

EXPERIMENTAL STUDY

Investigation of the effects of experimental cryptorchidism formed in prepubertal rats during the postpubertal and adult periods

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Abstract: *Objective:* Investigation of the effects of experimental cryptorchidism formed in prepubertal rats during the postpubertal and adult periods.

Material and methods: The abdominal cavities of 20 rats in the control group were just opened and closed without any further surgical procedure. The testicles of 20 rats in the experimental group were fixed to the anterolateral wall of the abdominal cavity under general anesthesia. At the 6th month, blood and testis tissue samples of rats were obtained and the rats were sacrificed and the experiment was terminated. Statistical analysis was done by SPSS 15.0 package software.

Results: The histopathological evaluation of testicular tissues of rats in the control group was in the normal limits. Of the experimental group, the testicular tissues had injury in nearly half of the rats, in which the testicles were fixed in the abdomen for three months that is the 1st subgroup. FSH levels were statistically significantly elevated in both experimental groups of rats ($p < 0.01$).

Discussion and conclusion: Cryptorchidism formed experimentally causes a partial damage in the testicular tissue of pubertal rats, however the damage increases gradually in the testicles of rats, in which the testicles were descended and left in the scrotum for another three months. The statistically elevated levels of FSH in both experimental groups of rats can be accounted for severe testicular damage. The timing in descending the undescended testicles to the scrotum is essential and should be performed before the puberty and as early as possible in avoiding the risk of infertility (Tab. 3, Fig. 5, Ref. 44). Full Text in PDF www.elis.sk.

Key words: testicle, infertility, cryptorchidism, rat.

Fertility is a vital issue for the maintenance of human being and other living creatures. Sustaining fertility is possible with the determination and treatment of the causes of infertility.

Cryptorchidism is a developmental insufficiency during the descent of the testicles from the abdominal cavity into the scrotal pouch. Undescended testis or cryptorchidism is one of the frequently encountered problems in boys.

It is encountered with a rate of 9–30 % in premature and 3–6 % in term boys, as well as in 1 % in the first age and remains the same in 0.7 % until puberty. The most important risks of long term complications in undescended testis are infertility and development of malignancy. Malignant degeneration was reported as 5–40 fold higher than normal (1, 2).

Development of testis starts at the 6–7th weeks of intrauterine life and continues until adolescent period in humans. Hormonal secretion starts at the 8th week in the fetal testis (1, 2).

Histopathological changes were observed in the testicles of boys with undescended testis as early as 1–2 years of age. These were the decrease in the Leydig cell counts, degeneration in Sertoli cells, decrease in germ cell counts and inadequate development of spermatocytes (1, 2). Fertility is affected much more in bilateral cases (3). Regular mechanism of testicular descent is a combination of complex hormonal and anatomical factors and the real process isn't known yet (4, 5).

It is well known that cryptorchidism affects male fertility through germ cell degeneration. However, being a real father is also affected from other factors such as fertility and age of the woman. Hormonal levels and semen quality have partial effects upon fertility potency (6, 7).

Descending of the testicles from abdomen into scrotal pouch in the late period does not lessen the risk of infertility, however it can stop the degeneration in the testis (8).

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Germ cells are absent and Leydig cells are hypoplastic in intraabdominal testes. The germ cell count is normal in cryptorchidic testes in the first year of life, but it decreases after the second year and results in infertility in adulthood.

While germ cell aplasia is seen at a rate of only 1 % in normal children of 1-2 years, it is encountered at 20 % in 2 years, 40 % in 6 years and 45 % in 10–11 years of age in children with cryptorchidism. Absence of spermatogenesis is not congenital, but it is thought to develop secondary to germ cell degeneration (9, 10).

Cryptorchidism affects 3–4 % of term male infants, and it is known that bilateral testicular descensus abnormalities may result in azospermia even though they have been corrected in early childhood (11).

It has been reported that a significant difference was not found in the “fertility index” between undescended testis and testis descended to the scrotum after intervention in patients of one year or under (12). But this difference was found rather significant in other groups of ages. Fertility was evaluated in a study in 91 patients with unilateral cryptorchidism who had orchiopexy in postpubertal period. Azospermia or oligospermia was determined in 83.5 % of patients (13).

