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Leydig Cell Hyperplasia in Cryptorchid Patients: Quantitative Evaluation of Leydig Cells in Undescended and Contralateral Scrotal Testes

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Summary. Leydig cell number was evaluated quantitatively in testicular biopsies from post-pubertal cryptorchid patients and normal controls. For this quantitative evaluation we used the following method. This is based on the determination of the total number of Leydig cells, Leydig cell clusters and seminiferous tubules in the entire histologic sections of each biopsy and the determination of the following indices; mean Leydig cells per tubule, mean Leydig cell clusters per tubule and mean Leydig cells per cluster. In addition, the numbers of Sertoli cells were counted, and Leydig-Sertoli cell ratio was also determined. These indices were correlated with each other. All indices were significantly elevated not only in undescended but in contralateral scrotal testes of the cryptorchid patients in comparison to those in normal controls. Between undescended and descended scrotal testes of the same individual patients, those indices were significantly higher in the descended scrotal testes than in the undescended ones. Thus, Leydig cell hyperplasia was noted in the testes of post-pubertal cryptorchid patients, and was more prominent in the contralateral scrotal testes than in the undescended ones.

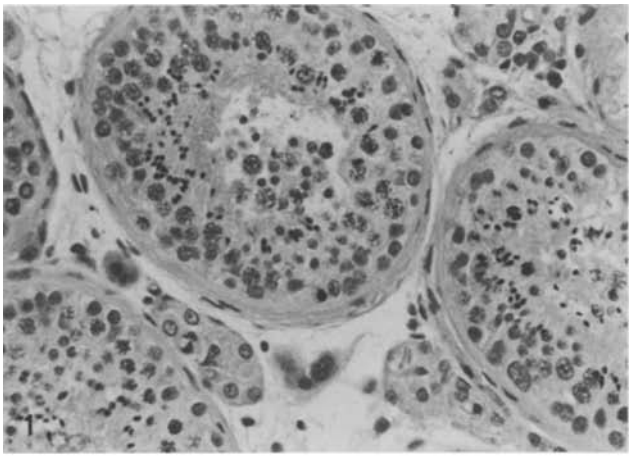
Key words: Human, Cryptorchidism, Leydig cell hyperplasia.

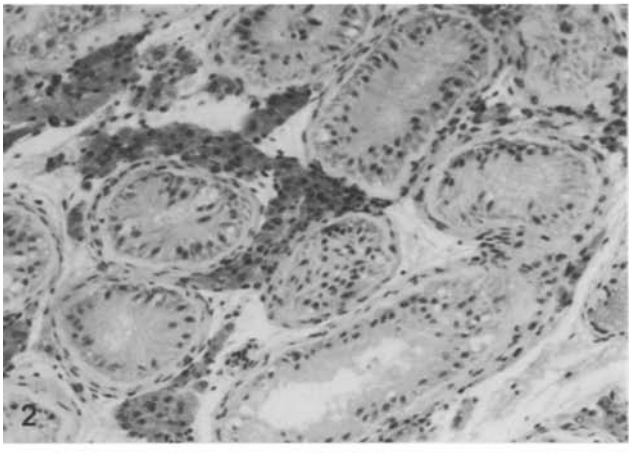
Introduction

It is well known that sterility and subfertility are frequently observed in cases of cryptorchidism [1, 2]. In previous light and electron microscopic studies on the testes of this disorder, various changes in each histological component such as germ cells. Sertoli cells, walls of seminiferous tubules and interstitial tissues have been described morphologically [3, 4]. However, the precise nature of the pathogenesis of the sterility in the disorder remains to be known, and only a small number of reports have appeared of Leydig cells in cryptorchid testes. Some authors [5–7] insisted that Leydig cells were not only morphologically but functionally normal in the cryptorchid testes; however, other reports on the endocrinological evaluation of cryptorchid testes have recently suggested that the cells may somehow be related to disturbed spermatogenesis in the cryptorchid testes [8]. Thus, objective quantitative analyses of functional structures from the testicular biopsy specimens are believed to provide some information concerning the pathogenesis of the cryptorchid testes. Leydig cell hyperplasia has been reported to occur in various pathological conditions of the testes such as X ray radiation [9], Vitamin E deficiency [9] and varicocele [10], and it has often been found in association with disturbed spermatogenesis. According to a previous study [11], Leydig cell hyperplasia was often noted in cryptorchid testes, even though other workers [5–7] have suggested that Leydig cells were morphologically normal in such testes. In most of these studies, however, the evaluation of Leydig cell hyperplasia was based upon rough and subjective data obtained only by microscopic observations of biopsied specimens. Weiss et al. [12] proposed a simple, objective method of determining the number of Leydig cells in the testis. Using this method here, attempts have been made to determine the number of Leydig cells in the undescended and contralateral scrotal testes of cryptorchid patients and those of normal controls, to compare the results obtained with one another.

