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Efficiency Of Hormone Stimulation Treatment Before Hypospadias Surgery: Experimental Study

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ABSTRACT

Aim: In severe forms of hypospadias the penis may be small and the surrounding tissues may be weak. There is a relationship between size of the penis and surrounding tissues and the success of the hypospadias surgery. As the penis size gets smaller and the surrounding tissues are inadequate; the chance of surgical success decreases. Hormone treatments are used to increase penile size and tissue development. However, there is no consensus on the choice of hormone preparation to be used, how long the treatment should be done, and the timing of the surgical treatment to be performed. The purpose of this study, is to observe structural and histopathologic changes of hormone stimulation therapy methods in the healthy (without hypospadias) animal model and as a result of these observations, decide on the ideal surgical time period after hormone stimulation.

Material and method: 50 Wistar Albino rats, which were 4-6 weeks old, were used. Rats were divided into 5 groups as parenteral human chorionic gonadotropin group, parenteral testosterone group, topical dihydrotestosterone group, parenteral control group and topical treatment control group. Penis diameter and length were measured weekly at the beginning of the application, throughout the study and after the application. Biopsies were taken from preputium for histopathologic examination at 0, 1, 3, 6, 7 and 8th weeks. Biopsies were evaluated for vascularity, epithelial thickness, inflammation and fibrosis.

Results: In the group of which parenteral human chorionic gonadotropin was given; an increase of 56% in penile length, 58% in penile diameter and 84% in number of vessels in preputium biopsies was achieved. Increases in penile length, diameter and number of vessels were statistically significant when compared to the control group.

In the parenteral testosterone-administered group, an increase of 62% in the length of the penis, 59,8% in the diameter of the penis and 109% in the number of vessels in the preputium biopsies was observed. Increases in penile length, diameter and number of vessels were statistically significant when compared to the control group.

In the topical testosterone-administered group, an increase of 46% in the length of the penis, 59% in the diameter of the penis and 100% in the number of vessels in the preputium biopsies was observed. Increases in penile length and number of vessels were statistically significant when compared to the control group.

Conclusion: This study has shown that human chorionic gonadotropin, parenteral testosterone and topical dihydrotestosterone treatments are useful in increasing penile size and tissue quality before hypospadias surgery. It was observed that parenteral testosterone was the most effective among these hormone stimulation treatment regimens. In addition, this study showed that hormone stimulation treatments did not exacerbate inflammation and fibrosis, but periodically suppressed. The best time for surgical repair starting from the end of drug administration was found as after the 4th week in parenteral human chorionic gonadotropin group, after the 6th week in parenteral testosteron group and after the 1st week in testosteron group.

Keywords: *Hypospadias, preoperative hormone stimulation, rat preputium, experimental study, surgical timing*

1. INTRODUCTION

The importance of glans penis size, tissue quality and preputium vascularization for successful hypospadias surgery has been demonstrated in many studies.[1-6] Preoperative hormone stimulation is accepted to improve these parameters in severe hypospadias cases. The most commonly used preparations in hormone therapy are; parenteral Human Chorionic Gonadotropin (HCG), parenteral testosterone and topical dihydrotestosterone (DHT). Due to the lack of well-planned, evidence-based randomized trials among similar patient groups; dose of these preparations, the duration of the treatment and the timing of the surgery to be done after the treatment are still controversial. The purpose of this study is to evaluate type of

drugs used for hormone stimulation therapy (HST), duration of use and size and histopathologic changes of rat penis after use of these drugs and in conjuction with these to determine the optimal surgery time after use of hormone preparations.

2. MATERIAL AND METHOD

This study, supported by the Cukurova University Scientific Research Fund, which is approved by Cukurova University (CU) Ethics Committee, was carried out at the ÇÜ Medical Sciences Experimental Research and Application Center by observing bioethical rules.

A total of 50 Wistar Albino rats weighing between 200-250 gr with 4-6 weeks of age were used. Rats were divided into 5 groups; parenteral and topical control group, parenteral HCG group, parenteral testosterone group, topical DHT group. Grouping of rats, drug administration dose and duration, biopsy and follow-up times are shown in Table 1.

Body weights, penile length and penile diameter of rats in all groups were measured once a week. Penile diameter measurements were made from the widest part of the penis by retracting the preputium (Figure 1A). The length measurement was obtained by measuring the distance from the penis tip to the abdominal wall on the ventral face (Figure 1B). At the 0, 1, 3, 6, 7 and 8 weeks; full-fledged preputium biopsies were obtained (Figure 2). The first doses of the drugs were also applied when the first preputium biopsies were obtained. Since frequent biopsy of the small penis will cause deformity and will affect penile length measurements; the number of biopsies were reduced (6 biopsies) and the observation period was extended (8 week).

