Ethane 1,2-Dimethane Sulphonate (EDS) and Unilateral Cryptorchidism (UNICR): Two Experimental Models in Investigation of Cellular Interactions in Testis

ETAN 1,2-DİMETAN SÜLFONAT (EDS) VE UNILATERAL KRİPTORŞİDİZM (UNİKR): TESTİSTEKİ HÜCRESEL ETKİLEŞİMLERİN ARAŞTIRILMASINDA İKİ DENEYSEL MODEL

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Summary

We aimed to investigate the cellular interactions in adult rat testis, using human chorionic gonadotrophin (HCG) besides the experimental models of ethane 1,2-dimethane sulphonate (EDS), a specific Leydig cell toxicant, and unilateral cryptorchidism (UNICR). The groups were as follows: UNICR induced only (Groups 1,11,111), UNICR induced 3 days after EDS injection (75 mg/kg) (Groups 1,3,5) and UNICR induced after HCG (Pregnyl, Organon) administration (100 IU/day) for 5 consecutive days (Groups 2,4,6). Animals were sacrificed at 7th (Groups 1,1,2), 14th (Groups 11,3,4) and 28th (Groups 111,5,6) days and their testes were histologically examined. While tubular and interstitial appearances were intact in scrotal testes, tubular degenerations and Leydig cell hyperplasia progressing depending on the time were seen in abdominal testes of UNICR induced groups. Wlxile tubular and interstitial appearances were intact in scrotal testes, tubular degenerations and Leydig cell hyperplasia were seen in abdominal testes of UNICR induced groups. In EDS+UNICR induction, tubules were generally intact besides additional scarce degenerative cells in scrotal testes on day 7 and 14, however, degenerative tubules in vaiying degrees were seen on day 28. In the abdominal testes of the same groups, diffuse tubular atrophy was observed and damaged Leydig cells and occational picnotic cells were encountered in interstitium. While tubular and interstitial appearances were intact in scrotal testes, tubular degenerations in vaiying degrees and Leydig cell hyperplasia progressing depending on the time were seen in abdominal testes ofHCG+UNICR induced groups. UNICR and HCG+UNICR had no effect on mast cells, however, EDS lead to the accumulation of these cells in peripheric and even in central interstitium. EDS was found to provoke the degenerative effects of UNICR in scrotal and abdominal testes. It was concluded that gonadotrophin administration is not enough for cellular proliferation and differantiation in testis and local factors could play role in these processes.

Key **Words:** Ethane 1,2- dimethane sulphonate, Unilateral cryptorchidism, Rat

T Klin J Med Res 1999, 17:80-86

Received: March 11, 1999

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Özet

Spesifik bir Leydig hücresi toksikantı olan ethane 1,2-dimethane sulphonate (EDS) ve unilateral kriptorşidizm (UNİKR) modelleri yanısıra insan koryonik gonadotropini (HCG) kullanılarak erişkin sıçan testisindeki hücresel etkileşimlerin araştırılması amaçlandı. UNİKR oluşturulan, EDS enjeksiyonundan (75 mg/kg) 3 gün sonra ve 5 gün ardarda HCG uygulandıktan sonra (100 IU) UNİKR uygulanan denekler 7, 14, 28 günlük periyodlarda sakrifiye edildi. UNİKR uygulamasında skrotal testislerdeki tubuler ve interstisyel görünüm normal iken abdominal testislerde zamana bağlı olarak ilerleyen tubuler dejenerasyon ve Leydig hücrelerinde hiperplazi görüldü. EDS+UNİKR uygulamasında skrotal testislerde 7 ve 14'üncü günlerde nadiren dejenere hücreler yanında genelde tubuller normaldi. 28. Günde değişik derecede dejenere tubuller bir aradaydı. Bu grupların abdominal testislerinde diffüz tubuler atrofi gözlendi, interstisyumda Leydig hücresi harabiyeti vardı ve yer yer piknotik hücrelere rastlandı. HCG+UNİKR uygulamasında skrotal testislerde tubuler ve interstisyel görünümler normalken abdominal testislerde değişik derecede tubuler dejenerasyonlar ve Leydig hücrelerinde zamana bağlı olarak artan hiperplazi gözlendi. UNİKR tek başına mast hücrelerini etkilemezken EDS bu hücrelerin periferik ve yer yer sentral interstisyumda sayıca artmasına yol açtı. HCG uygulamasında skrotal ve abdominal testislerde mast hücre akümülasyonu görülmedi. EDS"nin skrotal ve abdominal testislerde UNİKR'nin dejeneratif etkilerini provake ettiği saptandı. Sonuç olarak gonadotropin uygulamasının tesliste hücresel proliferasyon ve farklılaşma için yeterli olmadığı ve burada muhtemelen lokal faktörlerin rol oynadığı sonucuna varıldı.

