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MORPHOGENESIS

Age-Related and Individual Anatomical Variation in Testicular Topography in Human Fetuses

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Abstract—At present, male infertility remains an urgent medical concern. From year to year, despite advances in methods of diagnosis and treatment, medicine encounters an increasing number of infertile couples with male infertility playing a leading role. Prerequisites for fertility disorders very frequently appear in childhood. Urologists consider cryptorchidism a leading cause of male infertility. The aim of our study was to establish the relationship between testicular descent to the scrotum and the age of the fetus. Material and methods. The study was conducted using 195 specimens of male fetuses aged 4–10 months with 81.0–375.0 mm parietal-coccygeal length (PCL) using the methods of macromicroscopic, conventional, and microslide preparation under control of binocular loupes and morphometry. Results. At the beginning of the fetal period of human ontogenesis (fetuses 81.0–135.0 mm PCL), the right and left testicles are mainly located above the corresponding deep inguinal ring and they are less often located in a region of the iliac fossae. An analysis of topographic and anatomical features of the male reproductive glands in 5-month-old fetuses (136.0–185.0 mm PCL) revealed that the testicles were located within the large pelvis, with the lower end of both the right and left testicles located above the entrance to the deep inguinal ring at a distance that equals the length of the pelvic part of the gubernaculum testis—3.2 ± 0.3 mm (right) and 2.8 ± 0.2 mm (left). In 11 fetuses aged 7 months (231.0–270.0 mm PCL), the lower ends of the testicles and their gubernaculum testis are immersed in the corresponding deep inguinal ring. In eight fetuses, the testicles were within the deep inguinal ring. A combination of many factors contributes to the final migration of a testicle through the inguinal canal into the scrotum (fetuses: 270.0 cm–290.0 mm PCL), including muscle contraction of the anterolateral abdominal wall, an increase in intra-abdominal pressure, contractile capacity of the gubernaculum testis of the testicle, the vaginal process of the peritoneum, and the neuro-muscular system. We believe that the gubernaculum testis is a particularly significant factor in testicular descent to the scrotum. The gubernaculum testis is maximally developed prior to migration of a testicle through the inguinal canal (eighth month of antenatal development), as evidenced by the prevalence of smooth muscle cells over connective tissue elements. An analysis of testicular topography in fetuses aged 9 months (311.0–345.0 mm PCL) revealed that testicles were located in the scrotum in nine fetuses, near the superficial inguinal ring in six fetuses, within the inguinal canal in four cases, and in the deep inguinal ring in one case. In fetuses aged 10 months (346.0–375.0 mm PCL), testicles were located in the scrotum in 13 cases and within the inguinal canal in seven cases. According to our research, the fusion of layers of the vaginal process of the peritoneum occurs in fetuses aged 9–10 months, resulting in the disappearance of the communication of its cavity with the peritoneum. A delay in the fusion of the peritoneal vaginal process layers at the end of the fetal period is an anatomic prerequisite for the occurrence of congenital inguinal-scrotal hernias. Conclusions. It has been found that the rate of testicular descent to the scrotum does not always coincide with the corresponding stage of fetal development. An accelerated development of the gubernaculum testis in fetuses aged 5–8 months is a major factor of heterochronic development of a testicle and subsequent testicular descent into the scrotum.

Keywords: testicle, testicular descent, scrotum, fetus, morphogenesis

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INTRODUCTION

At present, male infertility remains an urgent medical concern. From year to year, despite advances in methods of diagnosis and treatment, medicine encounters an increasing number of infertile couples with male infertility playing a leading role. Diverse causes of male reproductive system disorders have been well studied (Latyshev et al., 2008; Khmara et al., 2015). Prerequisites for fertility disorders very frequently appear as early as in childhood. Urologists regard true and false cryptorchidism as a leading cause of male infertility (Lee and Houk, 2013). Gonad descent into the scrotum occurs in the prenatal ontogenesis. Perturbations in this period cause cryptorchidism, which is a risk factor for reproductive dysfunc-

tion and testicular cancer (Hutson et al., 2013; Toppari et al., 2014; Fantasia et al., 2015). Cryptorchidism is the most frequent congenital anomaly of the male reproductive system. The incidence of cryptorchidism is 2–5 per 100 male neonates and it affects 1–4% of full-term and 30% of preterm male infants (Chung and Brock, 2011; Kollin and Ritzen, 2014; Goel et al., 2015).