Histopathological examination

Histopathological examination and evaluation of tissue specimens were performed at the Department of Pathology, CU Faculty of medicine laboratory. Immunohistochemical samples prepared with Anti-Von Willebrand Factor Antibody and Hematoxylin Eosine (H & E) stained samples were evaluated with light microscopy by two pathologists. For biopsy specimens, in H & E stained preparations at 10 large growth fields (BBA), the vessels in each area were counted one by one and averaged. Immunohistochemical staining with Von Willebrand factor (vWF) was performed to verify the number of vessels. Vascularity, epithelial thickness, inflammation and fibrosis were evaluated in biopsy materials. Inflammation and fibrosis were semi-quantitatively scored between 0-3. No inflammation and fibrosis: 0, inflammation and fibrosis under 25% : 1 (mild), between 25-50% : 2 (moderate), inflammation and fibrosis more than 50% : 3 (severe). For epithelial thickness; if epithelium has 1-2 lines 1 point, 2-3 lines 2 points, 3-4 lines 3 points, 4-5 lines 4 points and 5-6 lines 5 points were given.

Evaluation of Results and Statistical Analysis

The results of the measurements and biopsies obtained by 8-week follow-up of the subjects were compared within the group and between groups according to weeks.

One-way analysis of variance (ANOVA) was used in repeated measures to compare the change of measurements with time in the study.

The averages obtained from the measurements of the experimental groups were compared with the Mann-Whitney U tests to see if the monitoring parameters significantly differed in the control groups from the groups treated with hormone stimulation therapy.

The results from the groups were analyzed using the SPSS 24 pocket program.

3. RESULTS

Parenteral HCG-parenteral control group assessment

In the parenteral HCG group, an average of 56% increase in penile length was detected. This increase was 35% in the parenteral control group and was statistically significantly higher in the HCG group (p:0,00).

An average increase of 58% in the HCG group and 56% in the control group was observed for penile diameter. The increase in penis size was statistically significantly higher in the HCG group compared to the control group (p: 0,026). In the measurements made on 4th and 5th weeks of HCG administration (weeks 3 and 4) and 4 weeks after last HCG administration (8th week of the study); the penile diameter was significantly higher than the control group (p:0.026, p: 0,001 and p: 0,02, respectively).

It was determined that the rate of increase of the number of vessels in HCG group was 84% on average. This increase was 17.6% in the parenteral control group and it was found statistically significant (p: 0,002). Number of vessels, was increased from the first administration and became statistically significant than control group at 2 weeks after last drug administration (6th week of study) (p: 0,04). The number of vessels continued to increase until the end of the study (Figures 3A, B).

The mean epithelium thickness was 2.2 in the first preputium biopsies and 3.4 in the final biopsies. When the increase in epithelial thickness compared with the control group; it was statistically significantly higher at 4 weeks after the last HCG administration (at the 8th week of the study) (P: 0,01) (Figure 4B). Inflammation during HCG administration was

significantly less than in the control group between 3 - 7 weeks, in other words at the 4th and 5th dose HCG administration, and the next 3 weeks (p: 0.02, p: 0.007, p: 0.02, respecctively). In the HCG group, fibrosis was significantly lower than the control group at the 4th and 5th doses of HCG administration and in the period covering the next 3 weeks (p: 0,001, p: 0.04, p: 0,006; respectively) (Figure 5).

When the changes introduced by parenteral HCG administration were evaluated together; it was found that the best time for surgical intervention was 4 weeks after the end of the treatment, with the penile length, diameter, number of vessels and epithelial thickness being the greatest (Figure 6).

Parenteral testosterone-parenteral control group assessment

In the follow-up of the penile lengths of subjects who received parenteral testosterone, an average length increase of 62% was detected. The most significant increase occurred in the weeks when the second and third doses of testosterone were administered. No increase was detected in the last three measurements. When penile length was compared with control group; statistically significant increase was observed from the week of the 3rd dose of testosterone (the 2nd week of the study) until the end of the study (p: 0,001).

In the follow-up of penile diameters, a mean diameter increase of 59.8% was detected. This increase was 56% in the control group and significantly higher in the testosterone group compared to the control group. The penile diameter was significantly higher at 1, 2 and 6 weeks after the last testosterone dose (3, 4 and 8 weeks of study) (p: 0,001, p:0, and p: 0,008; respectively).

