Urological Research 3, 25-30 (1975)

Effect of Human Chorionic Gonadotrophin on the Dog Seminiferous Tubule, with and without Experimental Unilateral Cryptorchism

Bernhard Ibach, Lothar Weißbach and Klaus Wesener

Urological Clinic and Institute for Clinical and Experimental Nuclear Medicine, University of Bonn, Bonn, FRG

Received: January 17, 1975

Summary. The effect of human chorionic gonadotrophin (HCG) on the testes of pubertal and postpubertal Beagle dogs was studied, in the one instance in bilaterally normotopic testes and in the other on the still scrotally located gonad of dogs which had been subjected to unilateral experimental cryptorchism. The area of the cross-sectioned seminiferous tubules served as a parameter for evaluating the effect of HCG. Administration of therapeutic doses of HCG produced, in animals with bilaterally normotopic testes, a marked diminution of the area of the seminiferous canals. This means that HCG application at normal dosage impairs the seminiferous tubules in the dog. In animals with unilateral cryptorchism, HCG application produced no significant change in the tubule area of the scrotally located testis beyond that which is ascertainable anyway on the orthotopic testis following translocation of the contralateral gonad to within the abdominal cavity. Hence, HCG does not influence the degenerative change in the orthotopic testis in experimental unilateral cryptorchism. Rather, an adverse effect of HCG on the seminiferous tubule is evident, independently of the hitherto published reports of successfully attained descent.

Key words: Cryptorchism, chronionic gonadotrophin effect, histomorphometry.

Introduction

Only suppositions have so far been expressed concerning the cause of undescended testis. One assumption is that a deficiency of maternal gonadotropin is responsible (2, 19), and this has been the sole justification for the now widely used human chorionic gonadotropin (HCG) treatment for testis dystopy, introduced into clinical practice by Shapiro (23, 24).

HCG has a principally ICSH effect (ICSH = interstitial cell stimulating hormone); it also contains slight amounts of FSH (follicle stimulating hormone), the ratio of ICSH to FSH being 1000 : 1 (15).

HCG preparations are applied clinically on the basis of mainly empirically gained criteria. Time of application and dosage were and still are very variable:
Karcher (11) recommends, for patients of ages from 2 to 3 years, 1000 to 1500 international units (IU) weekly over 6 weeks (maximum cumulative dose 18,000 IU); Bauer and Hasse (3) advise for patients between 5 and 6 years a dosage of 3 times 5000 IU with a week's interval (maximum cumulative dose 18,000 IU); Knorr (13) injected into patients aged from 6 to 9 years a dose of 1000 to 1500 IU in 12 applications spread over a 6-week period (maximum cumulative dose 24,000 IU); Ichikawa (10) holds that a maximum total dose of 118,000 IU can safely be administered.

The statistical data concerning degree of success in attaining a testis descensus and later fertility are correspondingly at variance. Gross and Jewett (6) undertook a comparative compilation of studies reporting achieved descent from the international literature; they found grades of success ranging from 0% to 90%. Seguy (22) observed that fertility capacity had been achieved in 27% of patients receiving HCG treatment; Pröscholdt (21) claimed, in respect of HCG-treated patients with unilateral cryptorchism, a favourable response regarding subsequent fertility in 6 of 15 cases, and Knorr (14) with similar patients gave a success quota of 7 out of 11.

Only recently have uniform rules regarding HCG therapy been formulated, to forestall detrimental

effects through administration during puberty and through overdosage (International Health Foundation, Geneva 1973):
Infants of up to 2 years of age should be given 250 IU twice weekly over a total period of 5 weeks. Infants of up to the age of 6 should receive, over the same period, 500 IU twice weekly. From this age on until secondary sex characteristics develop, 1000 IU twice weekly is the recommended dose.

Within the framework of a comprehensive study on unilateral cryptorchism we tested, among other things, the effect of HCG: 1. on the seminiferous tubules of the testes of normal puberal and postpuberal Beagle dogs[1] and 2. on the seminiferous tubules of the scrotally located testis of dogs of the same breed and age which had been subjected to unilateral, experimental cryptorchism.

In this paper solely the HCG effect on the tubules will be dealt with. The negative influence exerted by the translocated position of the cryptorchic gonad on the scrotally remaining one will only be touched upon in this connection (cf. the studies of Hecker et al. (7), Shirai et al. (25), Weißbach and Müller (28), Weißbach (29), Mönks (18) und Tittert (27)).

Materials and Methods

Pubertal and postpubertal Beagle dogs were used for the investigation. To reduce biological anomalies to a minimum the animals were selected, so far as possible, from a litter of pure incest strain.

The dogs of both age levels (test series I and II) were separated into two groups consisting of experimental and control animals. One group at a time was treated with HCG.

