# THE INFLUENCE OF HCG TREATMENT ON TESTICULAR APOPTOSIS AND FERTILITY INDEX IN AN EXPERIMENTAL CRYPTORCHIDISM MODEL

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## ABSTRACT

• ***Objective:*** *Histopathological outcome of hCG treatment and the correlation of testicular apoptosis caused by hormone treatment with fertility potential were evaluated in an experimental cryptorchidism model in rats.*

• ***Material and Method:*** *Three main groups of healthy, male, Sprague-Dawley rats, aged 22 days were were divided into three main groups Sham-operated (SO), experimental cryptorchidism (EC), and hormone treated EC groups (HT). Each main group was also divided into early and late orchidectomy groups to investigate early and late changes in the testis. Each group contained 10 rats. Eight and 30 days after first surgery, left orchidectomy was performed in all rats. All groups were compared to each other in terms of apoptosis (AI) and fertility index (FI). The relationship between AI and FI was evaluated with Pearson correlation coefficient.*

• ***Results:*** *AI in HT group was lower than EC group in both periods; the difference was statistically significant in late period while it was not so in early period.*

*FI in both EC and HT groups was significantly smaller than SO group in late period. Although FI was numerically smaller in HT than EC group in early and late periods, the difference was statistically insignificant. No consistent correlations between AI and FI could be shown.*

• ***Conclusion:*** *No significantly increased negative effect, neither a beneficial effect of hCG treatment in terms of AI and FI respectively could be shown in our study. Also, our findings suggest that AI at a certain time point is not a reliable indicator of future fertility potential of that individual.*

• ***Key Words:*** *Cryptorchidism, hCG, hormone treatment, apoptosis, fertility* ***Nobel Med 2010; 6(3): 73-78***

## DENEYSEL BİR KRİPTORŞİTİZM MODELİNDE HCG TEDAVİSİNİN TESTİKÜLER APOPTOZİS VE FERTİLİTE İNDEKSİ ÜZERİNE ETKİSİ


## ÖZET

• **Amaç:** Farelerdeki deneysel bir kriptorşitizm modelinde, HCG tedavisinin histopatolojik sonuçları ve hormon tedavisinin neden olduğu apoptozisin fertilite potansiyeli ile korelasyonu değerlendirildi.

• **Materyal ve Metod:** 22 günlük, sağlıklı, erkek, Sprague-Dawley fareleri ile 3 grup oluşturuldu: Sham operasyonu (SO), deneysel kriptorşidizm (DK) ve hormonla tedavi edilmiş DK grubu (HT). Her ana grup ayrıca testislerdeki erken ve geç etkileri araştırmak için erken ve geç orşiektomi gruplarına ayrıldı. Her grupta 10 fare var idi. İlk cerrahiden 8 ve 30 gün sonra, tüm farelere sol orşiektomi yapıldı. Tüm gruplar bir diğeri ile apoptozis (AI) ve fertilite indeksi (FI) açısından karşılaştırıldı. AI ve FI arasındaki ilişki Pearson korelasyon katsayısı ile değerlendirildi.

• **Bulgular:** HT grubundaki AI hem erken hem geç dönemde DK grubundan daha küçüktü. Fark geç dönemde istatistiksel olarak anlamlı iken erken dönemde değildi. FI, DK ve HT grubunda SO grubundan geç dönemde belirgin olarak küçüktü. Bununla beraber FI, HT grubunda erken ve geç dönemde sayısal olarak DK grubundan küçüktü, fark istatistiksel olarak anlamlı değildi. AI ve FI arasında anlamlı bir korelasyon gösterilemedi.

• **Sonuç:** Çalışmamızda, HCG tedavisinin AI ve FI üzerine, artmış bir olumsuz ya da yararlı bir etkisi gösterilemedi. Ayrıca bulgularımız, belli bir anda ölçülen AI'nin, bu kişinin gelecekteki fertilite potansiyelinin güvenilir bir göstergesi olmadığını düşündürmektedir.



