EXPERIMENTAL STUDY

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

Ozbag D1, Gumusalan Y1, Ozkaya M2, Ciralik H3, Tolun F4, Yuzbasigolu F5, Bakan V6, Arslan S7, Okumus M8

Department of Anatomy, Faculty of Medicine, Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey: davutozbag@hotmail.com

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.els.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.

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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 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 on 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 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 (Groups 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 testosteron, LH, FSH, estradiol, TSH levels were studied with EIA (Enzyme immunoassay) method. Testosteron, 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 hematoxiline-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

<table>
<thead>
<tr>
<th>Tab. 1. Experimental Design of Study.</th>
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<tr>
<td>Groups (n=40)</td>
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<tr>
<td>Group 1 (Control group) (n=10)</td>
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<td>Group 2 (Experimental group) (n=10)</td>
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<td>Group 3 (Control group) (n=10)</td>
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<td>Group 4 (Experimental group) (n=10)</td>
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Tab. 2. Differences between Group 1 and Group 2.

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Difference Between Group 1 and Group 2</th>
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<tbody>
<tr>
<td></td>
<td>Mean±Sd (ng/ml)</td>
<td>Mean±Sd (ng/ml)</td>
<td></td>
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<tr>
<td>FSH</td>
<td>6.7±3.5</td>
<td>13.6±3.5</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>LH</td>
<td>1.1±0.5</td>
<td>1.2±0.9</td>
<td>p&gt;0.05</td>
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<tr>
<td>TSH</td>
<td>1.02±0.9</td>
<td>0.19±0.19</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>TESTOSTERON</td>
<td>444.1±42.8</td>
<td>453.7±39.3</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>ESTRADIOL</td>
<td>1283.6±124.7</td>
<td>1109.9±155.5</td>
<td>p&lt;0.05</td>
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Tab. 3. Differences between Group 3 and Group 4.

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Difference Between Group 3 and Group 4</th>
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<tr>
<td></td>
<td>Mean±Sd (ng/ml)</td>
<td>Mean±Sd (ng/ml)</td>
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<tr>
<td>FSH</td>
<td>3±0.4</td>
<td>6.1±1.3</td>
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<tr>
<td>LH</td>
<td>2±0.8</td>
<td>0.91±0.39</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>TSH</td>
<td>1.5±0.11</td>
<td>1.8±0.2</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>TESTOSTERON</td>
<td>475.8±10.7</td>
<td>559±6.1</td>
<td>p&lt;0.05</td>
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<tr>
<td>ESTRADIOL</td>
<td>1286.1±12.3</td>
<td>1212.3±25.1</td>
<td>p&lt;0.05</td>
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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 spermatogenetic 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 spermatogenetic maturation (Experimental group; Group 4) (Hematoxylin-Eosin x200).

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 testes 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 retractyl 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 premature, 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 prematures 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 descensus 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 prematures 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 cryptorchid 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 than 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 mammalians for healthy sperm production (20).

Recently, germ cell degeneration has been demonstrated to take place by means of programmed cell death (apoptosis) characterized with internucleozomral 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) tfe irst-line treat-ment 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 orchiecotomy, 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 orchiecotomy 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 deci-sion 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 criptorchid 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 transforma-tion 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 criptorchidism 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 uni-lateral criptorchidism, while this rate was found as 33 % in males with bilateral criptorchidism. In terms of development of infer-

References