Male fertility is highly dependent on proper formation of the interstitium and seminiferous tubules necessary for spermatogenesis and well-timed testicular descent into the scrotum (Hauser et al., 2015; Komarova and Pichugova, 2017). Low fertility was revealed in cryptorchidism using spermograms from 48% of men with unilateral undescended testes and 78–80% of men with bilateral anomaly (Cortes et al., 2008; Hutson et al., 2010; Raigorodskaya et al., 2017).

Cryptorchidism is a risk factor for dysfunction of Leydig and Sertoli cells during the fertile period of life and leads to a decrease in testosterone levels (Chung and Brock, 2011; Goel et al., 2015).

The development of fetal surgery requires anatomists to investigate the patterns of structure and formation of organ topography and structures of various systems, including the male reproductive system, in the fetal period of human ontogenesis.

The aim of this study was to establish the relationship between testicular descent to the scrotum and the age of the fetus.

MATERIALS AND METHODS

The study was performed using 195 specimens of the male fetuses aged 4–10 months with 81.0–375.0 mm parietal-coccygeal length (PCL) and macromicroscopic, conventional, and microslide preparation under the control of binocular loupes and morphometry.

The material was collected from obstetric and gynecological departments of medical institutions of the city of Chernovtsy and Chernovtsy region. Specimens of fetuses weighing 500.0 g or higher were studied at the Chernovtsy regional pathoanatomical bureau according to the cooperation agreement. In addition, topographic and anatomical features of the male reproductive organs were investigated using specimens of 4–10-month-old human fetuses and the organocomplex of male genitourinary organs from fetuses of different age groups from the collection of the Museum of Human Anatomy, Bukovinian State Medical University, Chernovtsy. The Commission on Biomedical Ethics of Bukovinian State Medical University identified no violations of ethical and legal norms in the medical research.

The embryonic, prefetal, and fetal periods are systematized according to the classification by G.A. Shmidt (1968). The age of study objects was determined according to tables of V.M. Petten (1959) and B.P. Khvatov and Yu.N. Shapovalov (1969) based on the measurement of the parietal-coccygeal length (PCL). Macroscopic and microslide preparations of abdominal and pelvic organs of the fetuses of different age groups were used to study shape, structure, topography, and relationship of testicles and epididymis with peritoneum, adjacent organs, and fascial-cellular formations and vessels, and their morphometry was performed.

RESULTS

In 81.0–135.0-mm PCL fetuses, the right and left testicles in 11 cases were located over the deep inguinal ring and in a region of the iliac fossae in four cases. In two fetuses (105.0 and 115.0 mm PCL), the right testicle was located in region of the right iliac fossa and the lower end of the left testicle and its gubernaculum testis were immersed in the left deep inguinal ring. The 115.0-mm PCL fetus had separated location of epididymis relative to the testicles. In a 95.0-mm PCL fetus, the lower end of the right testicle was immersed into the deep inguinal ring and the left testicle was located above the entrance to the deep inguinal ring. In a 135.0-mm PCL fetus, the testicles were crescent-shaped, had a vertical location within the large pelvis, and contacted at the medial edges. Perineal ectopia of the testicles and epididymis, atresia of the tail of the right epididymis, and absence of testicular ducts were revealed in a 135.0-mm PCL fetus.

An analysis of topographic and anatomical features of the male reproductive glands in 136.0–185.0-mm PCL fetuses revealed that the testicles were located within the large pelvis; in 13 cases, the lower ends of both testicles were located above the entrance to the deep inguinal ring at a distance that is equal to the length of the pelvic part of the gubernaculum testis—3.2 ± 0.3 mm (right) and 2.8 ± 0.2 mm (left) (Fig. 1). In five fetuses, the testicles were located in the corresponding iliac fossae, near the deep inguinal rings. In two cases (160.0 and 180.0-mm PCL fetuses), the lower ends of the right and left testicles, together with their gubernaculum testis, were immersed in the corresponding deep inguinal ring. In a 170.0-mm PCL fetus, the lower end of the right testicle was immersed into the right deep inguinal ring, while the left testicle had an oblique location within the large pelvis and lay parallel to the left inguinal ligament. In a 175.0-mm PCL fetus, the testicles were located in the abdomen at the level of the middle part of the inguinal ligaments, while the right testicle was situated at 3.0 mm below the left testicle. In a 180.0-mm PCL fetus, the testicles were located horizontally at the boundary between the large pelvis and the small pelvic cavity. In one case (a 185.0-mm PCL fetus), the right testicle was adjacent to the anterior surface of the right psoas major muscle horizontally, above the deep inguinal ring, and the left testicle was in the middle of the inguinal canal.

