Relationship between the Development of the Gubernaculum and the Testicular Descent in the Rat Fetus: Macroscopic and Light and Electron Microscopic Observation

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(Received 14 September 1988/Accepted 27 December 1988)

ABSTRACT. The gubernaculum consists of the gubernacular cord connected with the vas deferens and the gubernacular bulb connected with the retroabdominal wall. The cord was first distinguished on day 15 of gestation. Its length reached the peak on day 16, followed by a reduction until day 18 to be almost constant thereafter. The bulb was first distinguished on day 16, and its length was steadily increased toward term. The testis began to descend with a reduced length of the cord. By day 19, the testis descended close to the caudal part of the bulb by the sharp bending of the cord. Microscopically, the bulb consisted at first of a centrally located mesenchymal mass and of a covering layer of myoblasts. Thereafter, the mesenchymal mass was gradually shifted toward the cranial part of the bulb, trailing loose mesenchymal tissue in the caudal part of the bulb. On day 19, the mass was decreased in cell population but increased in amount of collagen fibers. It is suggested that the testicular descent starts on day 16 together with the commencement of shortening of the gubernacular cord and enlargement of the gubernacular bulb. —KEY WORDS: gubernaculum, rat fetus, testicular descent.


Relationship between the developmental changes in the gubernaculum and the process of testicular descent into the scrotum has been studied by many authors [2, 3, 7, 8, 10]. In fetal life, the mesonephros is sustained by the mesonephrodiaphragmatic ligament at its cranial part and by the mesonephroinguinal ligament at its caudal part, both the ligaments consisting of peritoneal folds. It has been known that the mesonephroinguinal ligament is detached from the urogenital cord to become the genitoinguinal ligament [5]. In male, the gubernaculum testis develops from this genitoinguinal ligament [4]. The gubernaculum in the rat fetus is divided into two parts, cranial and caudal. The cranial part, connected with the vas deferens, is slender as the gubernacular cord and the caudal part, connected with the retroabdominal wall, is expanded as the gubernacular bulb. The testicular descent takes place by outgrowth and regression of mesenchymal tissues of the gubernacular bulb [9, 11].

The present work was undertaken to observe the development and the differentiation of the fetal gubernaculum using a dissecting, a light and an electron microscope. In particular, electron microscopic observation of changes in the gubernacular bulb can be expected to bring forth interesting information about the mechanism of the testicular descent.

MATERIALS AND METHODS

Rats of the Wistar strain were given a commercial diet (Labo MR Breeder) and water, both ad libitum, and maintained at 22±2°C in a 12: 12 photoperiod. Pregnancy was ascertained by the presence of sperm in the vaginal smear on the day following an
overnight mating, which was counted as day 0 of gestation.

Male fetuses were used on days 14–21 of gestation. Each fetus was decapitated and fixed in Bouin's fluid. Under a dissecting microscope, the alimentary canals were removed to expose the genital organs as shown diagrammatically in Fig. 1. The length of the cord and the length and width of the bulb of the left gubernaculum were measured with a micrometer set in the dissecting microscope. Then, the gubernaculum was removed and embedded in Paraplast (Sherwood Med. Industries) and sectioned at 5 μm. The sections were stained with hematoxylin and eosin or with Masson's trichrome.

For electron microscopic observation, another series of fetuses were sacrificed and the gubernacular bulb of each fetus was quickly removed and fixed in 2.5% glutaraldehyde in 0.2 M cacodylate buffer, pH 7.4, for 2 hr at 4°C. The tissue was rinsed in the same buffer for 15 min at 4°C, postfixed in 1% osmium tetroxide buffered in 0.2 M cacodylate for 1 hr at 4°C and rinsed once again. Following rapid dehydration, the tissue was embedded in Epon 812 (TAAB). Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with a JEM-100CX electron microscope.

**RESULTS**

**Measurements:** The gubernacular cord was first distinguished on day 15 of gestation and was measured from this day onward (Fig. 2). The length of the cord soon reached the peak on day 16, and was extremely decreased until day 18 to remain almost constant thereafter. The gubernacular bulb was first distinguished on day 16 of gestation and its length was steadily increased with its constant width toward term.

**Gross anatomy:** On day 15 of gestation, the gubernaculum was seen to be cord-like, and its cranial tip was connected with the vas deferens and its caudal tip was joined with the retroabdominal wall, on which no bulb-like swelling was observed (Fig. 3). The testis was located on the ventral side of the kidney.

On day 16 of gestation, the gubernacular bulb was first observed at the caudal part of the gubernaculum which was connected...
with the abdominal wall (Fig. 4). The gubernacular cord was much longer than that on day 15. The testis was placed on the lateral side of the kidney.

On day 17 of gestation, the gubernacular bulb was lengthened, but the gubernacular cord became extremely short (Fig. 5). The cord was joined with the vas deferens at nearly right angle, and the testis descended on the caudal part of the kidney.

On day 18 of gestation, the bulb was further elongated, while the cord became shorter (Fig. 6). The testis descended further.