The problems during the descent of testis in the fetal life and the persistence of testis in the abdomen, especially in human, result in severe infertility. Serious problems may occur if the testicles are not descended from the abdominal cavity into the scrotal pouch directly after birth. Therefore, the experimental cryptorchidism formed in prepubertal rats and its effects during the postpubertal and adult periods were studied in this study. Other factors and the measures that can be introduced to prevent infertility were also evaluated.

We aimed to investigate the effects of experimental cryptorchidism formed in prepubertal rats on the development of testis and hormonal values in postpubertal and adult periods.

Materials and methods

Forty wistar albino male rats, twenty-one days old, were included in this study with the permission from Kahramanmaras Sutcu Imam University Medical Faculty Local Ethics Committee on Experimental Animal Research. Probable parents were bred to obtain those twenty-one day old male rats and then only healthy juvenile male rats were selected following birth.

Rats were divided into the two groups as the Control and the Experimental group at the beginning of the experiment. Then they were anesthetized with a combination of ketamin HCL + xylazine HCL. Abdominal wall of the rats were dissected in the Experimental group after anesthesia. Testes of the rats were passed through the inguinal canal into the abdominal cavity and were fixed to the posterior surface of the anterolateral abdominal wall at the same level with the inferior border of the kidneys and then abdominal wall was closed. Anterior abdominal wall was only opened and closed in the Control group of rats (Tab. 1).

Rats in the Experimental group were divided into the two subgroups after three months. Blood and testis tissue samples were obtained from the first half (Group 2) under deep anesthesia and then they were sacrificed. Testes those had been already fixed to the

Tab. 1. Experimental Design of Study.

Groups (n=40)	Procedures
Group 1 (Control group) (n=10)	Abdominal cavity of rats was opened and closed at the beginning of the experiment and rats were sacrificed after three months from the beginning.
Group 2 (Experimental group) (n=10)	Testes of the rats were ascended from the scrotal pouch and fixed on the posterior aspect of antero-lateral abdominal wall at the beginning of the experiment and rats were sacrificed after three months from the surgery.
Group 3 (Control group) (n=10)	Abdominal cavity of the rats was only opened and closed at the beginning and third month of the experiment and rats were sacrificed at the end of sixth month.
Group 4 (Experimental group) (n=10)	Testes of the rats were ascended from the scrotal pouch and fixed on the posterior aspect of antero-lateral abdominal wall at the beginning of the experiment and descended again to the scrotal pouch at the third month and the rats were sacrificed at the 6th month.

posterior surface of anterolateral abdominal wall were re-descended surgically through the inguinal canal into the scrotal pouch in the second half (Group 4). On the other hand, the Control group rats were also divided into two halves. Blood and testis tissue samples were obtained from the first half under deep anesthesia (Group 1) and then they were sacrificed. The abdominal cavity was only opened and closed in the second half of the rats (Group 3) (Tab. 1).

Blood and testis tissue samples were obtained from the second halves of the Control and Experimental group rats (Group 3 and 4) under deep anesthesia and then they were sacrificed after six months from the beginning of the experiment (Tab. 1).

Blood specimens were collected in 8 mm tubes with gel. Specimens were centrifugated at 4000 rpm for 10 minutes after coagulation. The sera obtained were kept at -20 °C until study time in eppendorf tubes.

Serum testosterone, LH, FSH, estradiol, TSH levels were studied with EIA (Enzyme immunoassay) method. Testosterone, estradiol (Cayman Chemical Company, USA) and rat TSH ELISA KIT (Shibayagi Co. Ltd. Japan) and LH- RH EIA KIT (Phoenix Pharmaceutical Inc. USA) and rat FSH EIA KIT (ALPCO Diagnostics, USA) were used for the analyses.

Testis tissue samples were kept in 10 % formaldehyde. Tissues were embedded in paraffin blocks after a routine histological process. Sections of 5 µm were taken from paraffin blocks and stained with hematoxyline-eosine. They were evaluated under a light microscope and then photographs were taken.