The Beagle dogs of each experimental group were kept together in communal quarters and were fed a dry standard diet with water ad lib.

Test series I. At commencement the dogs were 21/2 months old.
Group Ia. This group consisted of 4 control animals and 5 experimental animals with unilateral cryptorchism.
Group Ib. 2 control animals and 4 experimental animals with unilateral cryptorchism were treated with HCG (Primogonyl ^{R}) (300 IU i.m. twice weekly over a period of 3 months; total dose 7200 IU).

Test series II. At commencement the dogs of this series were 24 months old.
Group IIa. This group comprised 5 control animals and 5 experimental animals with unilateral cryptorchism.
Group IIb. 4 control animals and an equal number of experimental animals with unilateral cryptorchism were treated with Primogonyl ^{R} (5000 IU i.m. twice weekly over 3 months; total dose 120,000 IU).

Under general anaesthesia (sodium pentobarbital) the ductus deferens and testis were mobilized and, after severance of the cremaster muscle and opening of the peritoneum, translocated into the abdominal cavity. Attachment to the lateral pelvic wall was done with Histoacryl ^{R} . After 3 months all animals were castrated under pentobarbital narcosis.

The ablated testes were fixed in Bouin's solution, embedded in Paraplast ^{R} , cut into 5 µ thick slices and stained with the PAS and HE techniques.

The average tubular area of 50 cross-sectioned tubules per testis was determined according to the 'hit' method. By this is meant a dot-counting process (26) applied in cytomorphometry, whereby the area to be measured is determined by integrative assessment using a grid placed on the eyepiece of the microscope (8, 9).

The points of intersection of the gridlines are spaced at a regular interval of a_o, enclosing an area of a_o^2. This area is termed grid-value A_o. In evaluating an area with the help of such a grid, marginal areas are encountered where only a part of the area to be assessed lies within the quadrangle a_o^2. This area is assessed in relation to the cen-

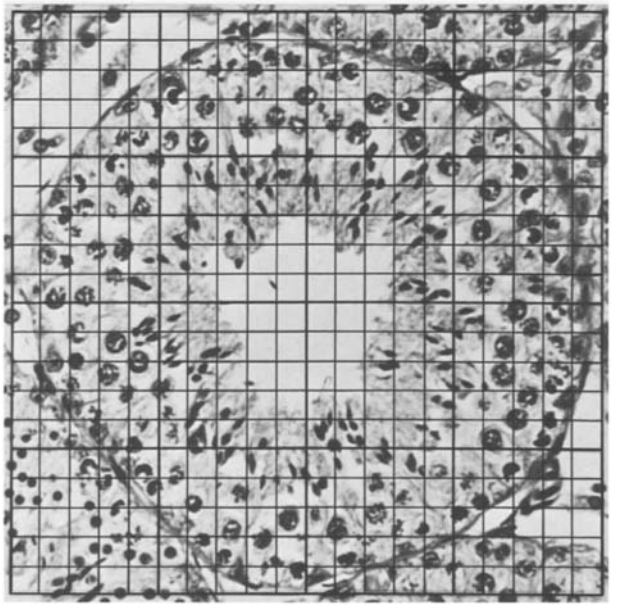


Fig. 1. Method of determining area of cross section of seminiferous tubule with aid of grid attached to lens (spacing a_o = 0.05 mm). Magn. x 480

1 We are grateful to the firm of Schering AG (Berlin) for kindly donating and caring for the Beagle dogs.

tre point of the square. Since the centre points collectively form a dotted grid with the same grid value, A_0, it does not matter for calculation purposes whether the centre points or the corner points (grid points) of the square are used. If the grid points only are considered, such a point is termed a hit if it lies within the outer delimitation of the area to be evaluated. A grid point projected onto the borderline of the area to be assessed should be registered as a half hit (26). Since, according to Sitte (26), the number of hits P_1, P_2..... P_n are spatially as valid as the areas A_1, A_2..... A_n, each recorded hit represents a fraction of an area. Hence the size of an area, by applying this method, can be estimated by simply counting the hits and multiplying by the grid value (cf. Fig. 1).

We have tested the reliability of the 'hit method' in a preliminary study. As compared with the planimetric evaluation we established a maximum error of 2.5%.

Results

The pubertal experimental animals of group I a show, in respect of the orthotopic testes compared with the control animals, a significant growth-deficit of the tubule area of 38%. Under HCG treatment (group I b) the growth deficit is, at 20%, not significant (cf. Table 1 and Fig. 2).

The postpubertal experimental animals of group II a show a substantial diminution of tubule area of 18%. Under HCG treatment (group II b) the difference amounts to merely 3%, which is not significant (cf. Table 2 and Fig. 3).

Discussion

In an earlier study we traced, by means of seminiferous tubule measurements on 84 animals, the normal testicular development of the Beagle dog (29).