The increase in the number of vessels was found to be 109% in the parenteral testosterone group and 17,6% in the parenteral control group and this difference was statistically significant (p: 0,004). The number of vessels started to increase after the first testosterone administration. The greatest increase was observed during the week of second testosterone dose. Four weeks after the last testosterone administration (6th week of study), it was statistically significantly higher than the control group (p: 0,04). The highest number of vessels was reached 5 weeks after the last testosterone administration (7th week of the study). The number of vessels decreased in the 8th week of the study, however, the number of vessels at 8 weeks was significantly higher than the control group (p: 0,005) (Figure 3C, D).

The average of the epithelial thickness scores was found as 2.2 at the first biopsy and 3.5 at the last biopsy. In the last biopsies of the rats in this group, it was determined that the epithelium thickness was 3-5 rows (Figre 4D). In the first and final biopsies of the rats in the control group, the epithelial thickness score was 2, with a maximum of 3 rows of epithelium. When the increase in epithelium thickness was compared with control group; statistically significantly higher status at 5th and 6th weeks (7th and 8th weeks of the study) after testosterone therapy was discontinued (p: 0,01 and p: 0,003; respectively). There was no statistically significant difference with the control group about the inflammation score in all biopsies taken during the study ($p<0,05$). The average of the fibrosis score in the biopsies taken when the first and second doses of testosterone administration were performed were similar with the control group. Fibrosis was significantly lower for 5 weeks (between 3rd and 7th weeks of study) starting from 1 week after the last testosterone dose (p: 0.001, p: 0.04, p: 0,006; respectively) (Figure 5E). In the final biopsy; fibrosis was similar to the control group again (p:0,1).

When the changes introduced by parenteral testosterone administration were evaluated together; the length of the penis, the diameter, the number of vessels and the epithelium thickness were the greatest and the optimal time for surgical treatment was found to be 6 weeks after the end of application (Figure 7).

Topical DHT-topical control group assessment

An average length increase of 46% was detected in the follow-up of penile lengths. This increase was 35% in the control group and was significantly higher in the DHT group compared to the control group (p: 0,001). The greatest increase in penile length was in the first 3 weeks of the study. There was no significant increase in penile length in the last 3 weeks. Penile length remained significantly high until the end of study starting from the fifth week of study, in other words starting from 1 week after stopping of DHT treatment (p: 0,002).

In the follow-up of penile diameters of the rats in the topical DHT group; there was a diameter increase of 59% at the last measurement. When the penile diameter was compared with the control group; a significant difference was detected only at the third week of the study (p: 0,01). There was no significant difference in diameter measurements at other weeks compared to control group.

It was determined that the rate of increase in the number of vessels was 100% in the control group and 45% in the control group and this difference was statistically significant (p: 0,000). There was a statistically significant increase in the number of vessels compared to the control group 1 week after the start of drug administration (p: 0,04) and continued to increase throughout the entire study (figure 3E-F).

Epithelium thickness score was 2.6 in the first biopsy and 3 in the last biopsy. In the last biopsies it was seen that the epithelium thickness had 3-4 rowsIn the last biopsies of the rats in the control group, the epithelium thickness score was 2.7, with a maximum of 3 rows of epithelium (Figure 4C). These increases in epithelial thickness were not statistically significant when compared with the control group ($p>0,05$). Inflammation scores were higher than the control group and this difference

was not statistically significant. The mean fibrosis score was similar to the control group for the first two weeks (p: 1 and p: 0,3; respectively). Fibrosis was found to be significantly higher at 3 weeks of drug administration compared to control group (P:0,04). Fibrosis remained similar to the control group in the next biopsies until the end of the study (p: 1, p: 0,7 and p: 0,06, respectively) (Figure 5F).

When the changes related with topical DHT administration were evaluated together; the ideal time for surgical intervention begins one week after the end of treatment, which is the common period in which the length, diameter of the penis and number of vessels were highest (Figure 8).

Table 1. Grouping of rats, drug administration dose and duration, biopsy and follow-up times. HCG: human chorionic gonadotropin, DHT: dihydrotestosterone, im: intramuscular

	Dose	Duration	Biopsy	Measurement
Group 1 Serum physiological, im	0,1 cc	Once a week / 5 weeks	0, 1, 3, 6, 7 and 8. weeks	Once a week / 8 weeks
Group 2 Serum physiological, topical	0,1 cc	Once a day/ 28 days	0, 1, 3, 6, 7 and 8. weeks	
Group 3 HCG, im	25 IU/ kg	Once a week / 5 weeks	0, 1, 3, 6, 7 and 8. weeks	
Group 4 Testosterone, im	2 mg/kg	Once a week / 3 weeks	0, 1, 3, 6, 7 and 8. weeks	
Group 5 DHT, Topical	0,1 cc	Once a day/ 28 days	0, 1, 3, 6, 7 and 8. weeks	