On day 19 of gestation, the epididymis came closer to the cranial part of the

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Fig. 3. Caudal part of the abdomen on day 15 of gestation. Alimentary canals removed. Testis (T) placed in the dorsal part of the abdomen. The gubernaculum (arrow) connected between the vas deferens (V) and with the retroabdominal wall. B: bladder, R: rectum. ×18.

Fig. 4. Caudal part of the abdomen on day 16 of gestation. The gubernacular bulb (large arrow) differentiated at the caudal end of the lengthened gubernacular cord (small arrow). Testis (T) situated on the lateral side of the kidney (K). ×18.

Fig. 5. Caudal part of the abdomen on day 17 of gestation. The cord (small arrow) becoming short. The bulb (large arrow) enlarged. The testis (T) beginning to descend to be placed on the caudal part of the kidney (K). ×14.

Fig. 6. Caudal part of the abdomen on day 18 of gestation. The bulb (large arrow) elongated and the cord (small arrow) shorter than on the previous day. The testis (T) descending further. ×14.
gubernacular bulb by the sharp bending of the gubernacular cord, which accelerated the testicular descent (Fig. 7). The vaginal process was formed around the caudal end of the bulb. The bulb and the vaginal process were developed progressively toward term (Fig. 8).

**Histological differentiation:** On day 16 of

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**Fig. 7.** Caudal part of the abdomen on day 19 of gestation. The bulb (large arrow) further enlarged and the cord (small arrow) bending sharply. The epididymis (E) close to the cranial part of the bulb. The vaginal process (P) beginning to develop. ×14.

**Fig. 8.** Caudal part of the abdomen on day 21 of gestation. The bulb (large arrow) and vaginal process (P) developing further. ×14.

**Fig. 9.** Transverse section of the gubernacular bulb on day 16 of gestation. The bulb consisting of a mesenchymal mass (M) covered by a cell layer (arrow). Light microscopy (LM). H. E. stain. ×200.

**Fig. 10.** Transverse section of the gubernacular bulb on day 17 of gestation. The mass (M) enlarged and the covering cell layer (arrow) clearly seen. LM. H. E. stain. ×150.
gestation, by light microscopy, the gubernacu-
lar bulb consisted of a condensed mesenchymal mass which was covered with a cell layer (Fig. 9). The gubernacu-
lar cord was sustained by a peritoneal fold, with bulged mesenchymal tissues in it.

On day 17 of gestation, the mass in the center of the bulb was enlarged and the covering cell layer was thickened (Fig. 10). By electron microscopy, the center of the bulb was composed of mesenchymal cells whose cytoplasm was narrow, containing a small number of mitochondria and rough endoplasmic reticula (rER) and sending off cytoplasmic processes by which the cells touched each other (Fig. 11). Cells in the covering layer were also mesenchymal in nature, but had wider cytoplasm containing myofilaments characteristic of myoblasts (Fig. 12).

On day 18 of gestation, the condensed mesenchymal mass located in the center of the bulb was shifted toward the cranial part of the bulb, trailing loose mesenchymal tissues in the caudal part of the bulb (Fig. 13). The cells in the mass became sharply slender and had well-developed rER (Fig. 14). The myoblasts in the covering layer were larger than those on the previous day, and the myofilaments included in them were increased in number, surrounded by ribo-
somes (Fig. 15). The cord was thickened with an increased amount of mesenchymal tissues in it.

On day 19 of gestation, the core mass of mesenchymal cells of the bulb became smaller and looser and its trailing tissues in the caudal part of the bulb also became remarkably looser (Fig. 16). The fibroblasts were shrunken with a minute amount of cytoplasm surrounded by many collagen fibers (Fig. 17). The myoblasts became elongated and their cytoplasm was filled with myofilaments whose cross striations were distinctly observed (Fig. 18). The cord was seen to be about the same as on day 18 of gestation.

After day 19 of gestation, the core mass of mesenchymal cells of the bulb was gradually decreased in size, leaving loose tissues of shrunken cells with much increased amount of collagen fibers. The layer of myoblasts developed further. Myoblastic syncytia be-
gan to be formed, but the nuclei were not yet shifted toward the cell membrane, just placed in the center of each syncytium.

DISCUSSION

According to Wensing [11], the testicular descent in the rat is affected by 3 successive histological changes as follows: 1) out-
growth of gubernacu lar mesenchyme, 2) subsequent regression of this mesenchyme and 3) development and growth of cremaster muscles. In the present study, the gubernaculum started to differentiate bet-
ween days 14 and 15 of gestation. The gubernacu lar bulb was first observed on day 16 of gestation. Swelling of the bulb took place by enlargement of the core cell-mass composed of undifferentiated mesenchymal cells. By day 18, the cells composing the core mass became slender and were special-
ized into fibroblasts surrounded by collagen fibers. The mass gradually migrated to the cranial part of the bulb, with an increased amount of collagen fibers among the com-
posing fibroblasts. These histological changes indicate that the development of the bulb is accompanied by such a rapid increase in amount of mesenchymal tissues, in agreement with Wensing’s observation of the outgrowth of gubernacu lar mesenchyme [11]. In the present study of prenatal period, a regressive sign of the gubernacu lar bulb was also observed as shrunken fibroblasts with much accumulated collagen fibers though the size of the bulb was still enlarged toward term.