• **Anahtar Kelimeler:** Kriptorşidizm, hCG, hormon tedavisi, apoptozis, fertilite **Nobel Med 2010; 6(3): 73-78**


## INTRODUCTION

Cryptorchidism in children is treated with hormonal and surgical methods. However, hormone treatment with exogenous hCG (human chorionic gonadotropin) or exogenous GnRH or LHRH agonists has been questioned since it provides lower success rates than surgical treatment,[1] and there exists controversial reports in the literature concerning the sperm parameters of the patients who received hormones for cryptorchidism during their childhood.[2,3]

Apoptosis (programmed cell death) is a physiologic phenomenon in testis. More than half of the spermatogenetic cells are eliminated through apoptosis before they become spermatozoa during normal spermatogenesis.[3-5] Dunkel et al found 6-fold more apoptotic spermatogonia in the biopsy specimens of the children who received hCG treatment as compared with those who did not receive hCG.[6] Some authors suggest that increased apoptosis may be the mechanism of infertility in cryptorchidism.[7-9] It has also been shown that hCG treatment causes inflammation in human and rat testis.[6,10] On the contrary, there are other studies that have concluded that hormone treatment in cryptorchidism may improve fertility potential.[2,11] Current controversy on the outcome of hormone treatment warrants further studies on this subject.

In this study, the histopathological outcome of hCG treatment in experimental cryptorchidism in rats in terms of apoptosis and fertility index, the course of the effects in a time span, and the correlation of apoptosis with fertility index were evaluated in the same setting.

## MATERIAL and METHOD

The study was carried out on 60 healthy male Sprague-Dawley rats, aged 22 days between April and June 2007 after obtaining the local ethics committee approval. The temperature of the environment was kept at 24±2 °C, and periods of 12 hours of daylight and 12 hours of darkness were provided. Each cage contained 4 rats.

Three main groups of rats were formed; i.e. Sham-operated (SO), experimental cryptorchidism (EC), hormone treated EC groups (HT). Each main group was also divided into early and late orchidectomy groups to investigate early and late changes in the testis. Sixty rats were distributed evenly into these 6 groups (Table 1). The rats in hormone treatment group received SC injections of 50 IU/kg β-HCG daily for 7 days starting on the first postoperative day after EC or sham operation was done. The scrotum was examined daily to confirm the left testis was in the abdomen at all times in EC and HT groups. Eight and 30 days after surgery, left orchidectomy was performed in all rats to investigate the early and late changes in the testis respectively.

**Surgical Technique:** Anesthesia was achieved by administering the mixture of 40 mg/kg of ketamine and 10 mg/kg of Xylocaine intraperitoneally. Through→

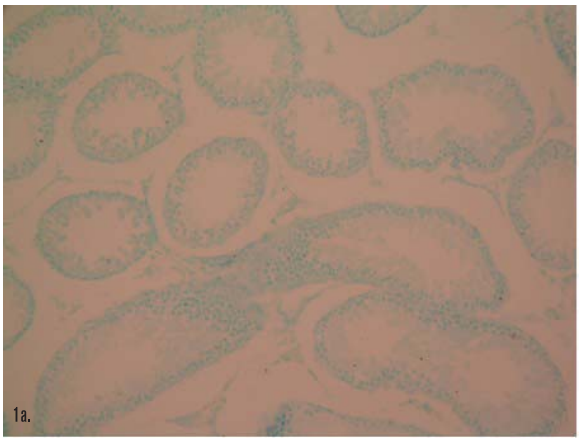
a 1-cm incision, left testis was freed completely and placed in the abdomen in EC and HT groups; and delivered out of the incision and replaced in the scrotum only after manipulating it in SO group. Inguinal canal was suture-closed in EC and HT groups. Rats were kept alone in the cages until they are fully awake after the surgery. Orchidectomy was also performed under anesthesia, and paraffin-embedded blocks were prepared from the specimens.

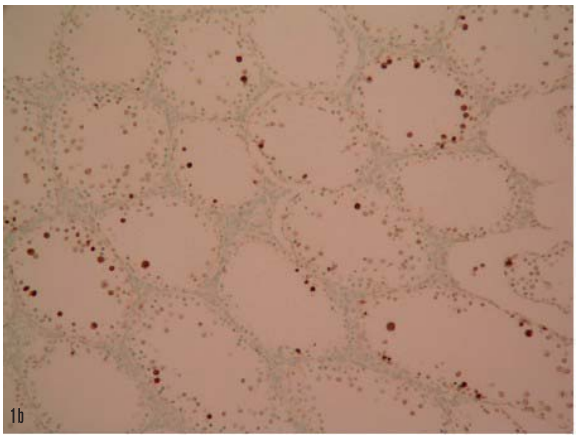
Histopathologic examination: Immunohistochemical (IHC) staining and hematoxylin-eosin were used to show apoptotic cells and spermatogonia, respectively. Apoptotic cells were labelled using TUNEL technique (ApopTag® Plus Peroxidase, In Situ Apoptosis Detection Kit, Chemicon International, Temecula, CA 92590) as in previous studies in the literature.[8,9,12,13] Two sections of 3-micrometer thickness from the paraffin-embedded blocks were laid on positive charged slides for each subject; one for negative control and the other for the test. Breast tissue of an adult breast-feeding Spraque-Dawley rat was removed 4 days after ablactation, used as the positive control specimen for apoptosis. IHC staining was completed following the instructions of the TUNEL kit. Apoptotic cells within 50 seminipherous tubules that were perpendicular to the cross-sectional area were counted, and the number of apoptotic cells per tubule was defined as apoptosis index (AI) (Figures 1a to 1c).[8,14] Positive control specimen was stained while the negative control specimen was not, which confirmed the accuracy of our technique.