Results

Macroscopical Evaluation

At the end of macroscopical evaluation of both the first and second halves of the Experimental group (Groups 2, 4), outer anatomical appearance of rat testes were observed to be different from

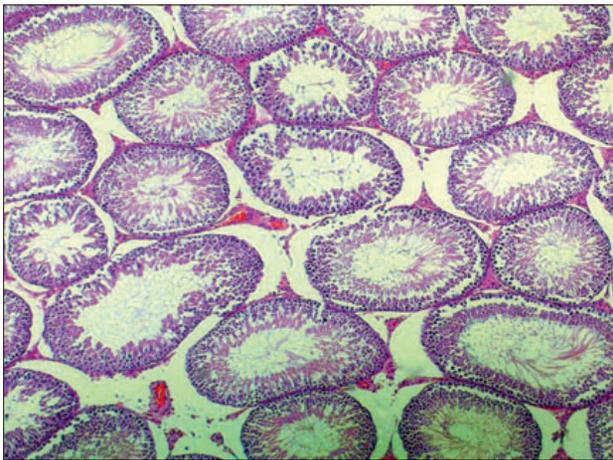


Fig. 1. The testicular tissue having seminiferous tubules with normal histology (Control groups; Group 1 and 3) (Hematoxylin-Eosin x100).

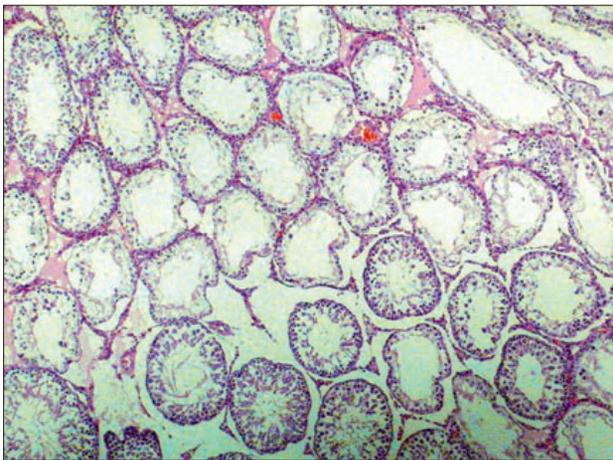


Fig. 2. Histological appearance of seminiferous tubules with normal structure at the lower half and with spermatogenic maturation at the upper half of the section. (Experimental group; Group 2) (Hematoxylin-Eosin x40).

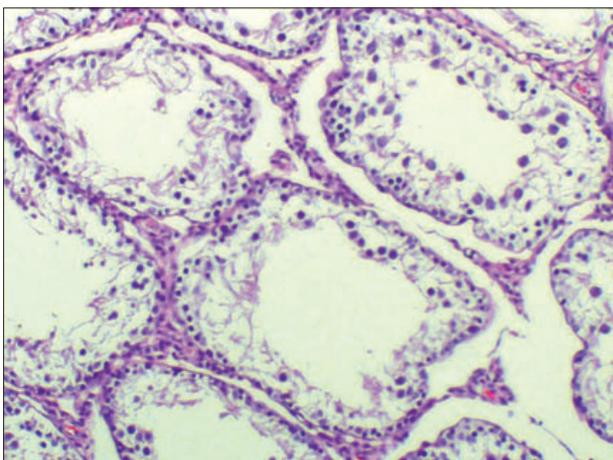


Fig. 3. Histological appearance of all seminiferous tubules with arrested spermatogenic maturation (Experimental group; Group 4) (Hematoxylin-Eosin x200).

Tab. 2. Differences between Group 1 and Group 2.

Hormones	Group 1 Mean±Sd (ng/ml)	Group 2 Mean±Sd (ng/ml)	Difference Between Group 1 and Group 2
FSH	6.7±3.5	13.6±3.5	p<0.05
LH	1.1±0.5	1.2±0.9	p>0.05
TSH	1.02±0.9	0.19±0.19	p<0.05
TESTOSTERON	444.1±42.8	453.7±39.3	p>0.05
ESTRADIOL	1283.6±124.7	1109.9±155.5	p<0.05

Tab. 3. Differences between Group 3 and Group 4.

