

## Plasma testosterone response at 1<sup>st</sup> and 4<sup>th</sup> day after short- and long-term hCG stimulation test

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**SUMMARY:** Adıyaman P, Öcal G, Berberoğlu M, Aycan Z, Evliyaoğlu D, Çetinkaya E. Plasma testosterone response at 1<sup>st</sup> and 4<sup>th</sup> day after short- and long-term hCG stimulation test. Turk J Pediatr 2004; 46: 309-314.

Human chorionic gonadotropin (hCG) stimulation test is a reliable dynamic test for the evaluation of testicular function during childhood. Several protocols have been recommended but their reliability is controversial. In order to decide the best timing to measure stimulated testosterone levels in short- and long-term hCG protocols, we evaluated 83 prepubertal patients in two groups. In group A, 34 patients with isolated micropenis and in group B, 49 inguinal cryptorchidic patients were enrolled. In group A short-term hCG protocol (3000 IU/m<sup>2</sup>/im/3 days) and in group B long-term hCG protocol (1500 IU/m<sup>2</sup>/im; thrice a week for 3 weeks) was administered. Blood samples were drawn at the initiation of the test and then at the 1<sup>st</sup> and 4<sup>th</sup> days after the last hCG injection. Each case's peak stimulated testosterone (T<sub>max</sub>) and the increment in plasma testosterone ( $\Delta$ T) were calculated and compared with the 1<sup>st</sup> and 4<sup>th</sup> day responses within the group.

In the short-term protocol the 4<sup>th</sup> day responses were higher than the 1<sup>st</sup> day responses ( $p < 0.01$ ). Interestingly, while four patients had insufficient responses at the 1<sup>st</sup> day, three had sufficient Leydig cell response at the 4<sup>th</sup> day. In the long-term protocol group, in contrast to the short-term group, the 1<sup>st</sup> day responses were higher than the 4<sup>th</sup> day ( $p < 0.01$ ).

According to our results, while performing hCG test in a patient, if a short-term protocol is planned, it is more convenient to check the 4<sup>th</sup> day testosterone response. On the other hand, in a long-term protocol it is best to check the 1<sup>st</sup> day response. We suggest that even if a patient's 1<sup>st</sup> day response is inadequate, the 4<sup>th</sup> day response should be checked in order to avoid misdiagnosis.

**Key words:** short- and long-term hCG stimulation test, testosterone response.

Assessment of testicular endocrine function is relevant to a wide range of disorders that affect male genital differentiation and male secondary sexual development<sup>1,2</sup>. During childhood, which is the quiescent phase of testicular activity, the human chorionic gonadotropin (hCG) stimulation test is widely used to evaluate testicular function<sup>1-15</sup>. This test is important for the diagnosis of circumstances in which there are questions about normal testicular function (e.g. primary testicular dysfunction, disorders of steroidogenesis, ambiguous genitalia, isolated micropenis, cryptorchidism, anorchia). There is no consensus regarding standard protocol of

hCG testing in the literature. Many different protocols exist regarding the dosing and duration of hCG and sampling time<sup>1-15</sup>.

The purpose of this study was to determine the best timing for stimulated testosterone levels in short- and long-term hCG protocols. The study was designed for the testosterone response to short- and long-term administration of hCG at the 1<sup>st</sup> and 4<sup>th</sup> day after the completion of stimulation.

### Material and Methods

A total of 83 prepubertal patients were enrolled in this study. A written consent was obtained from parents or legal guardians of the patients.

Patients were divided into two groups according to their clinical diagnosis. Group A included 34 patients with isolated micropenis and group B 49 inguinal cryptorchidic patients (32 unilateral, 17 bilateral). Then mean ages were  $8.25 \pm 3.99$  years in group A, and  $4.48 \pm 3.39$  years in group B. Penile stretched length and testicular localization were determined by physical examination before and after hCG testing. Testes residing within scrotum an appreciable portion of time during the physical examination was diagnosed as retractile testes, and these cases were excluded from the study. Patients with ectopic testes and virilization defects such as severe hypospadias, chordae, and bifid scrotum were also excluded from the study. hCG stimulation test was applied with two different protocols. While short-term hCG stimulation was applied to 34 patients with isolated micropenis (group A), we used long-term stimulation test in 49 patients with undescended testes (group B) in order to obtain both diagnostic and therapeutic advantage of long-term stimulation. Blood samples were drawn at the initiation of the test and at the 1<sup>st</sup> and 4<sup>th</sup> days after the last dose of hCG. Serum samples were extracted for the analyses of testosterone levels. Peak stimulated testosterone levels (Tmax) and the increment in plasma testosterone ( $\Delta T$ ) with hCG