Materials and Methods

In the present study, 9 post-pubertal cryptorchid patients aged 16 to 37 years (8 unilateral and one bilateral cryptorchids) were examined. Tissue specimens were obtained by an accepted biopsy technique from 10 undescended testes and from 4 contralateral scrotal testes (16, 19, 26 and 32 years old) and were immediately fixed in Carnoy's fluid for 2 h at room termperature. After fixation the tissue specimens were dehydrated in an ethanol series of ascending concentrations and embedded in paraffin. Sections were cut at a thickness of 4 μ, dewaxed in xylène, hydrated through an ethanol series of descending concentrations and then stained with hematox-





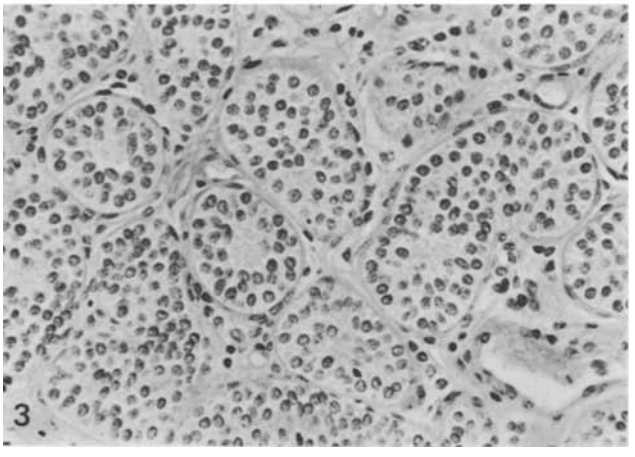


Fig. 1. The testis from a 33 year-old normal fertile man; Leydig cells are found to exist in clusters of several cells in the angular intertubular spaces

Fig. 2. The undescended testis from a 26 year-old unilateral cryptorchid; Leydig cells are observed to increase in number

Fig. 3. The undescended testis from a 9 year-old unilateral cryptorchid; typical mature Leydig cells are hardly observed

ylin-eosin for observation of the general structure. As normal controls, biopsy specimens were obtained from the testes of 6 healthy fertile men at vasectomy (26 to 50 years old). Tissue specimens from these normal controls were processed in the same way as that employed for the patients.

In all histologic sections from the biopsied specimens, the total number of seminiferous tubules, Leydig cells, Leydig cell clusters and Sertoli cells were counted. Then, the following indices were determined; Leydig cells per seminiferous tubule, Leydig cell clusters per seminiferous tubule and Leydig cells per cluster. In addition, the Leydig-Sertoli cell ratio was determined. Leydig cells per seminiferous tubule were calculated by dividing the total number of Leydig cells counted in the entire histologic sections by the total number of seminiferous tubules. Leydig cell clusters per seminiferous tubule were determined by dividing the total number of clusters by the total number of seminiferous tubules. Leydig cells per cluster were obtained by dividing the total number of Leydig cells in the clusters by the number of clusters counted in the entire sections. The Leydig-Sertoli cell ratio was estimated by dividing the total number of Leydig cells by the total number of Sertoli cells in the entire sections.

All these indices were subjected to statistical evaluation, and comparisons were made between data obtained in undescended and contralateral testes of cryptorchid patients and those of normal controls.

Results

In the testes of normal controls, Leydig cells were found to occur singly or in clusters of several cells in the angular intertubular spaces or in the perivascular spaces. Characteristically, these cells were polygonal or round in shape, and their cytoplasm was fine granular and eosinophilic. In the cytoplasm, an eccentrically located round or ovoid nucleus was noted which contained one or two prominent nucleoli (Fig. 1).