Anahtar Kelimeler: Etan 1,2- dimetan sülfonat, Unilateral kriptorşidizm, Sıçan

T Klin Araştırma 1999, 17:80-86

Evidence for local control of testicular function has evolved with the use of animal models of testicular damage (1). Among the models, the most popular ones are Ethane 1,2-Dimethane Sulphonate (EDS) treatment and unilateral cryptorhcidism (UNICR).

EDS has long been recognised to exert an unusual pharmacological action upon rat testis resulting in a temporary period of sterility 2-3 weeks after a single treatment (2, 3). In contrast to the infertility actions of other simple diesters of methane sulphonic acid, the antispermatogenetic effect of EDS is not attributable to the arrest of germ cell proliferation, but rather it appears to selectively impair the function of Leydig cells (2, 4). Primary biological effect of EDS appears to be limited primarily to Leydig cells, and resulting reversibl infertility can be prevented by prolonged administration of Luteinizing Hormone (LH) or Human Chorionic Gonadotrophin (HCG) (5). Such prolonged treatment of rats with HCG produces a significant rise in the volume of lipid droplet compartment of Leydig cells, along with a notable enhancement in their steroidogenetic capacity (6-8).

The induction of UNICR has long been recognised to disrupt spermatogenesis, which in turn is thought to alter, via local interactions, the morphology and secretory function of the Leydig cells (9, 10). The unilaterally cryptorchid rat model has special significance for the study of this paracrine interaction since, unlike the bilaterally cryptorchid model, changes in the abdominal testis can be directly compared with the scrotal testis in the same animal (9). The demonstration that some changes of Leydig cells occurred in the damaged testes in the unilateral models of spermatogenetic damage, whilst leaving the Leydig cells in the contralateral untreated testis unchanged, suggested a paracrine mechanism of regulating Leydig cell function (1).

The aim of the present study was to investigate the importance and efficiency of endocrine and paracrine regulation on cellular interactions between the tubular and interstitial compartments in rat testis, using EDS and UNICR treatments (with the durations of 7, 14, 28 days) along with HCG administration.

Materials and Methods

Adult male Wistar rats (BW, 300-350 g) were kept under standard animal house conditions and allowed food and water ad libitum. Fourty five rats (n:5) were equally divided into nine groups. The groups were as follows: UNICR induced only (Groups I,II,III), UNICR induced 3 days after EDS injection (75 mg/kg) (Groups 1,3,5) and UNICR induced after HCG (Pregnyl, Organon) administration (100 IU/day) for 5 consecutive days (Groups 2,4,6). Animals were sacrificed at 7th (Groups 1,1,2), 14th (Groups 11,3,4) and 28th (Groups 111,5,6) days and their testes were histologically examined. EDS was prepared in laboratory conditions as described previously (4) and administered by a single intraperitoneal injection. UNICR was induced surgically under ether anaesthesia by gently relocating the left testis into the abdomen, as described by Kerr et al. (10). The design of the experiments and the groups involved are summarized in Table 1. After cervical dislocation, scrotal and ab-

Table 1. The groups that induced UNICR, EDS plus UNICR, and HCG plus UNICR. (X: The days at which the animals sacrificed)