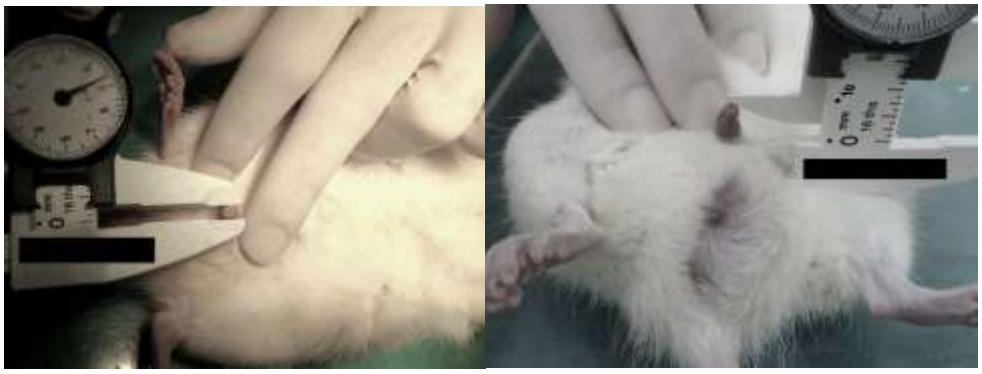


Figure 1. Measurement of penile diameter (A) and length (B)

Figure 2. Preputium biopsy removal

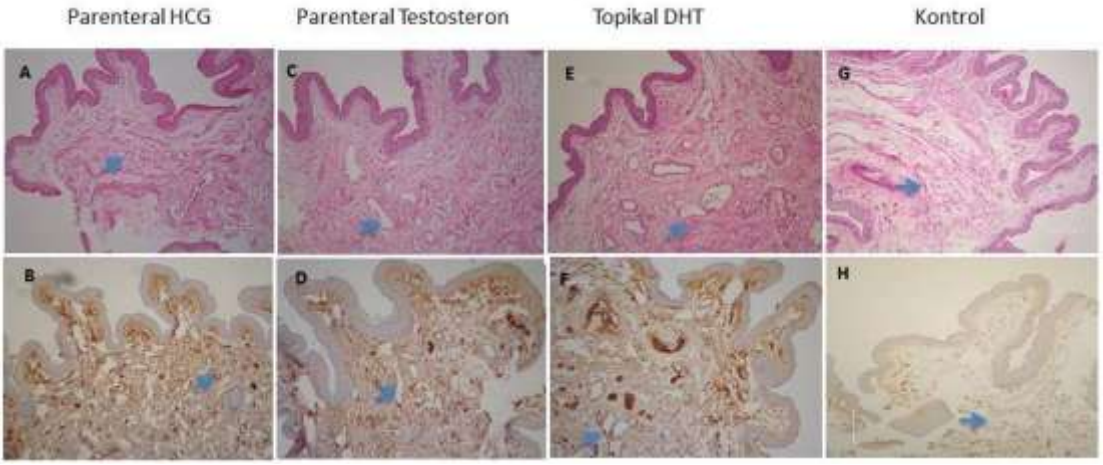


Figure 3. Evaluation of all groups in terms of vascular proliferation (H&Ex10, IHKx10)

In Figure 3, groups were evaluated for vascular proliferation and a small number of capillary vessels were observed in the control group (arrow), parenteral HCG, parenteral testosterone, and topical DHT groups showed increase in number of middle-sized vessels and dilatation (vessels are shown with arrows) (hematoxylin Eosin staining in the upper row and immunohistochemical staining with anti-vWF in the lower row.