We have discovered variation in testicular and epididymis topography in 6-month fetuses with 186.0–230.0 mm PCL. In eight fetuses, the testicles were located near the deep inguinal rings in a region of the

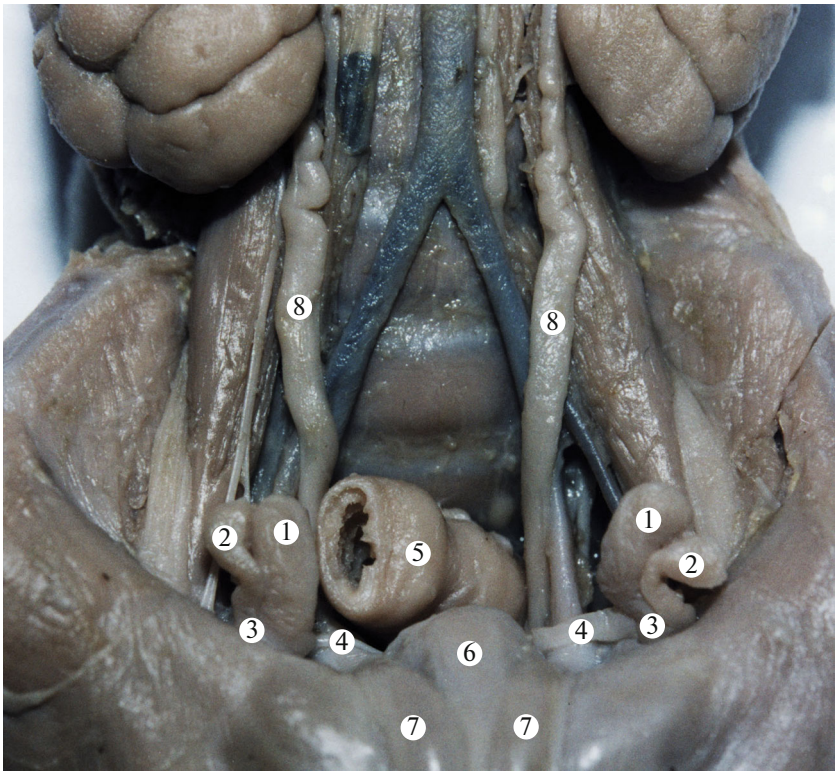

Fig. 1. Internal male reproductive organs of a 170.0-mm PCL fetus. Macroslide. Objective ×2.4. 1—testicle; 2—epididymis; 3—gubernaculum testis; 4—testicular duct; 5—rectum; 6—bladder; 7—umbilical artery; 8—renal duct.

iliac fossae. In 11 cases, the lower ends of both the right and left testicles and their gubernaculum testis were immersed in the corresponding deep inguinal ring. In five fetuses, the right testicle was in a region of the right iliac fossa and the lower end of the left testicle and its gubernaculum testis were immersed in the left deep inguinal ring. In five cases, the testicles were located in the scrotum. Pelvic ectopia of the left testicle was found in a 190.0-mm PCL fetus. In a 210.0-mm PCL fetus, the right testicle was located in the inguinal canal and had an oblique position, the left testicle was located almost vertically, anteriorly, externally, and slightly medially relative to the surface of the inguinal ring (Fig. 2). A single-chambered scrotum with a minor septum in the posterior portion was detected in a 230.0-mm PCL fetus, the right testicle was located in the right part of the single-chambered scrotum, and the left testicle was located 11.0 mm above the lower end of the right testicle. In addition, the medial surface of the left testicle was located adjacent to the dip under the medial part of the inguinal ligament.