According to Radhakrishnan et al. [9], the gubernacu lar bulb of the rat fetus consists of
Fig.11. Mesenchymal cell (M) in the core mass of the gubernacular bulb on day 17 of gestation. The cytoplasm containing a small number of mitochondria (large arrow) and rER (small arrow). A cytoplasmic process (double arrow) touching on this cell from another fellow cell. Electron microscopy (EM). ×11,800.

Fig.12. Myoblast (MY) in the covering layer of the gubernacular bulb on day 17 of gestation. The cell having a wider cytoplasm than the mesenchymal cell. The cytoplasm containing myofilaments (arrows). EM. ×14,800.

Fig.13. Transverse section of the gubernacular bulb on day 18 of gestation. The mass (M) shifted toward the cranial part of the bulb. LM. H. E. stain. ×150.

Fig.14. Mesenchymal cell differentiated into fibroblast (F) on day 18 of gestation. The cell sharply slender and rER (arrows) well developed. EM. ×8,000.
Fig. 15. Myoblast (MY) on day 18 of gestation. The cytoplasm wider than on the previous day and the myofibrils (arrows) increased in number. EM. ×17,000.

Fig. 16. Transverse section of the gubernacular bulb on day 19 of gestation. The mesenchymal mass (M) becoming loose and the tissues in the caudal part (C) becoming looser. LM. Masson’s trichrome stain. ×75.

Fig. 17. Mesenchymal cell (M) on day 19 of gestation. The cytoplasm extremely narrow, containing few cell organelles but surrounded by many collagen fibers (arrows). EM. ×8,000.

Fig. 18. Myoblast (MY) on day 19 of gestation. The cytoplasm filled with myofilaments (F) showing cross striations (large arrows). Sarcoplasmic reticulum (small arrow) observed. EM. ×11,200.
undifferentiated mesenchymal cells until day 18 of gestation. The myoblasts differentiate between day 18 and day 20 of gestation and develop into rhabdomyoblasts and striated muscles. In the present study, myoblast-like cells, covering the mesenchymal mass of the gubernacular bulb, were already observed on day 16 of gestation when the primordial bulb had been just made up. Myofilaments appeared in the cytoplasm of these myoblast cells on day 17 of gestation. Thereafter, the myoblasts were elongated with the increased number of myofilaments. On day 19 of gestation, the cytoplasm was filled with well-organized myofilaments showing distinct cross striations. After this time the myoblasts began to form syncytia, as origin of the cremaster muscle.

According to Gier and Marion [4], the process of testicular descent in the dog is divided into 3 distinct stages as follows: 1) nephric displacement, 2) transabdominal passage and 3) inguinal passage. In the present study, the testicular descent began between days 16 and 17 of gestation. At the same time, the gubernacular bulb began to develop and the gubernacular cord started to shorten. In the next stage, the epididymis approached the cranial part of the bulb. Thus the prenatal testicular descent seems complete by day 19 of gestation. Therefore, it can be said that the transabdominal passage of the testis takes place from day 16 to day 19 of gestation in relation to the outgrowth of the gubernacular bulb. Similar observations have been published in the dog and pig [1, 6].

The foregoing observation shows that the gubernaculum is first observed on day 15 of gestation as a cord-like structure and that the testicular descent begins after day 16 of gestation when the gubernacular bulb begins to develop remarkably with the increased mesenchymal volume and when the gubernacular cord begins to shorten suddenly. The testicular descent is complete by the sharp bending of the gubernacular cord on day 19 of gestation. Collagen fibers in the gubernacular bulb are increased in amount, in spite of the decrease in number of mesenchymal cells. The layer of myoblasts covering the bulb develops notably to become cremaster muscles later. It seems that, during prenatal period, the gubernacular bulb remains in preparation for its postnatal eversion into the inguinal canal, since it is surrounded by the primordial vaginal process just made up and since it is covered by the myoblastic layer which is destined to be the cremaster muscle after birth.

REFERENCES

要約

ラット胎仔の精巣導帯の成長と精巣下降の関係—肉眼、光顕および電顕的観察—藤掛・登・富樫宏史・山本雅子・有崎和義・植田安寛・江口保輝（麻布大学歯医学部解剖学第二講座）——精巣導帯は、精管に接着する索状部と後腹壁に接着する球状部からなる。索状部は胎児15日に初めて見られ、16日に最長となり、18日に向かって短縮した。球状部は16日に初めて見られ、胎仔の成長と共に下降し、胎児19日において球状部の鈍端により球状部の頭側端に接した。組織学的には、球状部は中心部に間葉細胞塊を有し、周囲に筋芽細胞層を有していた。間葉細胞塊は球状部の発達に伴い球状部の頭側端に寄り、尾側端は次第に疎性組織となった。胎児19日を境に間葉細胞塊は次第に疎となりが、膠原線維が増加した。以上の所見から、胎生期における精巣下降は、索状部の短縮および球状部の伸張が起こる胎児16日から始まるものと思われる。