Spermatogonia within 50 seminipherous tubules were counted on slides prepared from hematoxylin-eosin stained samples, and number of spermatogonium per tubule was defined as fertility index (FI).[15]

**Statistical Method:** Three main groups were compared to each other in terms of AI and FI; furthermore, each main group was also evaluated in terms of the differences between early and late periods. The relationship between AI and FI was evaluated with Pearson correlation coefficient.

The study data were processed using the SPSS (Statistical Package for Social Sciences) for Windows 11.0 software. ANOVA, Post-Hoc Scheffe test, paired samples t test for normally-distributed data, and Kruskal Wallis Variance Analysis, Mann-Whitney U test and Wilcoxon test were used for the data that were not normally-distributed. Results were evaluated in 95% confidence interval (CI) and threshold for significance was $p<0.05$. Correlations were evaluated with Pearson correlation coefficient. The significance of correlation coefficient was also assessed at 95% CI with $p<0.05$, and at 99% CI with $p<0.01$. →



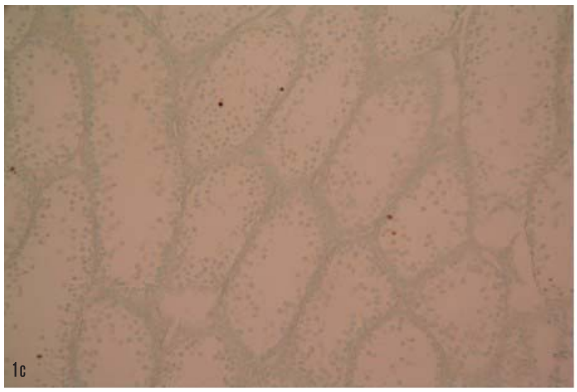




**Figure 1.** TUNEL staining for apoptosis. (Magnification for all photomicrographs is 100x) **1a.** Negative control slide. **1b.** Experimental cryptorchidism group (late period) **1c.** Sham-operated testis (late period)

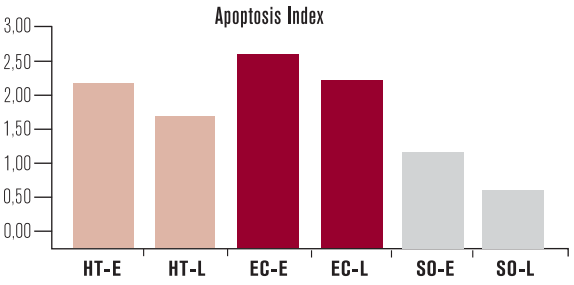



**Figure 2.** Mean AI values of all groups.

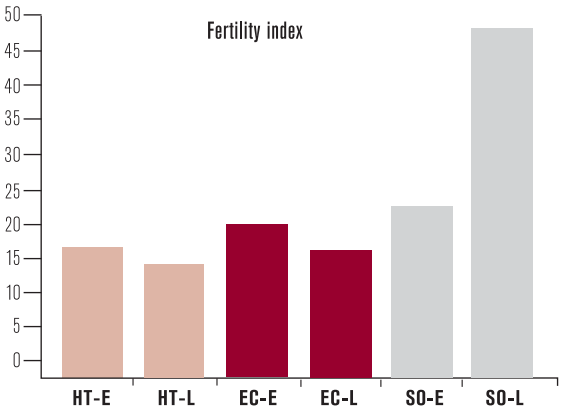



**Figure 3.** Mean FI values of all groups.

**Table 1:** Study groups

| Early orchidectomy (8th day after experimental cryptorchidism) | Late orchidectomy (30th day after experimental cryptorchidism) |
|---|---|
| SO-E: Control (Sham operated) n:10 | SO-L: Control (Sham operated) n:10 |
| EC-E: Experimental cryptorchidism n:10 | EC-L: Experimental cryptorchidism n:10 |
| HT-E: Experimental cryptorchidism+hCG n:10 | HT-L: Experimental cryptorchidism+hCG n:10 |