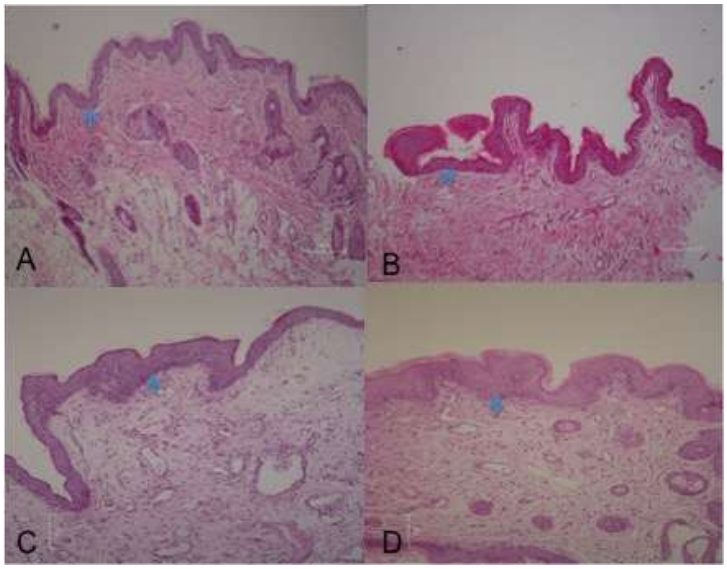
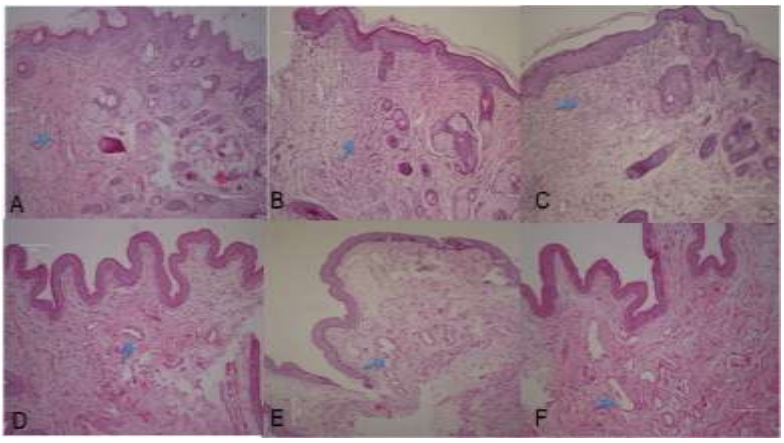


Figure 4. Evaluation of epithelial thicknesses of all groups (H&Ex10)

When the epithelial thickness is observed in all groups in figure 4; increased epithelial lining in groups of parenteral HCG (Figure 4B), topical DHT (Figure 4C), parenteral testosterone (Figure 4D) was observed compared to control group (Figure 4A).



Şekil 5. Evaluation of all groups in terms of fibrosis and inflammation (H&Ex10)

In Figure 5, samples prepared with H & E were evaluated for fibrosis and inflammation. In control group (Figure 5A, B, C); the severity of fibrosis and inflammation (indicated by arrows) in the first biopsies is increased compared to other biopsies. Figure 5A: inflammation and fibrosis score 1 (H&Ex10), Figure 5B: in the case of epithelium with dense crust on the surface; inflammation and fibrosis under the epithelium score 2 (H&Ex10), Figure 5C: inflammation and fibrosis score 3 (H&Ex10). In the parenteral HCG group (Figure 5D) inflammation and fibrosis score 1 (H&Ex10), In parenteral testosterone group (Figure 5E) inflammation and fibrosis score 0 (H&Ex10), In topical DHT group (Figure 5F) inflammation and fibrosis score 1 (H&Ex10).

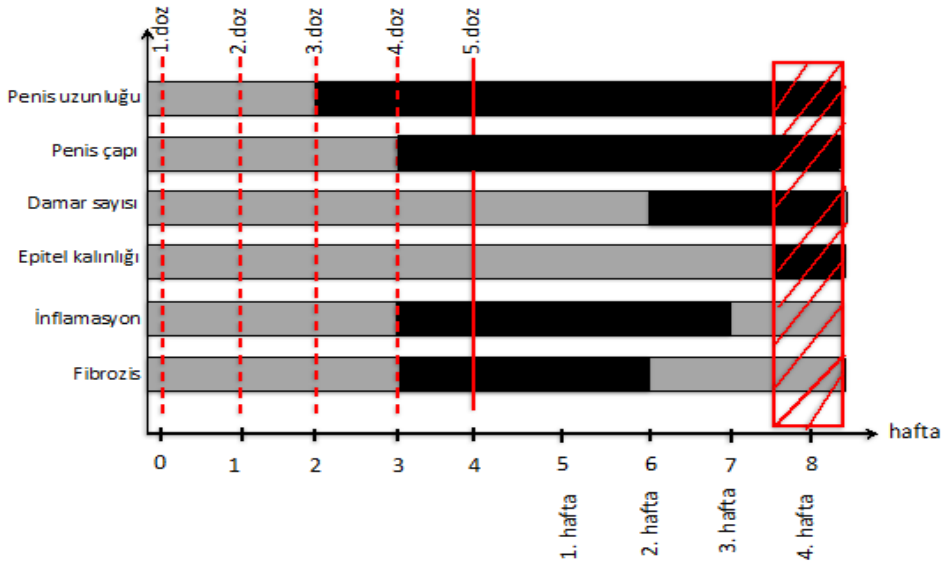


Figure 6. Changes in penile size and preputium biopsies with respect to time of rats to which parenteral HCG was administered.