Hormones	Group 3 Mean±Sd (ng/ml)	Group 4 Mean±Sd (ng/ml)	Difference Between Group 3 and Group 4
FSH	3±0.4	6.1±1.3	p<0.05
LH	2±0.8	0.91±0.39	p<0.05
TSH	1.5±0.11	1.8±0.2	p>0.05
TESTOSTERON	475.8±10.7	559±6.1	p<0.05
ESTRADIOL	1286.1±12.3	1212.3±25.1	p>0.05

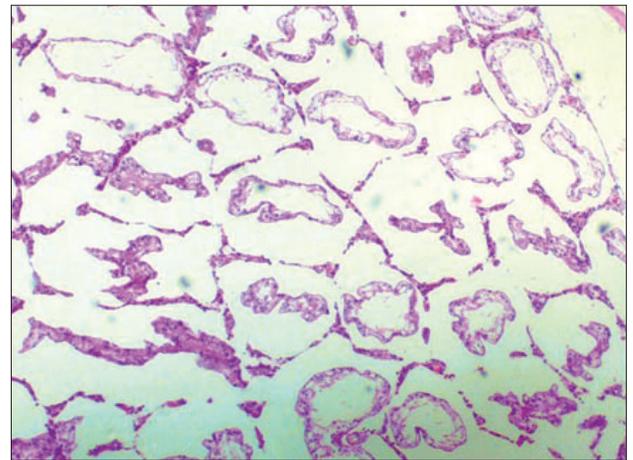


Fig. 4. Histological appearance of seminiferous tubules with only Sertoli cells (Experimental group; Group 4) (Hematoxylin-Eosin x40).

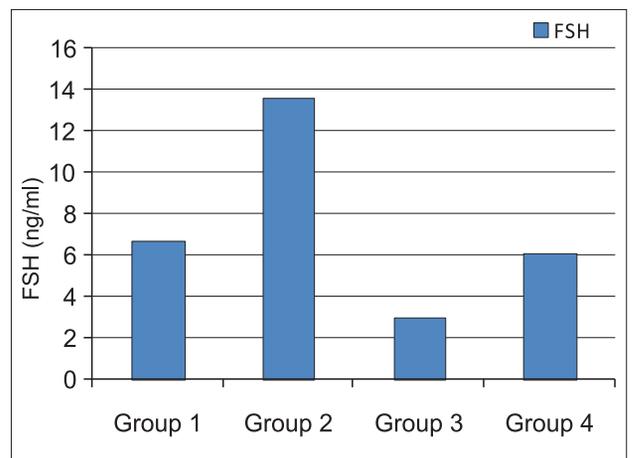


Fig. 5. The comparison of FSH level among the groups.

normal structure and dimensions of the testes decreased (atrophied testes). However, the testes of rats were in normal appearance and size in the Control groups (Groups 1, 3).

Histopathological Evaluation

Testis tissues were detected to have normal structure microscopically in both Control groups (Groups 1, 3) (Fig. 1). Atrophy was detected in nearly half of testis tissues of all rats, the testes of which were transferred to abdominal cavity from the scrotal pouch in prepubertal period and fixed to the posterior surface of anterolateral abdominal wall for three months and were then removed surgically. Seminiferous tubules with arrested spermatogenic maturation were seen in the first half of the Experimental group (Group 2) (Fig. 2) (Tabs 2, 3).

We found out that in nearly all testis tissues atrophy developed and seminiferous tubules with arrested spermatogenic maturation and Sertoli cells only were seen in rats, of which the testes were transferred to abdominal cavity from scrotal pouch and kept there for three months and then re-descended to scrotal pouch and kept in their regular site for another three months (Figs 3, 4)

We determined that experimental cryptorchidism, formed in pubertal rats by keeping the testicles in the abdominal cavity for three months, caused a partial damage in testicular tissues and the damage was found to persist in the testes that re-descended from the abdominal cavity to the scrotal pouch and were kept there for another three months (Figs 3, 4).

Biochemical Evaluation

The levels of FSH, LH, TSH, Testosterone and Estradiol in the sera of rats were found to be unchanged in the Control group, but a statistically significant increase was determined in FSH levels ($p < 0,01$) in both Experimental groups (Groups 2, 4) compared to the Control group (Tabs 1–3) (Fig. 5).

This increase in FSH levels can be explained by increasing damage in the testicular tissues of the Experimental group of rats. The mechanism is that a destruction of Sertoli cells in the seminiferous tubules prevents the release of FSH inhibitory substances and this in turn will cause an increase in FSH. The increase of FSH in the blood appears as the most important indicator of damage in the testicular tissues.