Prior to quantitative analyses, Leydig cells in the testes were generally observed in 70 cryptorchid patients who were 2 to 37 years old. In the undescended testes of postpubertal patients, numerous Leydig cells could be detected on rough and subjective estimation (Fig. 2). On the contrary, in the testes of pre-pubertal and even pubertal patients, typical mature Leydig cells were hardly found (Fig. 3). Therefore, attempts have been made to evaluate Leydig cell hyperplasia quantitatively in the testes of postpubertal patients. In Table 1 the age and location of the undescended testes as well as the degree of spermatogenesis (Johnsen's score) have been summarized.

Five testes of 6 normal controls showed normal spermatogenesis, whereas those of a 50 year-old man revealed a Johnsen's score of 9. In the contralateral scrotal testes of unilateral cryptorchid patients, cases 3, 4 and 8 exhibited almost normal spermatogenesis, while case 2 had a mild disturbance of spermatogenic activity. All the undescended testes, however, showed severe depletion of spermatogenesis (Johnsen's score 2 to 3).

In Table 2 the numbers of seminiferous tubules and Leydig cell clusters, the total number of Leydig cells and Sertoli cells which were counted in the entire histologic sections from each biopsy are shown. In Table 3 various indices of Leydig cell numbers for each biopsy are illustrated. As is apparent from the tables, no correlation could be noted between these indices and the ages of patients, cryptorchid sides and locations of the testes. Significant correlations

Table 1. Site, location and Johnsen's score of the testes

Cryptorchid patients						Normal controls			
Case	Age	Undescendence	Site	Location	Johnsen's score	Case	Age	Site	Johnsen's score
1	16	Undescended	L	Intra-canal	3	1	26	L	10
2	16	Undescended	L	Intra-canal	2	2	27	L	10
		Descended	R	Scrotal	8.5 (8 ~9)	3	31	L	10
3	19	Undescended	L	Intra-canal	2	4	33	L	10
		Descended	R	Scrotal	10	5	35	L	10
4	26	Undescended	R	Pre-pubic	2	6	50	L	9
		Descended	L	Scrotal	9.5 (9 ~10)				
5	29	Undescended	R	Intra-canal	2				
		Undescended	L	Intra-canal	2				
6	30	Undescended	L	Pre-pubic	2.5 (2 ~3)				
7	30	Undescended	L	Pre-pubic	2				
8	32	Undescended	L	Pre-pubic	2				
			R	Scrotal	10				
9	37	Undescended	L	Pre-pubic	2				

L: Left side; *R*: Right side

Table 2. Number of seminiferous tubules, Leydig cell clusters, Leydig cells and Sertoli cells in each histologic section

Cryptorchid patients							Normal controls					
Case	Age	Site	No. of S.T.	No. of clusters	No. of Leydig cells	No. of Sertoli cells	Case	Age	No. of S.T.	No. of clusters	No. of Leydig cells	No. of Sertoli cells
1	16	Ud	52	126	710	798	1	26	39	43	295	624
2	16	Ud	120	211	1,622	1,802	2	27	22	25	198	374
		D	62	98	669	778	3	31	43	38	159	544
3	19	Ud	91	147	1,047	1,050	4	33	64	64	299	729
		D	49	92	745	637	5	35	62	75	414	1,032
4	26	Ud	103	211	1,427	1,719	6	50	116	132	685	1,522
		D	81	190	1,551	1,102						
5	29	Ud	61	128	871	968						
		Ud	99	153	1,270	1,494						
6	30	Ud	107	174	1,178	1,122						
7	30	Ud	102	148	801	1,335						
8	32	Ud	126	195	1,420	1,036						
		D	113	220	1,792	2,240						
9	37	Ud	102	256	2,210	944						

S.T.: Seminiferous Tubule; *Ud*: Undescended Testis; *D*: Descended Testis

were noticed between Leydig cells per seminiferous tubule and clusters per seminiferous tubule ($r = 0.91$, $p < 0.01$) (Fig. 4a), and between Leydig cells per seminiferous tubule and Leydig cells per cluster ($r = 0.77$, $p < 0.01$) (Fig. 4b). A similar correlation was found to exist between Leydig cells per seminiferous tubule and Leydig-Sertoli cell ratio ($r = 0.92$, $p < 0.01$) (Fig. 4c).