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7	Day 14	Day 28
Groupl	UNICR						X		
GroupII	UNICR							X	
GroupIII	UNICR								X
Group 1		EDS				UNICR	X		
Group2	HCG	HCG	HCG	HCG	HCG	UNICR	X		
Group3		EDS				UNICR		X	
Group4	HCG	HCG	HCG	HCG	HCG	UNICR		X	
Group5		EDS				UNICR			X
Group6	HCG	HCG	HCG	HCG	HCG	UNICR			X

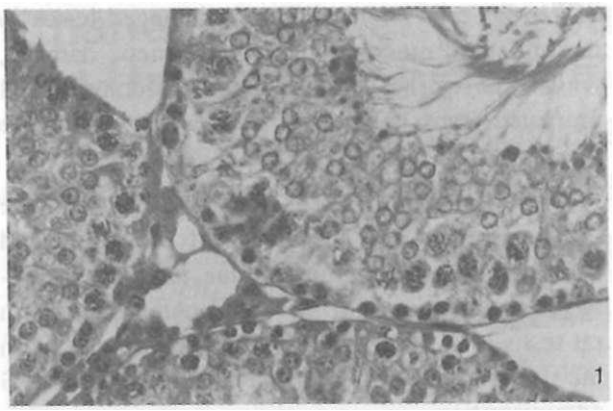


Figure 1. The micrograph taken from group III (UNICR 28 days) shows intact seminiferous tubules and interstitium in scrotal testis. (H&E, X 132).

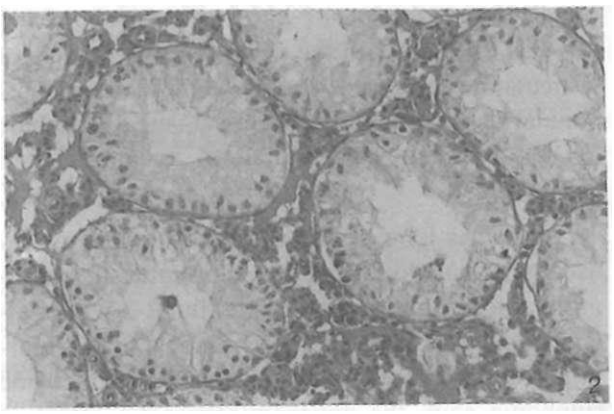


Figure 2. Degenerative seminiferous tubules and Leydig cell hyperplasia in interstitium of the abdominal testis of Group HI . (UNICR 14 days). (H&E, X 66).

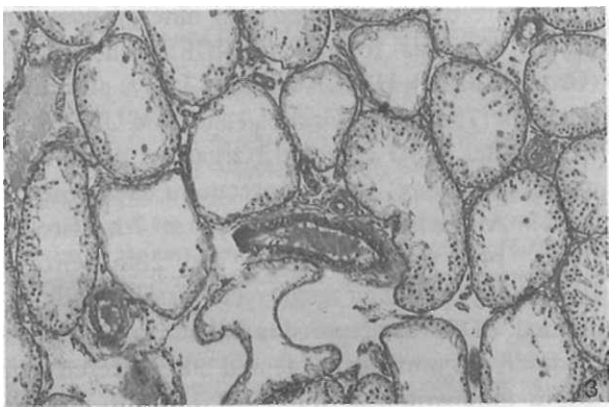


Figure 3. Degenerated and atrophic tubules that have thickened basement membranes in abdominal testis of Group 5 (EDS+UNICR 28 days). (PAS&H, X 33).

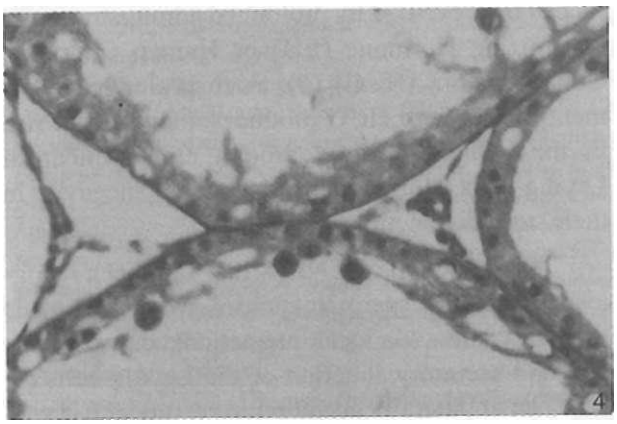


Figure 4. Eosinophilic degenerative cells and multinuclear giant cells in atrophic tubules and the interstitium devoid of typical Leydig cells showing some picnotic interstitial cells in abdominal testis of Group 5(EDS+UNICR 28 days). (H&E, X 132).