In 11 fetuses aged 7 months (231.0–270.0 mm PCL), the lower ends of the testicles and their gubernaculum testis were immersed in the corresponding deep inguinal ring. In eight fetuses, the testicles were within the deep inguinal ring. Vaginal process of the peritoneum was seen along the inguinal canal and projected through a fissure (the superficial inguinal ring) by 12.0 ± 0.5 mm. In four fetuses, the testicles were located in a region of the iliac fossae. In nine cases, the testicles were found within the inguinal canal. In eight fetuses aged 7 months, the testicles were in the scrotum. In particular, in a 245.0-mm PCL fetus, both testicles were located in the scrotum. In this case, the right oval-shaped testicle was located in the sagittal

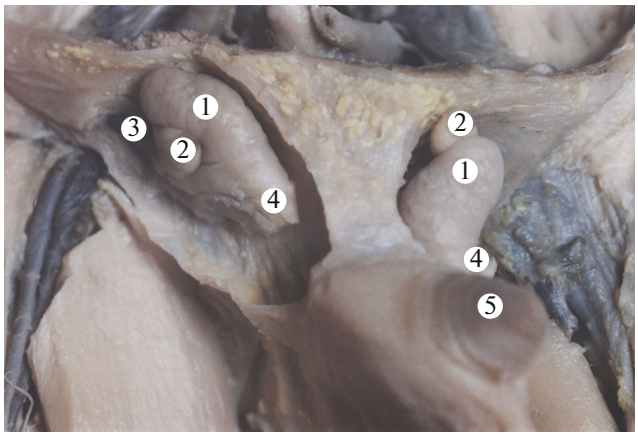

Fig. 2. Male reproductive organs of a 210.0-mm PCL fetus. Macroslide. Objective ×4.6. 1—testicle; 2—head of epididymis; 3—right spermatic cord; 4—gubernaculum testis; 5—penis.

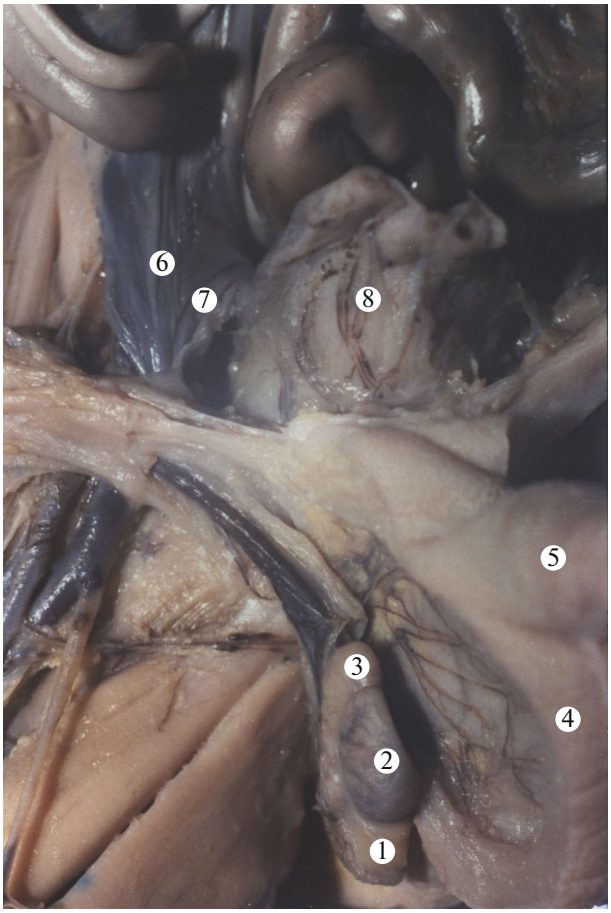

Fig. 3. Male reproductive organs of a 245.0 mm PCL fetus. Macroslide. Objective ×2.2. 1—gubernaculum testis of the right testicle; 2—right testicle; 3—epididymis of the right testicle; 4—scrotum; 5—penis; 6—testicular vessels; 7—right testicular duct; 8—bladder.

plane. Gubernaculum testis (6.4 mm long and 3.6 mm thick) projected from the lower end of the right testicle and was attached to the inner surface of the bottom of the scrotum (Fig. 3). The left testicle, oval-round, was located vertically in the sagittal plane. The lower end of the left testicle was located below by 3.0 mm compared to the lower end of the right testicle. The gubernaculum testis of the left testicle, cone-shaped, with a length of 5.0 mm and a thickness of 4.0 mm, also travelled from the lower end of the left testicle to the inner surface of the bottom of the scrotum.