**Table 2:** The statistical analysis of apoptosis index data

| | | | | p ‡ (Comparison between groups) | | |
|---|---|---|---|---|---|---|
| | HT | EC | SO | HT-EC | HT-SO | EC-SO |
| Early | 2.29±0.66 | 2.75±0.31 | 1.25±0.27 | 0.1055 | 0.0001* | 0.0001* |
| Late | 1.66±0.33 | 1.99±0.24 | 0.75±0.16 | 0.0362* | 0.0001* | 0.0001* |
| p† (Change in time) | 0.04* | 0.0001* | 0.0001* | | | |

*: statistically significant. †: paired samples t test ‡:ANOVA (Post Hoc Scheffe Test)

## RESULTS

Two rats in EC-E group died of anesthesia, and 1 rat in EC-L group died on the 15th postoperative day after the first surgery due to some unknown reason. No other early or late complications were observed. AI data of all groups and the corresponding p values are shown in Table 2.

AI was significantly greater in EC and HT groups than SO group in both early and late periods. AI in HT group was smaller than EC group in both periods; the difference was statistically significant in late period while it was not so in early period. AI was found to be significantly decreased with time in all 3 groups (Fig 2).FI data and the corresponding p values are shown in Table 3. FI was found to be increased significantly in SO group with time; however it decreased in EC and HT groups. FI in both EC and HT groups was significantly smaller than SO group in late period. Although FI was numerically smaller in HT than EC group in early and late periods, the difference was statistically insignificant (Fig 3).

The correlation coefficients between AI and FI and the corresponding p values are shown in Table 4. Insignificant negative and positive correlations between AI and FI were observed in early and late periods in SO and EC groups respectively. In HT group, positive and negative correlations were found in early and late periods respectively; the latter was statistically significant while the former was not.

## DISCUSSION

There are studies in the literature that suggest hormone treatment increases germ cell apoptosis in testis.[6,16] Heiskanen et al published their well-designed study with interesting results.[16] They took bilateral testis biopsies during orchidopexy in cases who received and did not receive hormone treatment prior to surgery, and stratified their findings on spermatogonial apoptosis according to the time passed after hormone treatment until the biopsy was taken, which, in turn, revealed that spermatogonial apoptosis increased first and returned to basal levels later on. Although it has been suggested that hCG causes acute inflammation-like effects in the testis, and this may be a negative impact on the future testicular function,[10] Karaman et al found that hormone treatment interfered with the histology of seminipherous tubule at the early stage, but this was reversible.[17] In our study, AI in all groups decreased significantly in time; interestingly, AI in HT was smaller than that of EC in both early and late periods, the latter being statistically significant. This finding may suggest that hormone treatment may actually have an effect that prevents or decreases apoptosis in EC model; at least hormone treatment might not be causing more apoptosis than EC alone does. The fact that AI in all groups including the SO group was greater in the early period may be explained by anesthesia stress, and that apoptosis in the early period of life may be increased physiologically. However, it is obvious that AI in both EC and HT groups were significantly greater than SO group in both early and late periods, which indicates an added influence of experimental cryptorchidism. A causal relationship between apoptosis and cryptorchidism has been suggested by other studies,[7,9] and this has been considered as a possible mechanism for infertility in adulthood;[18] such that, an increased apoptosis status may lead to an increased elimination of spermatogonia thereby leading to infertility later on.