dominal testes of all animals were fixed in Bouin's fluid. Paraffin wax sections (5(im) were stained with haematoxylin-eosin, periodic acid-Schiff reaction together with haematoxylin, Dominici techniques. Preparations were evaluated semiquantitavely with regard to tubular, interstitial and total degenerations (seminiferous tubules of normal to atrophic appearance: 0-4; Leydig cells of normal to hyperplasic appearance:0-3; interstitium of normal to infiltrated appearance showing widening and increase in cellular and interstitial structures, and mast cells of normal to incerased number in tunica vasculosa, peripheric and central interstitium:0-3).

Results

In UNICR groups (Group I, II, III), scrotal testes showed normal tubular and interstitial structures (Figure 1), while in abdominal testes there were tubular degenerations and Leydig cell hyperplasia progressing depending on the time (Figure 2). Despite scarcely seen degenerative cells on day 7 and 14, seminiferous tubules had healthy histological appearance in EDS plus UNICR groups (Group 1, 3, 5), however, on day 28 normal and degenerative tubules in varying degrees were observed all together. Additionally, eosinophilic degenerative cells and giant cells were seen in the lu-

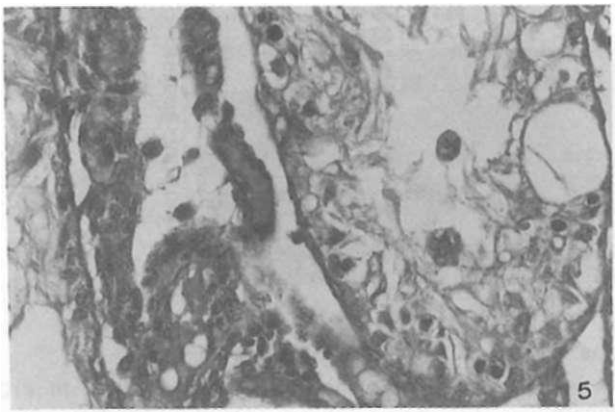


Figure 5. Multinuclear giant degenerative cells seen in the lumen of a seminiferous tubule in this tangential section of Group 1. (EDS+UNICR 7 days)(H&E, X 132).

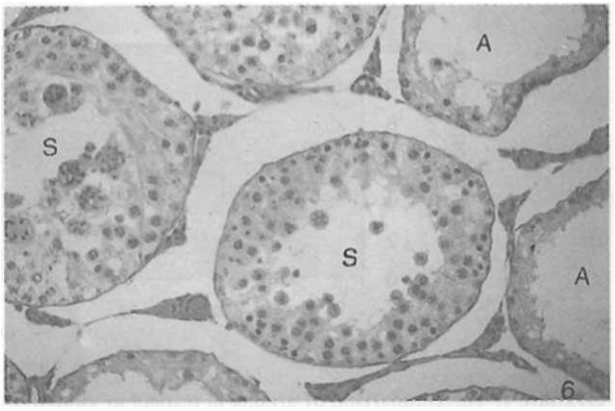


Figure 6. The tubules of atrophic appearance (A), and of decreased seminiferous epithelial thickness (S), besides multinuclear degenerative cells in Group 2 (HCG+UNICR 7 days). (H&E, X 66).