(Drug applications were shown with a dotted line, the last drug application was shown with a red straight line, parameters similar with control group were shown with a gray line and weeks in which significant changes were seen according to control group were shown with black color. The time interval in which all values were at their best point was shown with scarlet area in red frame. Horizontal axis represents weeks, while vertical weeks represent the periods since the end of the drug.)

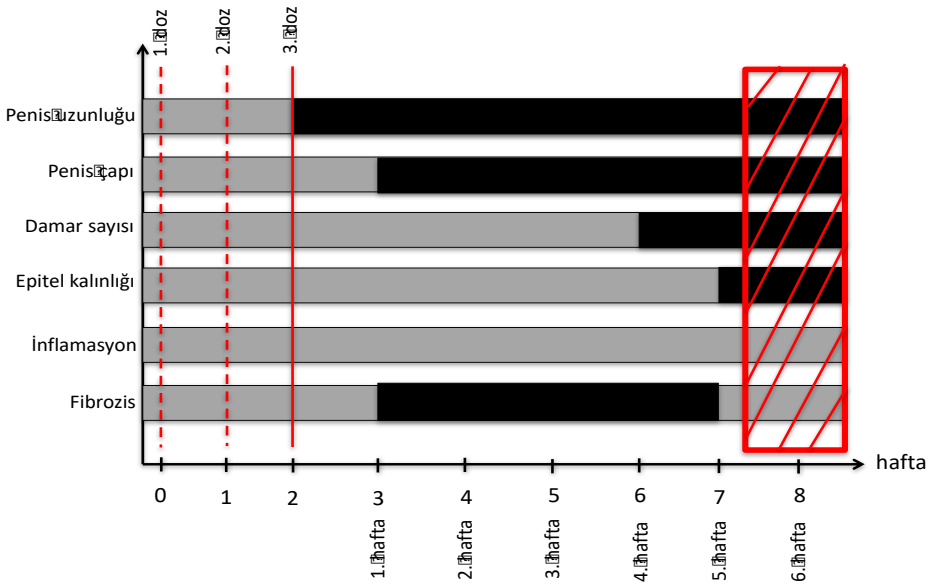


Figure 7. Changes in penile size and preputium biopsies of rats with respect to time to which parenteral testosterone was administered.

(Drug applications were shown with a dotted line, the last drug application was shown with a red straight line, parameters similar with control group were shown with a gray line and weeks in which significant changes were seen according to control group were shown with black color. The time interval in which all values were at their best point was shown with scarlet area in red frame. Horizontal axis represents weeks, while vertical weeks represent the periods since the end of the drug.)

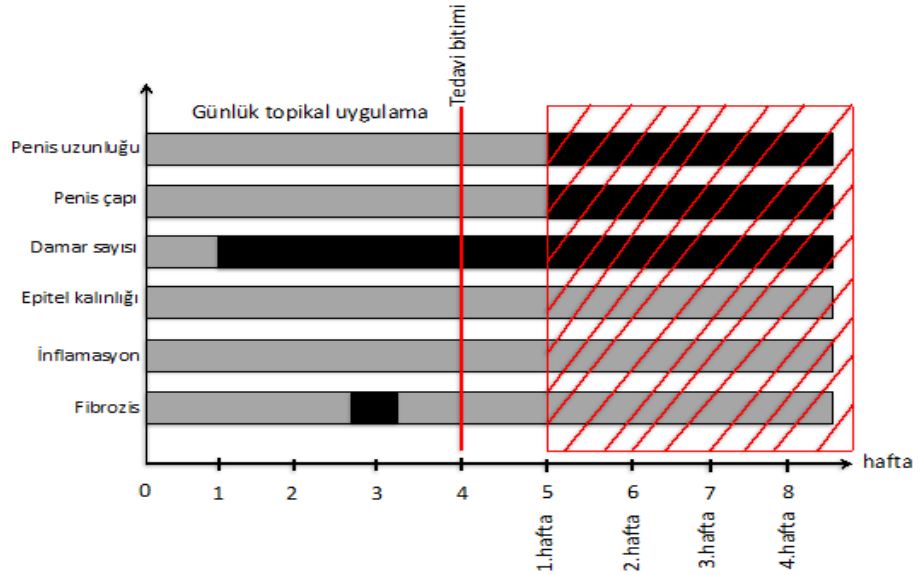


Figure 8. Changes in penile size and preputium biopsies of rats with respect to time to which topical DHT was administered.