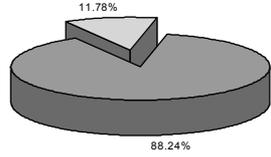
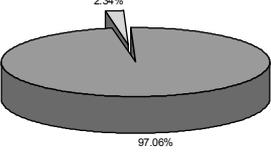
stimulation were compared within each group with the 1<sup>st</sup> and 4<sup>th</sup> day results after the last injection. Tmax was defined as the highest value during the test.  $\Delta T$  was calculated as the subtraction of baseline testosterone from Tmax. An increment of the plasma T concentration to greater than 100 ng/dl was considered normal<sup>16</sup>. Short-term Leydig cell stimulation was performed in group A using hCG 3000 IU/m<sup>2</sup> body surface area intramuscularly, every other day for three consecutive injections<sup>2</sup>. Long-term Leydig cell stimulation was performed in group B using hCG 1500 IU/m<sup>2</sup> body surface area, intramuscularly, thrice a week for three weeks<sup>8,17</sup>.

All samples were studied at Ankara University Endocrinology Laboratories with immunochemiluminescence method using ACS kit. Paired samples t test was used for statistical analyses using SPSS for Windows. Statistical significance was taken at the  $p \leq 0.05$  level. Data were presented as mean  $\pm$  standard deviation (SD).

**Results**

In the short-term hCG administered group (Group A) peak and delta testosterone levels were significantly higher at the 4<sup>th</sup> day in comparison to the 1<sup>st</sup> day values (Table I, Fig. 1). Mean baseline T levels rose from

**Table I.** Leydig Cell Response at 1<sup>st</sup> and 4<sup>th</sup> Day to Short-Term hCG Stimulation in Patients with Isolated Micropenis (n=34)

Basat T (ng/dl)	hCG stimulated testosterone (ng/dl)			Percentage of normal and abnormal Leydig cell response
	Days	Tmax (ng/dl)	$\Delta T$ (ng/dl)	
19.40 $\pm$ 22.31	1 <sup>st</sup> day	210.30 $\pm$ 136.40	190.91 $\pm$ 132.75	
	4 <sup>th</sup> day	272.96 $\pm$ 143.39	253.65 $\pm$ 141.74	
Significance		p<0.001	p<0.001	

Tmax: peak stimulated testosterone;  $\Delta T$ : increment in plasma testosterone.

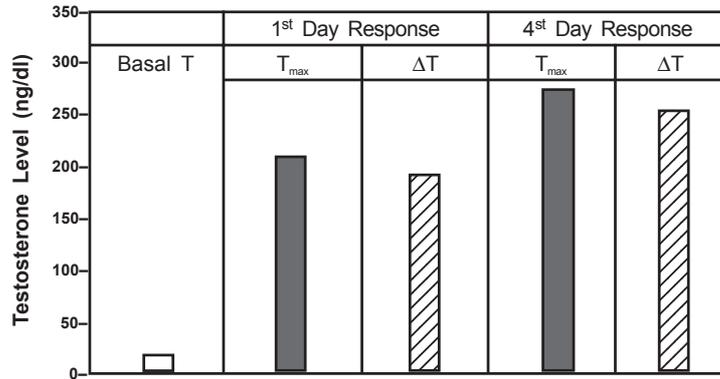


Fig. 1. The evaluation of Leydig cell function in short-term hCG stimulation test: comparison of 1<sup>st</sup> and 4<sup>th</sup> day testosterone responses. Significance: T<sub>max</sub> 1<sup>st</sup> day vs 4<sup>th</sup> day p<0.01; ΔT 1<sup>st</sup> day vs 4<sup>th</sup> day p<0.01). T<sub>max</sub>: peak stimulated testosterone; ΔT: increment in plasma testosterone.