In a 245.0-mm PCL fetus, the testicles were located in the middle of the deep and superficial inguinal rings distance, while the left vaginal process of the peritoneum projected 18.0 mm lower than the superficial inguinal ring and 12.0 mm lower than the right vaginal process of the peritoneum. In a 250.0-mm PCL fetus, the right testicle, oval-shaped, was located in the inguinal canal except for its lower end, which was located beyond the right superficial inguinal ring; the left testicle, bean-shaped, was located at the level of the left superficial inguinal ring. Pelvic ectopia of the left testicle, which was located within the small pelvis, behind the rectum, was detected in a 260.0-mm PCL fetus. The right testicle was located in the right half of the two-chambered scrotum.

We also revealed an individual variation of testicular topography in fetuses aged 8 months (271.0–310.0 mm PCL); namely, in ten cases, the right and left testicles were located within the inguinal canal (Fig. 4); in nine cases, the testicles were located in the scrotum.

In five fetuses aged 8 months, the left testicle was located beyond the inguinal canal in the superior part of the scrotum, and the right testicle was located within the inguinal canal. In four fetuses, the testicles were detected in the area of the corresponding deep inguinal ring. An analysis of a 280.0 mm PCL fetus identified testicular and epididymis dystopia: the right testicle and epididymis were located within the large pelvis and the left testicle and epididymis were located in the left scrotal cavity. Left testicular and left epididymis agenesis were detected in a 285.0-mm PCL fetus, while the right testicle and the right epididymis were located within the abdominal cavity near the deep inguinal ring.

An analysis of testicular topography in fetuses aged 9 months (311.0–345.0 mm PCL) revealed that the testicles were located in the scrotum in nine fetuses; the testicles were found near the superficial inguinal ring in six fetuses; they were found within the inguinal canal in four cases and in a region of the deep inguinal ring in one case. Testicular and epididymis dystopia was revealed in a 315.0 mm PCL fetus: the right testicle and its epididymis were located in the right iliac region and the left testicle with epididymis were located in the left half of the scrotum. In a 325.0-mm PCL fetus, the right testicle was located within the inguinal canal and the left testicle was in a region of the deep inguinal ring. Testicular agenesis and renal and vascular anomalies were revealed in a 320.0-mm PCL fetus.

In 13 fetuses aged 10 months (346.0–375.0 mm PCL), the testicles were in the scrotum (Fig. 5) and within the inguinal canal in seven cases.

DISCUSSION

At the beginning of the fetal period of human ontogenesis, the right and left testicles are mainly located above the corresponding deep inguinal ring and less often in the iliac fossae. In 120.0 and 125.0 mm PCL fetuses, the lower ends of the right and left testicles and their gubernaculum testis are immersed into the corresponding deep inguinal ring.

An analysis of topographic and anatomical features of the male reproductive glands in 5-month-old fetuses (136.0–185.0 mm PCL) revealed that the testicles were located within the large pelvis, the lower end

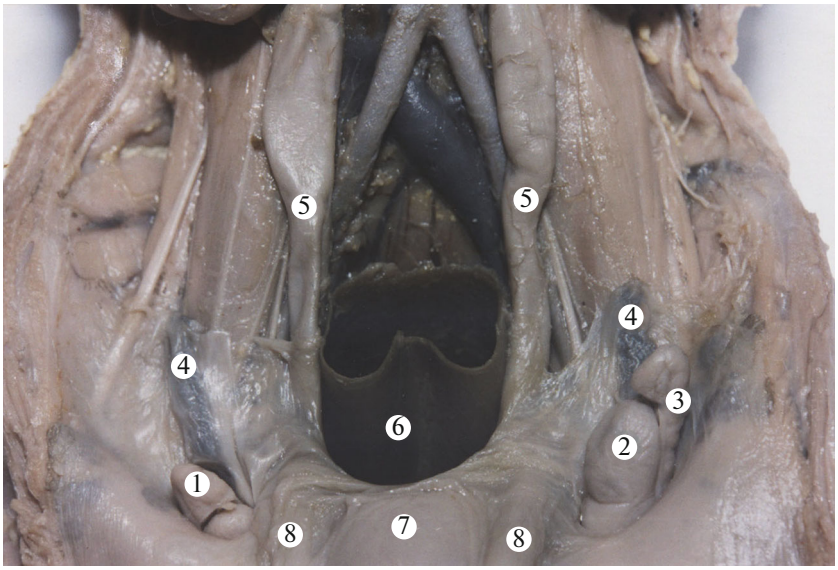

Fig. 4. Internal male reproductive organs of a 290.0-mm PCL fetus. Mactoslide. Objective ×2.4. 1—head of the right testicular epididymis; 2—left testicle; 3—epididymis of the left testicle; 4—testicular vessels; 5—renal ducts; 6—rectum; 7—bladder; 8—umbilical arteries.