(The last drug application was shown with a red straight line, parameters similar with control group were shown with a gray line and weeks in which significant changes were seen according to control group were shown with black color. The time interval in which all values were at their best point was shown with scarlet area in red frame. Horizontal axis represents weeks, while vertical weeks represent the periods since the end of the drug.)

4. DISCUSSION

Hormone stimulation with HCG, testosterone and DHT has been shown to increase penile length and glans size, warn neovascularization, facilitate surgical correction, reduce complication risk, and have tolerable side effects.[3,6,7,8] However, the choice of medications, doses, frequency of use and the timing of the surgery after HST are still controversial.[9,10,11]

In our study, the preparations used in clinical practice in HST were used at similar doses and frequency; changes they've made and the timing of the surgery to be applied after the end of the treatment were tried to be put forward. The ideal time for surgery is a common time interval in which penile length, diameter, vascularity and epithelial thickness increases and inflammation and fibrosis are minimum.

Although it is recommended that HCG should be given 1 or 2 times a week for a total of 5 weeks;[1,12] there are also clinicians who use a total of 6 doses over a 12-day period.[1,13,14] In our study, we administered HCG once a week for a total of 5 weeks.

It has been shown that penile length and diameter are increased, cord severity is decreased, cosmetic result is improved after repair, and complication rates are decreased in patients who have undergone preoperative HCG. Koff and Jayanthi found an increase in penile length from 38% to 150% (mean 94%) after preoperative 5-week HCG treatment in 12 children with hypospadias in a study conducted in 1999.[1] This increase was reported to occur in the proximal part of urethral meatus of the penis and that HCG response in children with hypospadias was very variable. In our study, we observed that HCG provides a 58% increase in penile diameter and an increase between 44% and 88% (mean 56%) for the length of the penis.

Studies with HCG are often compared with testosterone preparations, with a limited number of studies.[14,15,16] HCG and topical DHT (2%) were used preoperatively for 8 weeks and 4 weeks, respectively and topical testosterone was found to be superior in terms of surgical results and increase of penile tissue.[16] In our study, the superiority of topical DHT to HCG could not be demonstrated, the effects of parenteral testosterone on penile size and tissue quality were observed to be superior to HCG.

Increase in penile length, glans diameter, tissue quality, vascularization and size after parenteral testosterone treatment are expected changes. Use of vascularized and enlarged tissue in the preparation of free grafts and pedicle flaps used in the proximal hypospadias surgery facilitates surgery.[17]In parenteral testosterone administration, the treatment protocols are very variable, but response according to severity of hypospadias is also variable. It is known that in the testosterone treatment, adequate response is obtained in most of the cases up to 3 injections with 4 weeks interval. Depending on the size changes of the penis, there are also those who apply less than three doses.[7,18,19,20]

When Ishii et al. applied a fixed dose of testosterone (25 mg); the testosterone response was predicted to vary independently of the age, weight, and body surface area of the patient suggesting that this may be due to androgen receptor insensitivity.[21] In the clinical trial conducted by Snodgrass et al., It was necessary to increase doses up to 32 mg/kg according to the case of androgen insensitivity.[2] In our study, a total of three doses of testosterone (2 mg / kg / dose, once a week) were applied in the most common manner (2 mg / kg / dose, once a week) due to the use of healthy subjects (non- hypospadias) and having hormone responses in follow-up.

In the prospective, randomized trial of Asgari et al., in 2015; Children with proximal and midshaft hypospadias were given parenteral testosterone treatment once a month for 2 months and surgical repair was performed 1 month after the 2nd dose application.[7] Penile length and penile diameter were monitored for a total of 3 months from the beginning of the study and it was seen that penile growth was continued 1 month after the second dose of testosterone administration. At 3 months after the application; a total increase of 35% and 29% was achieved in the penile length and diameter of the cases, respectively. Gearhart and Jeffs also found a 50% increase for penile length in patients who received 2 doses of parenteral testosterone with 3 week intervals.[19] In our work an increase of 62% and 59% was observed in penile length and diameter, respectively in the i.m testosterone group. In many studies, the effect of testosterone on penile length was reported at 3-5 weeks and lasted for up to 3-12 months.[3,19,20,22] In our study, similar to clinical studies, maximum increase in penile length was observed after second testosterone application and this increase continued for 3 weeks after the end of the application. This finding supports that it should be waited for a while after hormone treatment.[7,9,15]