When the indices of the undescended and contralateral scrotal testes of the cryptorchid patients were compared with those of normal controls, all the indices determined of Leydig cell number were significantly higher in both the undescended and descended scrotal testes of the patients than in those of the normal controls ($p < 0.01$) (Fig. 5). In the undescended and contralateral scrotal testes, on the

Table 3. Indices of Leydig cell density in patient's biopsies

Cryptorchid patients							Normal control					
Case	Age	Site	Leydig cells/ S.T.	Leydig cells/ cluster	Clusters/ S.T.	Leydig cells/ Sertoli cell	Case	Age	Leydig cells/ S.T.	Leydig cells/ cluster	Clusters/ S.T.	Leydig cells/ Sertoli cell
1	16	Ud	13.7	5.63	2.42	0.89	1	26	7.56	6.86	1.10	0.47
2	16	Ud	13.5	7.68	1.75	0.90	2	27	9.00	7.92	1.14	0.52
		D	10.7	6.83	1.58	0.86	3	31	3.70	4.18	0.88	0.29
3	19	Ud	11.5	7.12	1.62	1.0	4	33	4.67	4.67	1.00	0.41
		D	15.2	8.10	1.88	1.17	5	35	6.68	5.52	1.21	0.40
4	26	Ud	13.8	6.61	2.05	0.83	6	50	5.91	5.19	1.14	0.45
		D	19.1	8.14	2.35	1.41						
5	29	Ud	14.3	6.80	2.10	0.90						
		Ud	12.8	8.30	1.55	0.85						
6	30	Ud	11.0	6.63	1.63	1.05						
7	30	Ud	7.85	5.76	1.45	0.60						
8	32	Ud	11.3	7.22	1.55	0.80						
		D	15.8	8.14	1.94	0.94						
9	37	Ud	21.7	8.60	2.51	2.34						

S.T.: Seminiferous Tubule; *Ud*: Undescended Testis; *D*: Descended Testis

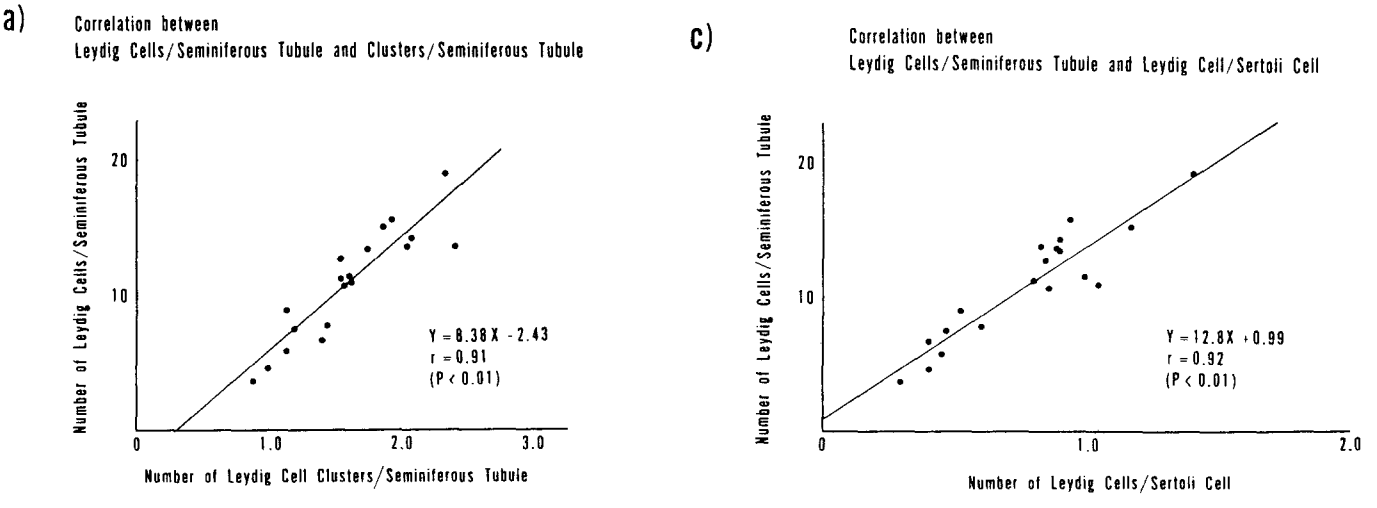


Fig. 4. Correlation of the various indices to the mean number of Leydig cells per seminiferous tubule

contrary, the mean values of all the indices tended to be higher in the latter testes than in the former ones (Fig. 5). When the undescended testes of the same individuals with unilateral cryptorchidism are compared with their contralateral scrotal ones, 3 of 4 cases (75%) (cases 3, 4 and 8) revealed a significantly higher value of the indices in the scrotal testes than in the undescended ones; the indices of Leydig cells per seminiferous tubule ($p < 0.01$), Leydig cells per cluster ($p < 0.01$) and Leydig cells per Sertoli cell ($p < 0.05$). The descended scrotal testes of these 3 cases showed nearly normal activity of spermatogenesis. In case 2, howev-

