability to dilate and shorten, allowing the testis to move through the inguinal canal more easily (El Zoghbi *et al*., 2007). The role of the gubernaculum in testicular descent highlights that the gubernaculum is a loose connective tissue, evolving with cell proliferation and secretion of extracellular components, hyaluronic acid, and collagen fibers (Fentener van Vlissingen *et al*., 1989). Gubernaculum tissue is the origin of the somatic mesoderm. The cause of gubernaculum swelling has been stated along with cell proliferation and extracellular matrix secretion. After the descent of the testicle, the gubernaculum changes from a very rich mucous tissue to a fibrous structure under the androgen hormone (Noden & Lahunta, 1985). The results obtained in the present study demonstrated that the gubernaculum tissue changed histologically according to fetal growth and decline in testicular. Thus, the gubernaculum was seen from mucoid connective tissue to lose connective tissue with collagen fibers. Also, according to the results, the evolution and growth of the gubernaculum were with increased development of cells and collagen fibers; in the last stages of testicular descent, this tissue becomes an atrophic state. According to previous studies, the above observations are consistent with other findings. In the present study, based on the obtained results of the macroscopic part of testicular descent, the growth and development of the gubernaculum were observed after the age of 40 days. The expansion of the gubernaculum was with the beginning of testicular migration; this expansion continued into the scrotal sac until the end of the third month. In microscopic observations, the growth and orientation of collagen fibers of the gubernaculum were seen from the age of 51 days; the arrangement and order of fibroblasts and collagens were associated with the onset of testicular migration order and collagen fibers until the end of the third month. Changes in the cell array and strings were observed after the age of 111 days in such a way that near the birth date, the gubernaculum converted into the atrophy tissue. It can be concluded that anatomical changes of the gubernaculum are associated with its histological changes during fetal development and testicular descent. Fentener van Vlissingen *et al*. (1989) stated that in the first stage of testicular migration, the tissue of the gubernaculum had growth, proliferation, and synthesis of hyaluronic acid and collagen, and in the second stage, the descent of the gubernaculum testis had a degenerative process. Noden & De Lahunta (1985) also found no contractile tissue in the gubernaculum to move gonads into the inguinal canal actively. On the other hand, Jiang *et al*. (2004) showed that the developing tissue of the gubernaculum consisted of two layers: the mesenchymal inner layer and the outer layer of muscle tissue. Also, the electron microscopic observations have shown that the cells of gubernaculum tissue have special arrangement, as well as order and discipline with myofibrils in the cytoplasm. In this study, in gubernaculum tissue with Masson's Trichrome Staining during the course of evolution, there was not any cellular or muscular cell with contraction property. The findings of this study were consistent with those of McGeady *et al*. (2006) and Noden & De Lahunta (1985) but inconsistent with Jiang *et al*. (2004). According to Bloom & Fawcett (1975), bands are stained with Tri-chrome Mallory (being blue) and Masson's Trichrome (being green). When these strings are not stretched, they can have a wavy path. Moreover, the role of fibroblasts in determining the arrangements of collagen fibers is mentioned, which is supposed to be characterized by the orientation of collagen fibers by the path of pressure in the tissue. However, researchers claim that changing the highly ordered arrangement of the filaments found in some parts of the body is hard based on the mechanical strength of the tissues. In the present study, the collagen fibers in the gubernaculum were shown by Masson's Trichrome Staining; also, the arrangement of fibroblast cells and collagen fibers was noted at the beginning of testis migration. It was further shown that the position and orientation of collagen fibers were associated with the arrangement patterns of fibroblasts. However, scientists have differing views on the fact that these strings are tightly associated with cells and that the cells partially govern the strings' directions. It has been proposed that the manner of production of collagen fibers allows fibroblasts to regulate collagen orientation; however, this remains only a hypothesis. Weil *et al*. (1885) stated that, during descent, the gubernaculum becomes shorter in a way comparable to the change that young connective tissue undergoes during scar formation; this is consistent with the findings of the present study. Thus, in the last age of the fetus, we saw the change of young connective tissue into old and atrophic connective tissue. However, Wyndham (1943) found very little connective tissue in the gubernaculum and rejected the theory that the testis is pulled down by scar contracture of the gubernacular fibers; this is inconsistent with the findings of the present study. Backhouse (1981) spotted, after the descent of the testis (or even if it fails to descend), a marked reduction in the intercellular fluid, leading to a decrease in the gubernacular bulk as its mesenchymal cells differentiate towards fibroblasts; this is consistent with the findings of the present study. Histologically, Wensing (1973) noted that the collagen fibers were arranged in a loose and delicate meshwork in the pig gubernacular before and during the period of testicular descent. After the descent, a condensation of the fibers with a reduction in the size of the mesh was seen; also, after the birth of the fetus, the meshwork was replaced by coarse bundles of parallel fibers. Shrinking or involution of the gubernaculum after descent noted by most investigators is probably a result of the accumulation of collagen with the disappearance of the cellular elements and may facilitate the descent of the testis inferior to the external inguinal ring. The present study found atrophy changes in the gubernaculum tissue. The generality of the above result is consistent with the observations obtained on the gubernaculum of the goat embryo, with the difference that the gubernaculum of the goat embryo is parallel and regular during the descent of the testis, and at the end of the testicle descent, it becomes an atrophied and compressed tissue.