19.40±22.31 ng/dl to 210.30±136.40 ng/dl at the 1<sup>st</sup> day, and to 272.96±143.39 ng/dl at the 4<sup>th</sup> day. ΔT values at the 1<sup>st</sup> and 4<sup>th</sup> day were 190.91±132.75 ng/dl and 253.65±141.74 ng/dl, respectively (p<0.01). Thirty patients (88.2%) of this group gave normal Leydig cell response at the 1<sup>st</sup> day. First day results implicated insufficient Leydig cell response in four patients (11.8%). Interestingly three of them gave sufficient Leydig cell response to hCG at the 4<sup>th</sup> day after the last injection. In this group only one case out of 34 patients was diagnosed as gonadal insufficiency.

As a function of long-term hCG stimulation, the increase in serum testosterone levels was evident at both the 1<sup>st</sup> and 4<sup>th</sup> days, despite the magnitude of the rise being higher at the 1<sup>st</sup> day when compared to the 4<sup>th</sup> day, in contrast with the short-term protocol (Table II, Fig. 2). Forty-six patients of this group had sufficient (93.9%) and three 1<sup>st</sup> insufficient (6.1%) Leydig cell response to hCG stimulation at both 1<sup>st</sup> and 4<sup>th</sup> days. In our study baseline testosterone level was 19.40±22.31 ng/dl. Plasma stimulated testosterone at the 1<sup>st</sup> and 4<sup>th</sup> days were 530.31±342.44 ng/dl and 404.44±249.13 ng/dl,

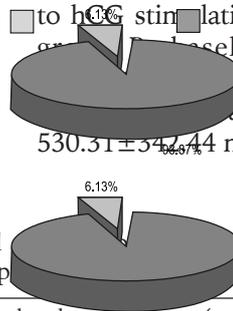


Table II. Leydig Cell Response at 1<sup>st</sup> and 4<sup>th</sup> Days in hCG Stimulation in Patients with Cryptorchidism

Basal T (ng/dl)	Days	T <sub>max</sub> (ng/dl)	ΔT (ng/dl)	Percentage of normal and abnormal Leydig cell response
	1 <sup>st</sup> day	530.31±342.44	515.23±343.64	Sufficient response: 93.9% Insufficient response: 6.1%
19.40±22.31	4 <sup>th</sup> day	404.44±249.13	393.38±249.43	Sufficient response: 93.9% Insufficient response: 6.13%
	Significance	p<0.01	p<0.01	

T<sub>max</sub>: peak stimulated testosterone; ΔT: increment in plasma testosterone.

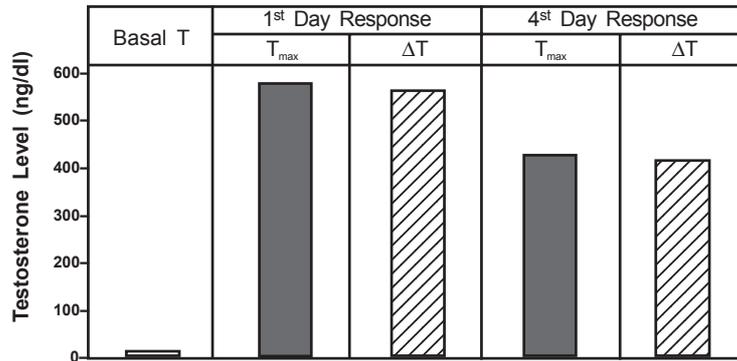


Fig. 2. The evaluation of Leydig cell function in cryptorchidic patients with long-term hCG stimulation test: comparison of 1<sup>st</sup> and 4<sup>th</sup> day testosterone responses (Significance: T<sub>max</sub> 1<sup>st</sup> day vs 4<sup>th</sup> day  $p < 0.01$ ;  $\Delta T$  1<sup>st</sup> day vs 4<sup>th</sup> day  $p < 0.01$ ). T<sub>max</sub>: peak stimulated testosterone;  $\Delta T$ : increment in plasma testosterone.

respectively ( $p < 0.01$ ).  $\Delta T$  values at the 1<sup>st</sup> and 4<sup>th</sup> day were  $515.23 \pm 343.64$  ng/dl and  $393.38 \pm 249.93$  ng/dl, respectively ( $p < 0.01$ ). Testicular descent into scrotal area occurred in 44 of 49 patients (89.8%). Five patients (3 out of 5 had Leydig cell dysfunction) required surgical treatment for cryptorchidism after hCG treatment.