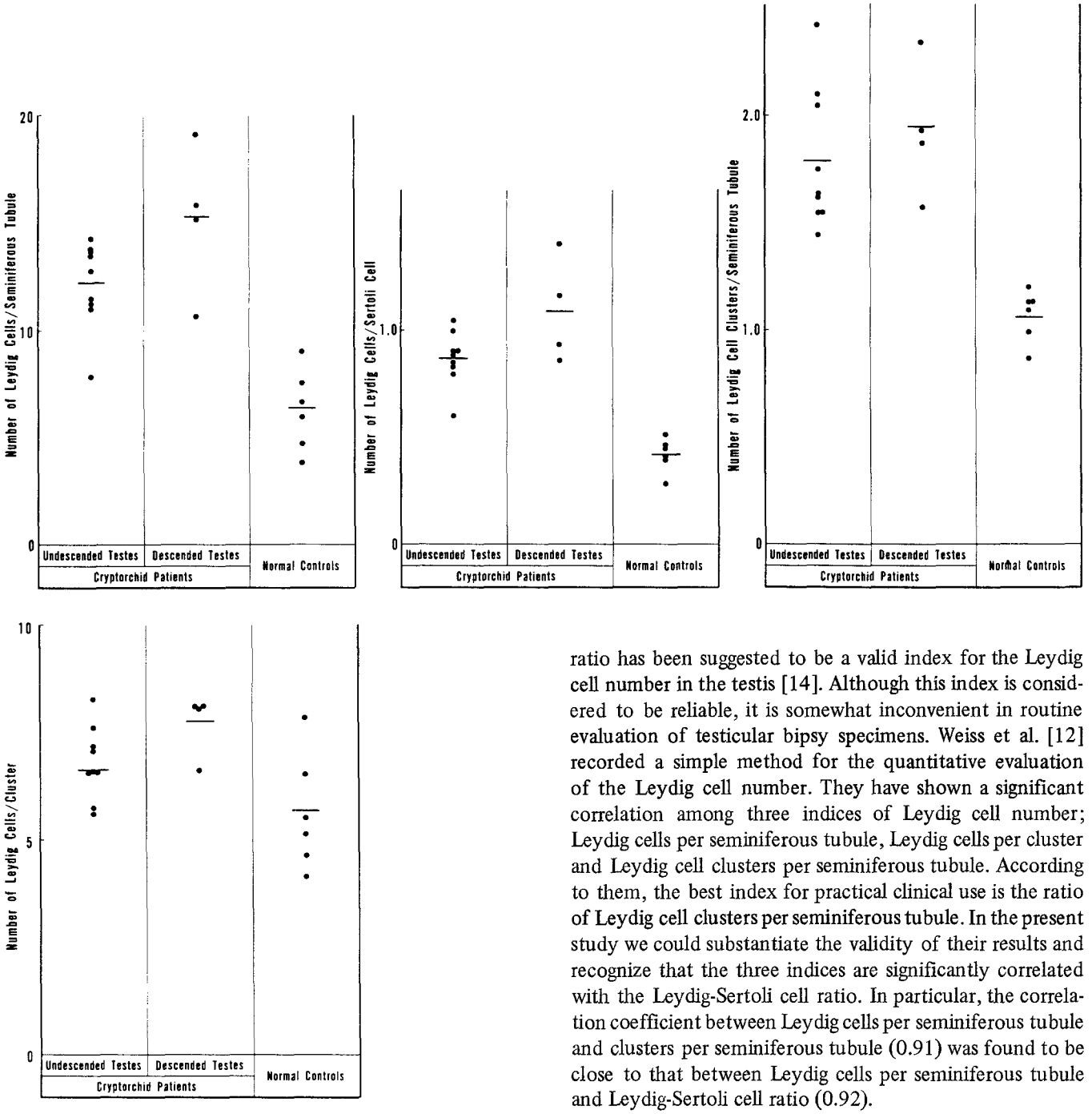


Fig. 5. Comparison of each index in cryptorchid patients and normal controls

er, all the indices were higher in the undescended testis than in the contralateral scrotal one, whereas the scrotal testis of this case exhibited disturbances of spermatogenesis.