Bastos et al., showed in a group of patients who used preoperative daily topical 1% testosterone propionate that; there was increase in the number of blood vessels and increase in blood volume density when they made Immunohistochemical staining of post-operatively removed preputium biopsy with von Willebrand Factor (vWF).[6] After topical testosterone administration in the rat transplant model of circumcision skin, immunohistochemical staining for vWF showed marked increase in vascular density and less accumulation of collagen.[5] Another study showed reduced inflammation and fibrosis which was thought to be due to neovascularization in VEGF-treated subjects.[23] In our study, a 109% increase in the number of vessels in the prepusium biopsies was detected in the parenteral testosterone group. This increase was about 5 times that of the control group and was statistically significant. In topical DHT, this increase was 100%.

It was predicted that bleeding will increase during surgery due to increased vascularity in HST treated patients.[5,24] In our study, an increase in the amount and duration of hemorrhage after biopsy was observed in all HST-applied rats. These rats should be followed-up after 3rd-4th weeks for bleeding after procedure. After the biopsy was taken, there was usually no

bleeding in the rats in the control group. In a few subjects with bleeding, it was observed that the amount of bleeding was very small and stopped shortly.

The positive effects of HST on inflammation, fibrosis and wound healing have been demonstrated in studies. In an experimental model, in which human preputium was transplanted into rabbits and topical DHT was applied, vWF staining was increased and fibrosis was decreased.[5] According to this study; better circulation means better oxygenation. Decreased inflammation and fibrosis in well-oxygenated tissues are expected and exaggerated scar formation is reduced.[6,23] In our study, inflammatory scores of parenteral testosterone administration group were lower than control group, but this difference was not statistically significant. Fibrosis was low in the early period of the study and was similar to the control group 6 weeks after the last testosterone administration. In the topical DHT, inflammation findings were similar to the control group, whereas fibrosis was higher in the third week of study. Similar findings with the control group were obtained in the other phases of the study. In addition to studies showing positive effects of HST on inflammation and fibrosis, there are studies that claim to have adverse effects. Gilliver et al. argued that testosterone preparations negatively affected the skin repair process, inhibited re-epithelization and increased early fibrosis, and that estrogen was more successful in wound healing.[26,27] Hassan et al. have shown that after the hypospadias model was constructed; the onlay grafted rats showed increased inflammation and fibrosis when topical or parenteral testosterone was administered early in the postoperative period.[25] In the Gorduza study, they observed that the complication rates were lower in untreated patients when the complication rates of patients who received testosterone and / or HCG and did not receive hormone stimulation were examined. This result has been associated with increased inflammation. However, this observation was not statistically significant.[13]

There are studies showing that topical DHT improves the success of the surgery and significantly reduces complications.[6,18,20,24] Topical forms of DHT gelatin 2.5-3% are applied on the penile shafts and glans 1-2 times per day.[24] The duration of application ranges from 3 weeks to 3 months. It is recommended to wait at least one month for surgery after DHT treatment.[6,7,24] Chalapathi et al compared the topical and parenteral forms of testosterone in a clinical trial with a protocol which was similar to the protocol we used in our study. In this study, a 75% length increase in the parenteral testosterone group and a 60% increase in the topical DHT group were observed for the penile length. No statistically significant difference was observed between two groups.[20] In our study, an increase of 62% in the parenteral testosterone group and 46% in the topical DHT group was found for penile length of the rats and no statistically significant difference was observed between two groups.

When topical DHT administration and parenteral testosterone administration were compared; although similar results were obtained in terms of penile length and vascularization, increased penile diameter, increased epithelial thickness and fibrosis suppression in parenteral therapy were more evident. It can be said that topical DHT is more effective because of the positive effects of parenteral testosterone on tissue quality.

Surgeons who apply HST have a waiting period ranging from 1 to 24 months for surgical repair after HST, but the general trend is to wait for 4-12 weeks.[3,4,15,18] Similarly in our study; increased penile size to the ideal level, increased vascularity, improved temporary suppression of inflammation and fibrosis were detected to occur at 4 weeks after drug application in HCG group, 5-6 weeks in parenteral testosterone group and 1 week in topical DHT group.

The biggest shortcoming of clinical trials is the lack of standardization. The absence of a well-defined appropriate experimental model also limits the performance of experimental studies. Few experimental studies are available in the literature. Weaknesses of our work: Our experimental model is not a hypospadias model. It is known that a significant proportion of proximal hypospadias patients have androgen receptor insensitivity. An endocrine normal rat may not fully represent the hypospadias patient with androgen receptor insensitivity.

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