In conclusion, what has attracted the attention of almost all researchers in the descent of the testicle is the shrinking, depression, and, finally, the disappearance of the gubernaculum after the descent. In general, it can be said that from the beginning of the period of testicular descent until its completion, the tissue of the gubernaculum undergoes cellular changes such as deformation, increase, and secretion of connective fibers. The connective fibers also start with order and increased tissue density and, finally, end with disorder and tissue compression.

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HEJAZI, S. ; RASEKH, M.; AL HAWZ, I.; SABET, A. & MAROUFI, M. M. Histogénesis del gubernaculum durante el descenso testicular en el feto de cabra (*Capra marghoz*) *Int. J. Morphol., 42(3)*:871-875, 2024.

RESUMEN: El descenso testicular es un proceso complejo que solo ocurre en los mamíferos. El papel del gubernaculum durante este proceso se ha explicado principalmente por su capacidad de dilatarse y contraerse. En este trabajo, se investigaron los cambios en la estructura de las fibras y células del gubernaculum en diferentes etapas del descenso testicular y edades en fetos de cabra. Se recolectaron muestras de embriones, agrupándose de manera que se obtuvieron 60 fetos de macho cabrío a partir de 100 hembras preñadas (*Capra marghoz*). Las muestras se clasificaron según la longitud media (CRL) de los embriones utilizados, dividiéndose en seis grupos de edad. Los tejidos del gubernaculum se tiñeron utilizando la técnica de Tricrómico de Masson para observar las fibras de colágeno bajo microscopía óptica. En el presente estudio, se observó el crecimiento y la orientación de las fibras colágenas del gubernaculum a partir de los 51 días de edad. La disposición y el orden de los fibroblastos y colágeno se asociaron con el inicio de la migración testicular, observándose las fibras colágenas hasta el final del tercer mes. Además, se detectaron cambios en las matrices y cadenas de células después de los 111 días de edad. Cerca de la fecha de nacimiento, el gubernaculum se convirtió en tejido atrofiado. En conclusión, desde el inicio hasta la finalización del período de descenso testicular, el tejido del gubernaculum sufre cambios celulares, como deformación y aumento de secreción en las fibras conectivas.

PALABRAS CLAVE: Histogénesis; Gubernaculum; Feto caprino; Descenso de testículos.

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Corresponding author:
Sajjad Hejazi
Department of Anatomy
Faculty of Veterinary Medicine
Near East University
Nicosia
CYPRUS

E-mail: Sadjad.Hijazi@neu.edu.tr

ORCID: https://orcid.org/0000-0001-7937-4125