On the other hand, it has been suggested that gonadotropin treatment increases fertility potential by→

facilitating gonocyte-Ad spermatogonium transformation, thereby increasing the fertility potential of that individual by increasing the number of the cells in the adult spermatogonium pool.[19] Hadziselimovic et al investigated the semen analyses of a group of adults who underwent orchidopexy for undescended testis and a simultaneous testicular biopsy. They showed that those who received hormone treatment after the surgery had better semen parameters (number of spermatozoa, motility, the percentage of morphologically normal sperms) than those who were treated by surgery alone.[2] Although this is a very well-designed study, the number of cases is small, and its conclusion has not been corraborated by others yet. In a more recent study, it has been found that fertility index on the biopsy specimen of the patients with undescended testis who received neoadjuvant GnRH treatment was significantly higher than those treated with orchidopexy alone, and this beneficial effect was more prominent when gonadotropin treatment was given earlier.[11]

In order to evaluate the link between apoptosis and fertility, FI was assessed in the same setting as AI in our study. FI decreased in EC and HT groups (p>0.05) while it increased in SO group (p=0.0001), as one might expect it to be. It can be speculated that there should be a negative correlation between AI and FI, if there was a causal relationship between apoptosis and infertility, that is, the greater the AI is, the smaller the FI should get. Nonetheless, no consistent correlation between the 2 parameters could be revealed in our study. In SO group, which may be considered as healthy controls, there were negative correlations between AI and FI in both periods, yet, statistically insignificant. Furthermore, a positive correlation was found, although statistically insignificant, between the 2 parameters in HT-L group. Our findings suggest that AI at a certain time point is not a reliable indicator of future fertility potential of that individual. The number of subjects in our study might have been insufficient to reveal an existing correlation between AI and FI. As a matter of fact, apoptosis is a physiologic phenomenon and it can be influenced by a number of factors. We suggest that apoptosis might be a dynamic process that it could be detected at different rates within a relatively short time frame. Certainly, this possibility needs to be investigated.

Although FI appears to be numerically smaller in both HT-E and HT-L groups than the corresponding EC groups, the difference was statistically insignificant. Therefore, no significant negative impact of hCG treatment on FI, that was more than that of EC alone, neither a positive effect could be shown in our study. It should be remembered that our design was based on an experimental cryptorchidism model in which not all of the mechanisms in the pathophysiology of the

**Table 3:** The statistical analysis of fertility index data

| | | | | p ‡ (Comparison between groups) | | |
|---|---|---|---|---|---|---|
| | HT | EC | SO | HT-EC | HT-SO | EC-SO |
| Early | 18.16±3.7 | 19.61±1.91 | 22.03±1.14 | 0.4641 | 0.0075* | 0.1313 |
| Late | 14.78±3.22 | 17.74±2.93 | 49.25±1.35 | 0.0779 | 0.0001* | 0.0001* |
| p† (Change in time) | 0.29 | 0.1 | 0.0001* | | | |

*: statistically significant. †: paired samples t test ‡:ANOVA (Post Hoc Scheffe Test)

**Table 4:** The correlations of apoptosis index and fertility index in early and late periods.

| | | Early period | | Late period | |
|---|---|---|---|---|---|
| | | Fertility index † | p | Fertility index † | p |
| Apoptosis index | HT | 0.130 | >0.05 | -0.842 | <0.01(*) |
| | EC | 0.118 | >0.05 | 0.435 | >0.05 |
| | SO | -0.510 | >0.05 | -0.410 | >0.05 |

†: Pearson Correlation Coefficient *Correlation Coefficient highly significant (p<0.01).

congenital cryptorchidism in humans were involved. In our study, basically the untoward effects of gonadotropin treatment with regard to the AI and FI were investigated in a testis that had descended normally, and then placed surgically in the abdomen in order to mimick the physical environment of an undescended testis. Therefore, ours is a drug safety study rather than a clinical outcome measure.

On the other hand, Lee et al, in their study investigating the relationship between unilateral cryptorchidism and infertility, reported that a considerable number of patients with unfavorable semen parameters achieved paternity.[20] Obviously, fertility potential and paternity are different concepts, and care must be taken when interpreting the results of a study investigating fertility potential, as in our study, and it should be remembered that every individual with unfavorable semen parameters may not necessarily be infertile; that is to say, researchers should not keep from investigating the beneficial effects of hormone treatment in cryptorchidism merely relying on the suggestions based on the studies that report hormone-related untoward effects, which are probably transient, in order not to forfeit the overall potential gain.

## CONCLUSION

The negative effects of experimental cryptorchidism on the testis in terms of AI and FI have been observed in our study. However, no significantly increased negative effects of hCG treatment could be shown. As for the prognostic capability of AI, our findings suggest that→

AI at a certain time point is not a reliable indicator of future fertility potential of that individual. Clinical studies in large series consisting of subjects with congenital cryptorchidism could provide accurate data on the clinical outcome of hormone treatment in cryptorchidism in terms of fertility.

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