mina of tubules. Degenerative tubules showed thickened and ondulated basement membranes (Figure 3). Abdominal testes of these groups showed a diffuse tubular atrophy (Figure 4) and same eosinophilic degenerative cells and giant cells were accompanied (Figure 5) besides increased fibrillar and cellular structures in interstitium. Leydig cell destruction was the common finding in all scrotal and abdominal testes, and occasional picnotic interstitial cells were encountered (Figure 4). In HCG plus UNICR administered groups, the tubular and interstitial appearance was similar to those of groups I, II, and III, while tubular degenerations in varying degrees (desquamations, decreased epithelial thicknesses, atrophy, degenerative cells in tubules) (Figure 6) and Leydig cell hyperplasia increasing depending on the time were observed in abdominal testes. UNICR and UNICR plus HCG induced groups were found to be ineffective for mast cells, but EDS caused the accumulation and proliferation of these cells in peripheric and even in central interstitium (Figure 7). Semiquantitavely scored degenerations of all groups were compared in Graphics 1-4.

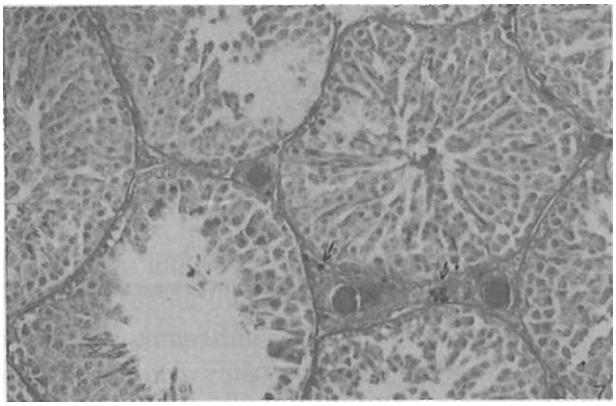


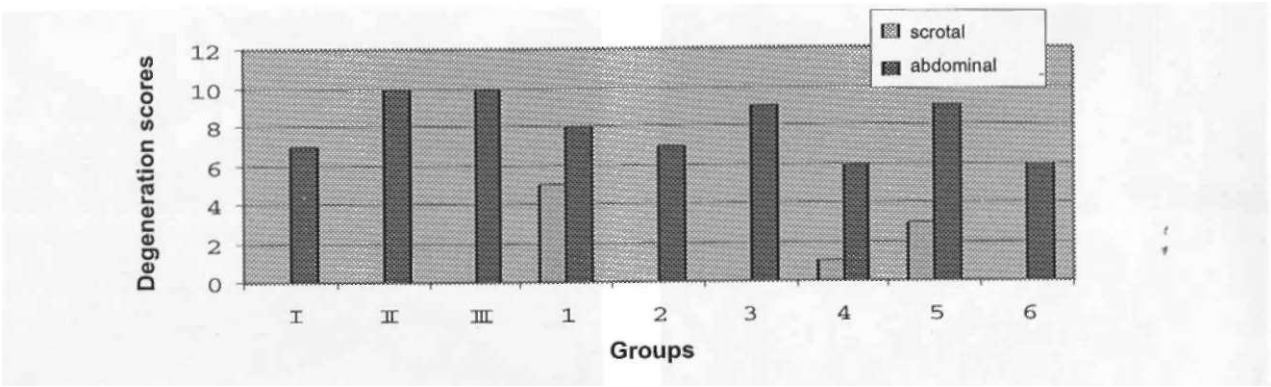
Figure 7. Mast cells in central interstitium (arrows) between the tubules of which epithelial thicknesses are decreased in scrotal testis. (Group 5) (dominici, X 66).