Discussion

In this study, we aimed to establish the effects of experimental cryptorchidism formed in the prepubertal period at postpubertal and adult periods, and to find out what can be done on this subject, which is an important cause of infertility. The requirement for seriously controlling the location of testes in the scrotal pouch in newborn boys is a known reality. The testes should be descended by hormonal treatment or a surgical intervention as soon as they are noticed not to exist in the scrotal pouch. Any delay of descent of testis may cause not only infertility but also adverse effects such as development of malignant tumors of testes. We suggested that a simple control whether or not testes are in the scrotal pouch can prevent these adverse effects.

The mechanism of undescended testis is not clear at present and it is one of the most frequent pathologies requiring surgical intervention in pediatric urology. It is for this reason that the priority for hormonal or surgical treatment is also widely debated. Hormonal treatment is suggested in patients with bilateral or retractile testes. Undescended testes must be descended surgically before 1 year of age because of the initiation of microscopical changes independent on receiving or not a hormonal treatment (14).

The incidence of cryptorchidism is related to the week and weight at delivery. Its incidence is almost 60–70 % in boys with a delivery weight less than 1500 g. While this ratio is 33 % in pretermatures, it decreases to 3 % in term males. Its incidence in one-year-old boys was found as 1.58 % in a study in 1986 in England (15).

Undescended testes are reported to be in 30 % in pretermatures in literature (16). In many other studies, similar rates were reported in newborn boys born earlier than the 37th week of gestation and with low birth weight less than 2500 g. Studies of large series were made to determine the natural history of descent of testis and the factors affecting postnatal descent (17, 18).

Transinguinal descent of testes takes place between the 24th and 28th week and is completed in the third trimester. Processus vaginalis becomes obliterated completely before the delivery and also gubernaculum is atrophied and transforms to a ligament. However, processus vaginalis has persisted to stay open in 90 % of undescended testes. The testes descend into the scrotal pouch at the last 2/3 period of gestation. The testes have already descended into the scrotal pouch at birth in more than 95 % of term babies and in 79 % of preterm babies. However, the descent of testes may be completed during the first year of life (19).

Isolated cryptorchidism is one of the most common congenital anomalies at birth. Cryptorchidism is encountered in 3% of newborn boys. Unilateral cryptorchidism is more frequently (1.6–1.9 %) compared to bilateral cryptorchidism. (20). The descent of testes into scrotal pouch is usually completed in the second trimester. However, a high cryptorchidism rate in pretermatures points out that the descent will be completed until delivery (20, 21).

These data shows that there is a significant relation between the descent of testes and prematurity, low birth weight, young according to gestational age and twin delivery (18). According to results of studies, 70–77 % of cryptorchidic testes descended spontaneously in the first three months after delivery.

The main cause of germ cell degeneration is a thermal environment. Testicular temperature is normally less than body temperature. Intraabdominal testes (35–37 °C) are warmer when compared to the normal scrotal testes (33 °C) (22). Timely diagnosis, accurate and appropriate treatment protects the patient from possible future problems such as infertility and malignancy. Testes migrate from the abdominal cavity (37 °C) into the scrotal pouch (33 °C), which has an environment with a lower temperature in many mammals for healthy sperm production (20).

Recently, germ cell degeneration has been demonstrated to take place by means of programmed cell death (apoptosis) characterized with internucleosomal fragmentation as a response to thermal stress (19, 21–23). In this stage, thermal shock transcription fac-

tor 1 is activated and induces apoptosis of primary spermatocytes, which results in infertility in male mice (24).

The timing for an operation is important for the surgical treatment of undescended testes. Structural degeneration in the early period of germ cells was observed in the previous studies. Various structural changes were established on an electron microscopy after 1 year and on a light microscopy after 3-4 years of age. Progressive macroscopical changes become marked in the primary school period. As the current approach, the operation is suggested to be performed between 12th and 18th month period even though the operation time is different in many centers (25, 26)

The incidence of cryptorchidism decreases to 1% approximately at 1 year of age and this ratio continues at the same level until adult age (27).

Deterioration of germ cell maturation is a well-known result of cryptorchidism. It is believed that the risk for subfertility is decreased when the testes are descended into scrotal pouch with early surgical intervention before histopathological changes were formed. But there are many data that orchiopexy does not lessen this risk. Previous reports focused on histopathological changes occurred in undescended testes at the 1st year of life. The reason for this was the probability of early surgical intervention to protect spermatogonial functions (28).