## Discussion

Our data indicates that while performing hCG stimulation test, if a short-term protocol is planned, it is more convenient to check the 4<sup>th</sup> day testosterone levels after the last injection. On the other hand, if a long-term protocol is planned, it is more convenient to check the 1<sup>st</sup> day testosterone levels after the last hCG injection.

Testicular endocrine function is known to occur during fetal life in infancy and throughout puberty, when it is responsible for the transition from childhood to adult life<sup>18</sup>. Defect of fetal testicular function can present clinically as abnormalities in the formation or growth of the external genitalia, i.e. as ambiguous genitalia or micropenis<sup>1,13,18</sup>. In pediatric patients, basal gonadal steroids are frequently undetectable in the plasma before puberty and gonadal function can be detected by hCG testing<sup>10,13,15</sup>.

In clinical prepubertal andrology, hCG testing can be applied as a non-invasive and highly sensitive method<sup>13</sup>. hCG test has a diagnostic significance but has not been standardized yet; several protocols have been reported<sup>1,3,5-8</sup>.

It has been clearly shown that several injections give much more valuable information than that of single injection<sup>8</sup>. Leydig cell desensitization may occur following multiple hCG injections, hence the rationale of alternate day injections<sup>8</sup>.

In this study we preferred alternate day injection in short-term (every other day for 3 consecutive injections) and long-term (thrice a week for 3 weeks) hCG testing. In the short-term hCG administered group of our study (patients with micropenis), mean peak and delta testosterone levels were significantly higher at the 4<sup>th</sup> day when compared to the 1<sup>st</sup> day. Testosterone response at the 1<sup>st</sup> day was insufficient in four cases and normal in the others. During the mid-childhood period, which is characterized by low gonadotropin, low sex steroid production, and a highly sensitive hypothalamic-pituitary-gonadal axis to feedback inhibition, a prolonged hCG stimulation may be necessary<sup>14</sup>. Patients with low testosterone increases, like our four unresponsive cases, were subjected to a prolonged test. Interestingly, three of four unresponsive patients had sufficient Leydig cell response to hCG at the 4<sup>th</sup> day after the last injection. Thus, prolongation of the hCG test was not needed because the testosterone response of these three patients was sufficient at the 4<sup>th</sup> day. Forest et al.<sup>6,7,9</sup> proposed that maximal T values are seen 72 to 120 hrs after the first injection and that the first injection induces a progressive and modest rise in the testosterone levels. The

second injection given one day later had little additional effect on response. The authors also added that in the prepubertal boys in whom several hCG injections were administered, T levels reached comparable levels after four or seven injections. These results are parallel to our findings during the short-term protocol. A cumulative effect may have resulted in the higher values at the 4<sup>th</sup> day.

It is known that Leydig cell functions may be impaired in some cases of cryptorchidism<sup>10,11,15,20,21</sup>. In our study 46 patients of our cryptorchidic group had sufficient (93.9%) and three patients insufficient (6.1%) Leydig cell response to the long-term hCG stimulation. The magnitude of the mean testosterone rise was higher at the 1<sup>st</sup> day when compared to the 4<sup>th</sup> day, in contrast with short-term protocol. This finding is in concordance with the literature<sup>3</sup>. We suggested that the prolongation of the hCG test period may be responsible for that result. hCG stimulation is preoperatively efficacious in causing the nonscrotal palpable undescended testes to become palpable in the scrotum<sup>11</sup>. Testicular descent into scrotum occurred in 44 of our 49 inguinal cryptorchidic patients (89.8%). hCG administration was ineffective in promoting testicular descent in five patients (3 of them had testicular insufficiency).

In conclusion, we emphasize the importance of deciding the best timing to draw samples for determination of stimulated testosterone levels in hCG stimulation test. It was interesting that the convenient sampling time was different in short-term and long-term protocols. Our results indicated that in short-term stimulation protocols, it is more convenient to check the 4<sup>th</sup> day stimulation response. In contrast to this, with administration of long-term stimulation protocols, it appears to be best to check the 1<sup>st</sup> day response. On the other hand, we also point out that even if a patient's 1<sup>st</sup> day response is inadequate, the 4<sup>th</sup> day response should be checked to avoid misdiagnosis. Unnecessary prolonged hCG administration may thus be prevented.

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