Discussion

Leydig cell hyperplasia has often been reported to exist in the testes of cryptorchid patients [11] and experimental cryptorchidism in animals [13]. In these testes, however, Leydig cell number has been evaluated almost entirely on the basis of rough and subjective data obtained by microscopic observation of biopsy specimens. The Leydig-Sertoli cell ratio has been suggested to be a valid index for the Leydig cell number in the testis [14]. Although this index is considered to be reliable, it is somewhat inconvenient in routine evaluation of testicular bipsy specimens. Weiss et al. [12] recorded a simple method for the quantitative evaluation of the Leydig cell number. They have shown a significant correlation among three indices of Leydig cell number; Leydig cells per seminiferous tubule, Leydig cells per cluster and Leydig cell clusters per seminiferous tubule. According to them, the best index for practical clinical use is the ratio of Leydig cell clusters per seminiferous tubule. In the present study we could substantiate the validity of their results and recognize that the three indices are significantly correlated with the Leydig-Sertoli cell ratio. In particular, the correlation coefficient between Leydig cells per seminiferous tubule and clusters per seminiferous tubule (0.91) was found to be close to that between Leydig cells per seminiferous tubule and Leydig-Sertoli cell ratio (0.92).

Leydig cell hyperplasia has occasionally been reported to exist in undescended testes. To the best of our knowledge, however, no hyperplasia in the contralateral scrotal testes has ever been described. In the present study, Leydig cell hyperplasia was quantitatively determined not only in the undescended but in the descended scrotal testes of the cryptorchid patients. Furthermore, Leydig cell hyperplasia tended to be more pronounced in the descended scrotal testes than in the undescended ones.

Leydig cells have been reported to be not only functionally but morphologically normal in cryptorchid testes [5, 6]. In the cryptorchid patients, however, hormonal abnormalities have recently been described by several authors. In animals surgically made cryptorchid, a diminution in amounts of extractable androgen was reported in the undescended testes of rats [15]. In dose, Eik-Nes [16] noted a lower concentration of testosterone in venous

blood from the undescended testes as compared with that from the contralateral scrotal testes. According to some authors, the activities of enzymes directly involved in steroidogenesis declined in the Leydig cells of undescended testes of rats [17]. In humans, Raboch et al. [18] reported a diminution in plasma testosterone level in postpubertal cryptorchid patients. Using his specific technique, Kodaira [19] noticed that androgen production was suppressed in the undescended testes of human cryptorchid patients. Hadžiselimović [8] studied the ultrastructural changes in Leydig cells of the testes of cryptorchid patients and demonstrated morphologically the impaired function of Leydig cells in such testes. Thus, all these reports have shown morphologically and endocrinologically that androgen production declines in undescended testes. If these reports are taken into consideration, Leydig cell hyperplasia noted here in the cryptorchid patients does not necessarily imply hyperfunction of Leydig cells, even though the possibility of a hyperstimulated state of these cells cannot be ruled out. According to several authors [20, 21], an increase in plasma LH level has been noted in post-pubertal and adult cryptorchids. From what has been discussed above, it may be postulated that Leydig cell hyperplasia noted in the present study does not represent hyperfunction in androgen production, but reflects, a compensatory or secondary reaction possibly induced by elevation of plasma LH level resulting from primary damages of Leydig cells in the cryptorchid testes.

It is interesting to note that in unilateral cryptorchids Leydig cell hyperplasia tended to be more pronounced in the contralateral scrotal testes than in the undescended ones. Since the number of cases examined is relatively small, further investigations into more cases with detailed hormonal surveys are needed, in order to interpret such phenomena more completely.

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Reviewer's Comment

The observation of increased number of the Leydig cells in contralateral descended testes of young adult patients with unilateral cryptorchidism as stated above is an important finding. This underlines the known fact that both testes in unilateral cryptorchid patiens are damaged. However, since it is known that intratesticular testosterone is within the normal range in both undescended as well as contralateral descended gonads in adult patients [1], these hyperplastic Leydig cells, although maximally stimulated, obviously could not increase their testosterone production adequately. Thus, the electron microscopic observations [2] of pathological Leydig cell cytoplasm point to the Leydig cell damage. F.H.

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