Discussion

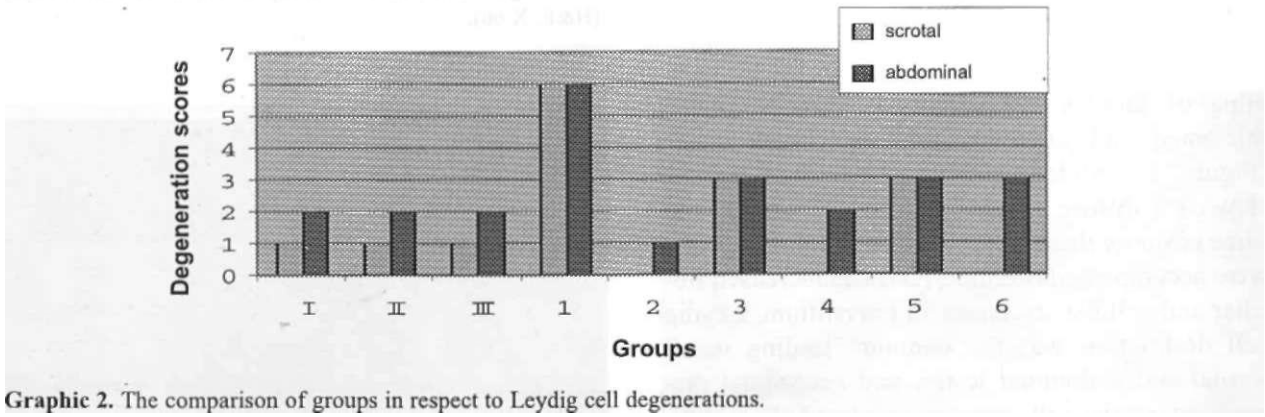
Among the wide range of chemicals that impair testicular function and induce infertility, ethane 1,2-dimethane sulphonate (EDS) is unique, since it appears to selectively and temporarily impair the function of Leydig cells without leading any systemic effect (5). Thus, EDS offers a unique opportunity to study the factors that control Leydig cell numbers and functions.

In UNICR model, interstitial and tubular changes in the abdominal testis can be directly compared with the scrotal testis in the same animal (9). The eosinophilic poligonal cells and multinuclear giant cells that we observed within degenerative tubules have been thought to be degenerative spermatids or fused spermatogenetic cells (11).

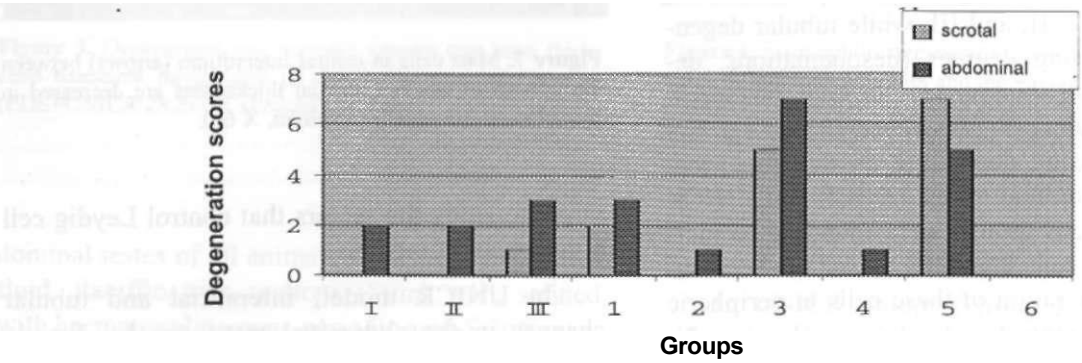
Heterogen degenerative appearance exhibited in some groups could be due to different stages of spermatogenetic cycle (12). According to our observations, depending on tubular atrophy increase in fibrillar and cellular structures, testicular intersti-



Graphic 1. The comparison of groups in respect to tubular degenerations.