Ceylan et al. reported that (except ectopy) the first-line treatment option in undescended testis should be hormonal, if this is unsuccessful, then the testes should be descended into scrotal pouch surgically. They also reported that the treatment of choice in postpubertal undescended testes is orchiectomy, orchiopexy may be performed following a testicular biopsy. This procedure may not support spontaneous fertility, however it may provide a source for IVF in the future in case of testicular dysfunction or orchiectomy in addition to its cosmetic benefit (8). Tellal et al. reported that semen parameters were disconcerted with the rate of 89 % in unilateral group and 100% in bilateral group in males who had orchiopexy at a mean age of 13. Surgical treatment decision should be made in the first year of life before these changes took place (29).

The risk of malignant degeneration in undescended testes is higher in cryptorchid and contralateral normally localized testis than in the normal population. This risk may be lowered with orchiopexy (3). The incidence for development of malignancy in normally localized testes was reported as 1/100000, however it was 1/2550 in cases with undescended testes (30). Biopsies made recently from cryptorchid testes confirmed an inadequate transformation to type A spermatogonies, which forms the first step of postnatal spermatogenic development (31, 32). Gonocytes are present initially, but after then they undergo degeneration and cause insufficiency in total germ cell count. Although it is controversial, these degenerated cells may be a source of carcinoma in situ (33).

It was declared that being a father may be at risk in patients who had previous bilateral cryptorchidism during childhood. Also it was shown that paternity wasn't correlated with orchiopexy age. In another study, paternity was positive in 87 % of males with unilateral cryptorchidism, while this rate was found as 33 % in males with bilateral cryptorchidism. In terms of development of infert-

ility in cases with cryptorchidism, other risk factors were shown to cause increased FSH and low sperm count. An additional risk factor in orchiopexy was also shown as the site of parenchymal testicular suture (27, 34, 35).

An important factor in infertility is the unilateral or bilateral existence of cryptorchidism. In studies conducted on male adults who were operated in childhood due to cryptorchidism, the rate of true paternity in bilateral cryptorchid males (65.3 %) was significantly lower than the unilateral cryptorchid males (89.7 %) and control (93.2 %) group (6, 7).

Low inhibin B levels secreted from sertoli cells and regulating the secretion of FSH and high FSH levels are associated with high infertility risk (36).

We established atrophy and degeneration in vast majority of cryptorchid testes of rats reached to puberty in our study. Also we found the atrophy and increase in cell degeneration of most of the testes of cryptorchid rats in postpubertal period. We think that these changes may result in infertility. Additionally, we think that increased levels of estrogen and FSH are important in demonstrating the tissue injury in testes.

Higher inhibin B and lower FSH levels were determined in unilateral cryptorchid males who had orchiopexy operation in 2nd year of life demonstrating that they benefited from an early surgical treatment (37). Higher estrogen and FSH levels explain a lower inhibin B level and testicular cell damage in our study. Results of our study are parallel to other authors' studies in terms of FSH and estrogen levels and testicular damage.

Another important risk related to cryptorchidism is testicular malignancy. It is presumed that this risk has become 3-10 fold higher than normal recently (38, 39). Even though it has been thought that germ cell degeneration and testicular dysplasia could lead to malignancy, its mechanism is still debatable (38, 39). The effects of orchiopexy operation performed in the first year of life should be investigated both for infertility and malignancy. In our study, we think that risk of malignancy will be probably high in testicular cell degeneration in postpubertal and adult period of rats, which had experimentally formed cryptorchidism in prepubertal period.

The timing for the descent of undescended testes into the scrotal pouch is very important and descending the testes into the scrotum as soon as possible before puberty is essential in lowering the risk of infertility.

In conclusion, we think that if cryptorchidism is not treated at the earliest stage in prepubertal and pubertal period, it may cause degeneration in testicular tissues. Also this degeneration may continue and increase at the postpubertal period. Thus, the testes should be descended into the scrotal pouch at the earliest possible time in cryptorchid males in prepubertal, pubertal, postpubertal or adult period in avoiding testicular malignancy.

References

1. **McAninch JW.** Disorder of the testis, scrotum and spermatic cord. In: Tanagho EA, Smith's General Urology. McGraw-Hill 15th ed. Int Ed 2000; 684-698.