of both the right and left testicles was located above the entrance to the deep inguinal ring at a distance that is equal to the length of the pelvic part of the gubernaculum testis.

We have discovered a variation in testicular and epididymis topography in 6-month-old fetuses. In most cases, during this period of ontogenesis, the lower ends of both testicles with gubernaculum testis are immersed in the corresponding deep inguinal ring. In a smaller part of the studied samples, the testicles were located in a region of the iliac fossae, near the deep inguinal rings.

In most 7-month-old fetuses, the lower ends of the testicles and their gubernaculum testis were immersed into the corresponding deep inguinal ring, and the testicles were within the deep inguinal ring in a part of the fetuses. At this stage of development, in several cases, the testicles were found within the inguinal canal, and the testicles were located in the scrotum in eight fetuses aged 7 months.

In fetuses aged 8 months, both testicles were located within the inguinal canal in 53% of cases and the testicles were located in the scrotum in 47%. In 9-month-old fetuses, the testicles were mainly located within the inguinal canal or in the scrotum. In 65% of 10-month-old fetuses, the testicles were in the scrotum, while they were within inguinal canal in 35% of cases.

The final process of testicular descent through the inguinal canal into the scrotum (270.0 cm–290.0-mm PCL fetuses) is influenced by a combination of many factors, including muscular contraction of the anterolateral abdominal wall, an increase in intra-abdominal pressure, contractile force of the gubernaculum testis (ligament) and the vaginal process of the peritoneum, and the neuro-muscular system. We believe that the gubernaculum testis plays a particularly important role in testicular descent to the scrotum among the above factors. The gubernaculum testis prior to migration of a testicle through the inguinal canal (the eighth month of antenatal development) is maximally developed, as evidenced by the prevalence of smooth muscular cells over connective tissue elements. The gubernaculum testis has well-developed vascular and nervous networks. The fibers and cells of the retroperitoneal por-

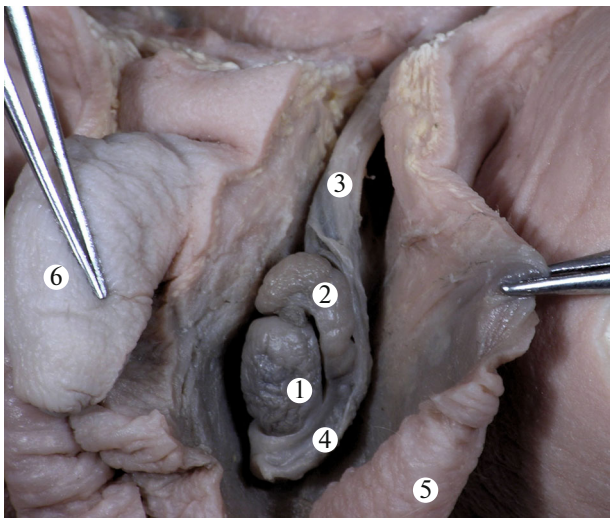

Fig. 5. Male reproductive organs of a 375.0-mm PCL fetus. Mactoslide. Objective ×1.8. 1—left testicle; 2—epididymis of the left testicle; 3—left spermatic cord; 4—gubernaculum testis of the left testicle; 5—scrotum; 6—penis.

tion of the gubernaculum testis are closely connected with those of walls of the inguinal canal. Taking into account active movements of the fetus observed during this period and resulting in contraction of muscles of the anterior abdominal wall, which receive nerve fibers from the same with gubernaculum testis source, there is no doubt that the gubernaculum testis plays an active role in migration of the testicles into the scrotum.

According to our research, the process of fusion of the peritoneal vaginal process layers occurs in 9–10-month-old fetuses, resulting in disappearance of the communication between its cavity and the peritoneum. Delayed fusion of the peritoneal vaginal process layers at the end of the fetal period is an anatomic prerequisite for the occurrence of congenital inguinal-scrotal hernias.