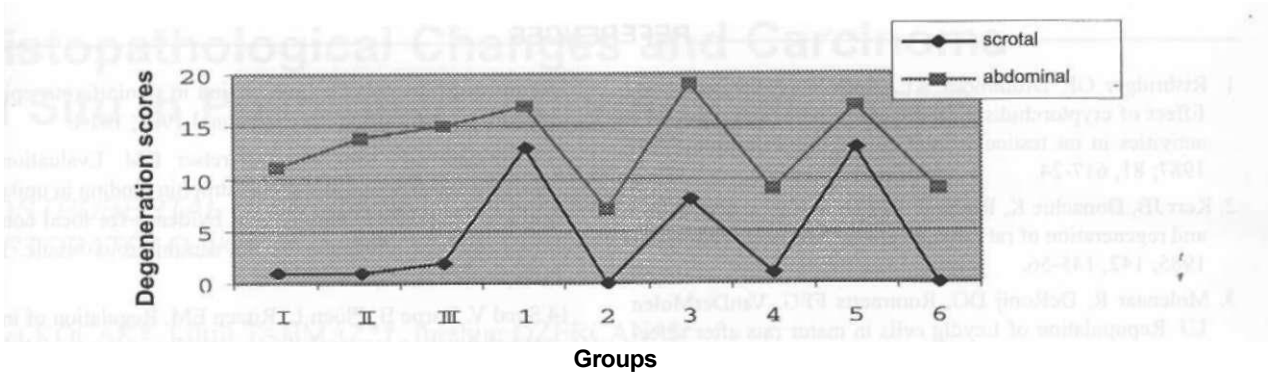


Graphic 2. The comparison of groups in respect to Leydig cell degenerations.



Graphic 3. The comparison of groups in respect to interstitial degenerations.

tium was observed wider, and basement membranes of the tubules were thickened. In abdominal testes of UNICR induced groups, because of impaired interaction between seminiferous tubules and interstitium, cyclic changes of Leydig cells were disappeared, and probably under the effect of another paracrine factor/s, the hyperplasia of Leydig cells became one of the most important changes in the testes (13, 14). Additionally, the effect of HCG on Leydig cells has been impaired.



Graphic 4. The comparison of groups in respect to total degenerations.

This may be due to increased capillary permeability (15). On the other hand, it had been reported that the concentration of 17|3-estradiol and the number of estradiol receptors have been increased in abdominal testes. 17(3-estradiol inhibits the testosterone production function of Leydig cells. Therefore, increased LH exerts a trophic effect on Leydig cells, while testosterone level of sera and intratesticular fluids decrease (16, 17).

Tubular atrophy was diffuse in abdominal testes, while Leydig cells were not encountered on day 14 in EDS plus UNICR groups, as expected. EDS provoked the testicular damage of UNICR induction. The observed occasional degeneration in scrotal testes of group 5 was thought to be due to defective testosterone synthesis developed in EDS administration which makes the tubular degeneration more severe. Furthermore, tubular atrophy is the result of inhibition developed in increased abdominal temperature. When it comes to HCG plus UNICR induction, there was a healthy spermatogenesis and intact interstitium in scrotal testes, but the degeneration of abdominal testes was seen to progress depending on the time. Increased temperature and capillary permeability existed in intaabdominal millieu lead to impairment of preventing effect of HCG on both scrotal and abdominal testes. Moreover, even if HCG passes from capillary wall to testicular interstitium, gonadotrophin uptake of the cells was reported to decrease (18).

The reason of the observation of Leydig cell response in abdominal testes, but not in scrotal ones, can be explained by the impaired paracrine interactions between tubular and interstitial compartments due to changed microenvironment (19). According to Kort et al, intraabdominal cryptorchidism soon leads to irreversibl damage to the testes resulting in azospermia (20). On the other hand, it had been reported that an increased number of mast cells (mastocytosis) was recognised in the interstitium of the idiopathically infertile men, and suggested that mastocytosis is a secondary change induced by interstitial fibrosis (21). This may be valid also for our findings, so increased cellular and interstitial compartments could be lead the accumulation of mast cells. According to the hypothesis that there is a paracrine regulation of mast cells in testicular interstitium; in normal adult rats Leydig cells influence the mast cell precursors or block the stimulatory functions, as a result of the inhibition of mast cells (22, 23). Thus, mast cell proliferation and accumulation that we observed in EDS plus UNICR groups appeared because of the lost of this inhibition by the destruction of Leydig cells.

There are a number of ways in which the present work could be extended. Taken together, the present results confirm the view that EDS is a specific toxicant of Leydig cells, and a very valuable model when combined with UNICR induction and HCG administration for investigation of cellular paracrine interactions of tubular and interstitial compartments of adult rats. Besides gonadotrophic hormones, local factors are playing very important roles in regulation of testicular functions.

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