2. **Cuz DAJ, Das K.** Undescending testes. *Ind J Pediat* 2004; 71: 1111–1115.
3. **Palmer JM.** Undescended testicle. *Endocrinol. Metab Clin North Am* 1991; 20: 231–240.
4. **Akand M.** Testiküler inis, kriptorsidizm ve inguinal herni. *Derleme, Infertilite*; 135–139.
5. **Elder JS.** The undescended testis. Hormonal and surgical management. *Surg Clin North Am* 1998; 68: 983–105.
6. **Lee PA.** Fertility in cryptorchidism. Does treatment make a difference? *Endocrinol Metab Clin North Am* 1993; 22: 479–490.
7. **Puri P, O'Donnell B.** Semen analysis of patients who had orchidopexy at or after seven years of age. *Lancet* 1988; 2: 1051–1052
8. **Ceylan K, Yilmaz Y, Yildiz A, Kus A, Gönülalan H.** Kriptorsidizm. 164 olgunun; birlikte bulunan anomali, komplikasyon, tedavi modalitesi, hasta yaşı açısından irdelenmesi. *TAD* 2006; 4 (3): 24–26.
9. **Mengel W, Hienz HA, Sippe WG et al.** Studies on cryptorchidism: a comparison of histological findings in the germinative epithelium before and after the second year of life. *J Pediatr Surg* 1974; 9: 445–450.
10. **Hadziselimovic F.** Fertility and cryptorchidism. *Am J Dis Child* 1985; 139: 963–964.
11. **Raman JD, Schlegel PN.** *J Urol* 2003; 170 (4 Pt 1): 1287–1290.
12. **Abe T, Aoyama K, Gotoh T et al.** Cranial attachment of the gubernaculum associated with undescended testes. *J Pediatr Surg* 1996; 31: 652–655.
13. **Backhouse KM.** The natural history of testicular descent and mal-descent. *Proc R Soc Med* 1966; 59: 357–360.
14. **Ozksacık S, Yazıcı M, Oztan MO, Etensel B, Culhacı N, Gursoy MH.** Does sepragel (hylan b) Have any Preventive Effect on Two-Stage Orchidopexy in the First Stage? *ADÜ Tıp Fakültesi Dergisi* 2006; 7 (1): 11 – 14.
15. **John Radcliffe Hospital Cryptorchidism Study Group:** Boys with late descending testes: the source of patients with retractile testes undergoing orchidopexy. *BMJ* 1986; 293: 789.
16. **Fentener van Vlissingen JM, Koch CA, Delpech B, Wensing CJ.** Growth and differentiation of the gubernaculum testis during testicular descent in the pig: changes in the extracellular matrix, DNA content, and hyaluronidase, beta-glucuronidase, and beta-N-acetylglucosaminidase activities. *J Urol* 1989; 142: 837–845.
17. **Akre O, Lipworth L, Cnattingius S et al.** Risk factor patterns for cryptorchidism and hypospadias (see comments). *Epidemiology* 1999; 10: 364–369.
18. **Hadziselimovic F.** Treatment of cryptorchidism with GnRH. *Urol Clin North Am* 1982; 9: 413–420.
19. **Uyanık B.** Sağlıklı Yenidoganlarda Ortalama Penis Boyu ve Ürogenital Anomali Sikligi, *Uzmanlık tezi, İstanbul* 2006.
20. **Muller U, Donlon T, Schmid M et al.** Deletion mapping of the testis determining locus with DNA probes in 46,XX males and in 46,XY and 6,X,dic(Y) females. *Nucleic Acids Res* 1986; 14: 6489–6505.
21. **Frey HL, Rajfer J.** Role of the gubernaculum and intraabdominal pressure in the process of testicular descent. *J Urol* 1984; 131: 574–579.
22. **Tapanainen JS, Tilly JL, Vihko KK et al.** Hormonal control of apoptotic cell death in the testis: gonadotropins and androgens as testicular cell survival factors. *Mol Endocrinol* 1993; 7: 643–650.
23. **Mieusset R, Fouda PJ, Vaysse P et al.** Increase in testicular temperature in case of cryptorchidism in boys. *Fertil Steril* 1993; 59: 1319–1321.