CONCLUSIONS

This study has shown that the rate of testicular descent into the scrotum does not always coincide with the corresponding stage of the fetus development.

An accelerated development of the gubernaculum testis in fetuses aged 5–8 months is a major factor contributing to heterochrony of testicular development and subsequent testicular descent into the scrotum.

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Conflict of interest. The authors declare that they have no conflict of interest.

REFERENCES

Chung, E. and Brock, G.B., Cryptorchidism and it's impact on male fertility: a state of art review of current literature. *Can. Urol. Assoc. J.*, 2011, vol. 5, no. 3, pp. 210–214.

Cortes, D., Kjellberg, E.M., Breddam, M., and Thorup, J., The true incidence of cryptorchidism in Denmark, *J. Urol.*, 2008, vol. 179, no. 1, pp. 314–318. doi 10.1016/j.juro.2007.08.158

Fantasia, J., Aidlen, J., Lathrop, W., and Ellsworth, P., Undescended Testes: A clinical and surgical review. *Urol. Nurs.*, 2015, vol. 35, no. 3, pp. 117–126.

Goel, P., Rawat, J.D., Wakhlu, A., and Kureel, S.N., Undescended testicle: an update on fertility in cryptorchid men, *Indian J. Med. Res.*, 2015, vol. 141, no. 2, pp. 163–171.

Hauser, R., Skakkebaek, N.E., Hass, U., Toppari, J., Juul, A., Andersson, A.M., et al., Male reproductive disorders, diseases, and costs of exposure to endocrine-disrupting chemicals in the European Union, *J. Clin. Endocrinol. Metab.*, 2015, vol. 100, no. 4, pp. 1267–1277. doi 10.1210/jc.2014-4325

Hutson, J.M., Balic, A., Nation, T., and Southwell, B., Cryptorchidism, *Semin. Pediatr. Surg.*, 2010, vol. 19, no. 3, pp. 215–224. doi 10.1053/j.sempedsurg.2010.04.001

Hutson, J.M., Southwell, B.R., and Li, R., The regulation of testicular descent and the effects of cryptorchidism, *Endocr. Rev.*, 2013, vol. 34, no. 5, pp. 725–752. doi 10.1210/er.2012-1089

Khmara, T.V., Khmara, A.B., and Kuftyak, V.V., Clinical and anatomical aspects of lowering the testicles in the scrotum, *Klin. Anat. Oper. Khir.*, 2015, vol. 14, no. 2, pp. 26–28.

Kollin, C. and Ritzén, E.M., Cryptorchidism: a clinical perspective, *Pediatr. Endocrinol. Rev.*, 2014, vol. 11, no. 2, pp. 240–250.

Komarova, S.Yu. and Pichugova, S.V., Germinal epithelium of the testis in children with cryptorchidism in the ultrastructural aspect, *Vestn. Eksp. Klin. Khir.*, 2017, no. 3, pp. 218–224. doi 10.18499/2070-478X-2017-10-3-218-224

Latyshev, O.Yu., Lavrova, T.R., Mirakov, K.K., Samsonova, L.N., Okulov, A.B., and Kasatkina, E.P., Cryptorchidism—testicular dysgenesis syndrome manifestation, *Vestn. Reprod. Zdor.*, 2008, nos. 3–4, pp. 2–7.

Lee, P.A. and Houk, C.P., *Cryptorchidism, Curr. Opin. Endocrinol. Diabetes Obes.*, 2013, vol. 20, no. 3, pp. 210–216. doi 10.1097/MED.0b013e32835ffc7d

Raigorodskaya, N.Yu., Sedova, L.N., Zakharova, N.B., Bolotova, N.V., and Morozov, D.A., Sexual development and the formation of the reproductive system of boys operated for unilateral inguinal and abdominal cryptorchidism, *Byull. Med. Internet-Konf.*, 2017, vol. 7, no. 2, pp. 553–556.

Toppari, J., Rodprasert, W., and Virtanen, H.E., Cryptorchidism—disease or symptom?, *Ann. Endocrinol.* (Paris), 2014, vol. 75, no. 2, pp. 72–76. doi 10.1016/j.ando.2014.04.010

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