24. **Billig H, Furuta I, Rivier C, Tapanainen J et al.** Apoptosis in testis germ cells: developmental changes in gonadotropin dependence and localization to selective tubule stages. *Endocrinology* 1995; 136: 5–12.
25. **Mieusset R, Bujan L.** Testicular heating and its possible contributions to male infertility: a review. *Int J Androl* 1995; 18: 169–184.
26. **Nakai A, Suzuki M, Tanabe M.** Arrest of spermatogenesis in mice expressing an active heat shock transcription factor. 1. *EMBO J* 2000; 19: 1545–1554.
27. **Yılmaz Y, Özen IO.** İnmemis Testis Kliniginde Güncel Yaklaşımlar. *STED*; cilt 13, sayı 6. 2004; 211–214.
28. **Basaklar AC.** Inguinoskrotal bölge hastalıkları. *Yenidoganın cerrahi hastalıkları. Palme Yayıncılık Ankara* 1994; 376–399.
29. **Andersson S, Geissler WM, Wu L et al.** Molecular genetics and pathophysiology of 17 beta-hydroxysteroid dehydrogenase 3 deficiency. *J Clin Endocrinol Metab* 1996; 81: 130–136.
30. **Attah AA, Hutson JM.** The role of intra-abdominal pressure in cryptorchidism. *J Urol* 1993; 150: 994–996.
31. **Tellaloglu S, Kadioglu A, Kilicaslan I, Alipour M, Kadioglu T, Ziyilan O.** Cryptorchidism. Is orchidopexy always preventive treatment for infertility. *Acta Chir Hung* 1994; 34: 195–201.
32. **Farrer JH, Walker AH, Rajfer J.** Management of the postpubertal cryptorchid testis: a statistical review. *J Urol* 1985; 134: 1071–1106.
33. **Huff DS, Hadziselimovic F, Snyder HM et al.** Histologic maldevelopment of unilaterally cryptorchid testes and their descended partners. *Eur J Pediatr* 1993; 152 (Suppl 2): S11–14.
34. **Güllüoğlu I.** Orkiopeksi operasyonunda başarıyı etkileyen faktörler. *Uzmanlık tezi, T.C. Sağlık Bakanlığı Sıslı Etfal Eğitim ve Arastırma Hastanesi, İstanbul* 2004.
35. **Huff DS, Hadziselimovic F, Snyder HM et al.** Postnatal testicular maldevelopment in unilateral cryptorchidism. *J Urol* 1989; 142: 546–548.
36. **Taskinen S, Hovatta O, Wikstrom S.** Early treatment of cryptorchidism, semen quality and testicular endocrinology. *J Urol* 1996; 156: 82–84.
37. **Skakkebaek NE, Berthelsen JG, Giwercman A et al.** Carcinoma-in-situ of the testis: possible origin from gonocytes and precursor of alt types of germ cell tumours except spermatocytoma. *Int J Androl* 1987; 10: 19–28.
38. **Miller KD, Coughlin MT, Lee PA.** Fertility after unilateral cryptorchidism. Paternity, time to conception, pretreatment testicular location and size, hormone and sperm parameters. *Horm Res* 2001; 55: 249–253.
39. **Attah AA, Hutson JM.** The role of intra-abdominal pressure in cryptorchidism. *J Urol* 1993; 150: 994–996.
40. **Coughlin MT, Bellinger MF, Lee PA.** Age at unilateral orchiopexy. Effect on hormone levels and sperm count in adulthood. *J Urol.* 1999; 162: 986–988.
41. **Lee PA, Coughlin MT, Bellinger MF, Inhibin B.** Comparison with indexes of fertility among formerly cryptorchid and control men. *J Clin Endocrinol Metab* 2001; 86: 2576–2584.
42. **Carmona Campos E, Regueiro Lopez JC, Prieto Castro R et al.** Cryptorchidism and testicular cancer. *Actas Urol Esp* 2000; 24: 49–51.
43. **United Kingdom Testicular Cancer Study Group.** Aetiogy of testicular cancer: association with congenital abnormalities, age at puberty, infertility, and exercise. *BMJ* 1994; 308: 1393–1399.
44. **Haughey BP, Graham S, Brasure J et al.** The epidemiology of testicular cancer in upstate New York. *Am J Epidemiol* 1989; 130: 25–36.

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