Table 1. Mean seminiferous tubule area per control-animal testis and orthotopic experimental-animal testis, and total mean value of Series I. Group I a without HCG treatment, group I b with HCG treatment. CA: control animals; EA: experimental animals; $\bar{x}$: total mean value; s: standard error

Group I a

CA No.	Area (μm^2)	EA No.	Area (μm^2)
2814	15123	2809	11829
2817	15499	2826	12164
2812	21380	2818	9527
1827	17996	2823	9210
		2828	11566
$\bar{x} \pm s$	17500 ± 2884		10868 ± 1371

Group I b

CA No.	Area (μm^2)	EA No.	Area (μm^2)
2734	9372	2737	10557
2733	13869	2738	8803
		2735	10664
		2736	7271
$\bar{x} \pm s$	11621 ± 3180		9324 ± 1613

Table 2. Mean seminiferous tubule area per control-animal testis and orthotopic experimental-animal testis, and total mean value of Series II. Group II a without HCG treatment, group II b with HCG treatment. CA: control animals; EA: experimental animals; $\bar{x}$: total mean value; s: standard error

Group II a

CA No.	Area (μm^2)	EA No.	Area (μm^2)
1854	30431	1882	33010
1860	32673	1896	24464
1876	31880	1897	26158
1918	32171	1901	25932
1920	34152	1904	22478
$\bar{x} \pm s$	32261 ± 1347		26408 ± 3971

Group II b

CA No.	Area (μm^2)	EA No.	Area (μm^2)
1627	27339	1628	27481
1648	30050	1629	23663
2120	23735	1637	26796
2125	27382	1648	26863
$\bar{x} \pm s$	27127 ± 2592		26201 ± 1719

According to this study, puberty begins as from the 5th postnatal month and the testis reaches full maturity with the 9th month. Thus the animals of Series I were in the phase of pubertal development at the time of orchidectomy. The HCG dosage corresponds, at a total dosage of 7200 IU, to that generally used for treating infants.

When comparing the seminiferous tubule area of treated and untreated pubertal control animals we discovered a significant deficit in growth following HCG treatment, of 34% (cf. Table 3a). If, however, the experimental animals of both groups are compared, no significant difference in growth (14%) can be confirmed (cf. Table 3a).

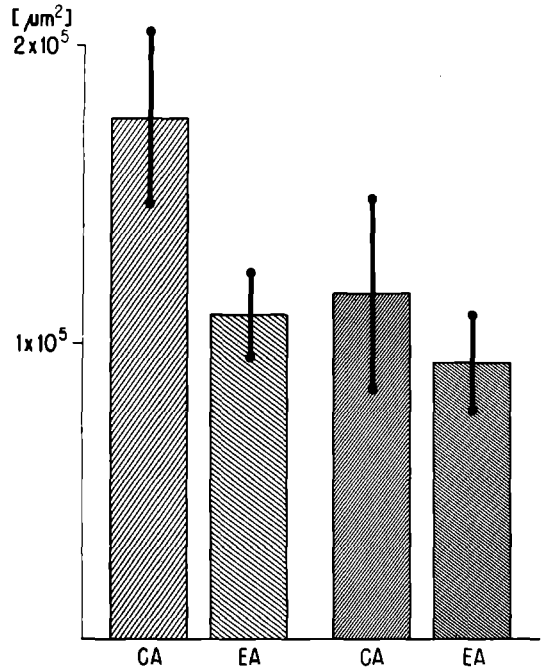
The postpubertal animals of Series IIb received a total dosage of 120,000 IU HCG. This corresponds to that cited as maximal dosage for humans (10) and was chosen by us for this reason, so that a possible influence of HCG could be established with certainty.

In comparing the mean values of the average tubule area of treated with nontreated postpuberal control animals, we find a significant deficit of 16% (cf. Table 3b). If, however, the experimental animals of both series are compared, here too - as in Series I - no significant difference (1%) can be ascertained (cf. Table 3b).

Hence, our results in both test series (pubertal and postpubertal animals) are seen to correspond. From this, the following conclusions can be drawn:

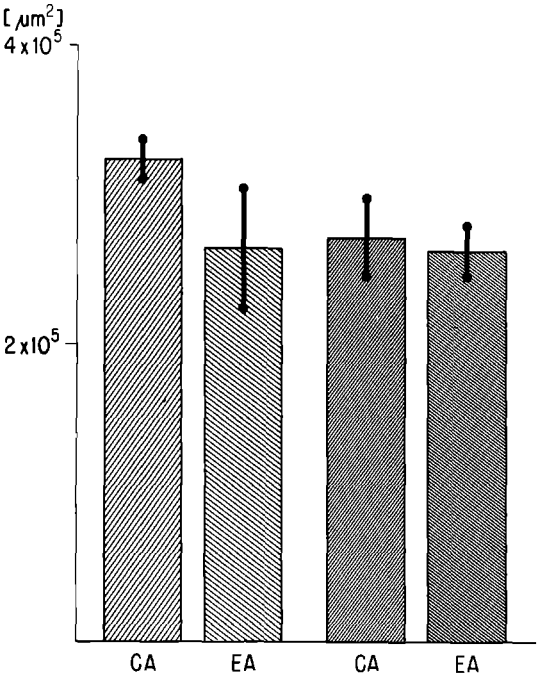
1. If a comparison is made between nontreated and HCG treated control animals - i.e. dogs with bilateral normotopic testes - then the significant diminution of tubule area in both series (pubertal: 34%, postpubertal: 16%) denotes an impairment caused by HCG (see Table 3b).

Whereas, according to Knorr (12) and Maier and Spann (17), HCG does not adversely affect the germinative tubule system, Nowakowski (20) holds that doses of over 10,000 IU, while Maddock and Nelson (16) maintain that doses of over 20,000 IU, could well lead to impairment of the tubules. Bay et al. (4) Bay (5) opine that HCG should not be administered to patients over the age of 10, due to the possibility of provoking lesions of the epithelium and calcification of the seminiferous canals.



	CA without HCG	EA without HCG	CA with HCG	EA with HCG
$\bar{x}$ [µm²]	17 500	10 868	11 621	9 324
Δ [µm²]	6632		2297	
Δ [%]	38		20	
Significance $\alpha = 0{,}01$	*			

Fig. 2. Comparative histogram of tubule area of control animal testes and of orthotopic, experimental animal testes of treated and nontreated pubertal Beagle dogs



	CA without HCG	EA without HCG	CA with HCG	EA with HCG
$\bar{x}$ [µm²]	32 261	26 408	27 127	26 201
Δ [µm²]	5853		926	
Δ [%]	18		3	
Significance $\alpha = 0{,}01$	*			

Fig. 3. Comparative histogram of tubule area of control animal testes and of orthotopic, experimental animal testes of treated and nontreated postpubertal Beagle dogs

2. In both age groups the difference in respect of evaluated areas of tubules as between control and experimental animals is diminished by HCG administration (pubertal: 20 %, compared with 38 % in non-treated animals, postpubertal: 3 % as compared with 18 % in non-treated animals - see Table 3 a, b). Therefore this effect stems not from a relative increase of tubule area in the experimental animals but rather from a diminution of tubule area in the HCG-treated control animals, as described in point 1.

3. The values for HCG treated and nontreated experimental animals do not significantly differ from one another in both age groups (pubertal: 14 %, postpubertal: 1 % - see Table 3 a, b). This means that the impairment of the orthotopic testis - possibly conditioned by the influence of the dystopic testis (7, 25) - is neither prevented nor enhanced by HCG.

Summarizing points 1, 2 and 3 the following can be stated:

Therapeutic administration of HCG has no reducing effect on the already diminished seminiferous tubule area in unilateral cryptorchidism. In bilateral scrotally located testes (control animals) HCG treatment causes a diminution of tubule area which does not significantly differ from that found in the scrotally situated testis of animals with unilateral cryptorchism (pubertal: 6 %, postpubertal: 3 % - see Table 3 a, b).

In the light of our observations on the influence of HCG application on the testis, it seems dubious whether an earlier HCG therapy as recommended by the International Health Foundation (Geneva 1973) can improve the fertility prognosis in children with undescended testis. For this reason, particular attention should be paid to the results of treatment with LH-RH (luteinizing hormone-releasing hormone) and to the effect of this hormone on the germinative epithelium (1).

Table 3 a and b. Comparison of mean seminiferous-tubule areas of non-treated and HCG treated control and experimental animals of two age-groups (pubertal and postpubertal). CA: control animals without HCG treatment, EA: experimental animals without HCG treatment, CA: control animals with HCG treatment, EA: experimental animals with HCG treatment, *: significance a = 0.01

	CA	EA	EA
CA	34*	38*	47*
CA		6	20
EA			14

	CA	EA	EA
CA	16*	18*	19*
CA		3	3
EA			1

References

1. Bartsch, G., Frick, J.: Therapeutic effects of luteinizing hormone releasing hormone (LH-RH) in cryptorchidism. Andrologia 6, 197 (1974)
2. Bierich, J. R.: Moderne Gesichtspunkte zum Problem des Hodenhochstandes. Internist 8, 42 (1967)
3. Bauer, U., Hasse, W.: Die Retentio testis im Kindesalter. Med. Wschr. 24, 7 (1970)
4. Bay, V., Carstensen, E., Matthaes, P.: Die operative Behandlung der Hodendeszensusstörungen. Tägl. Prax. 10, 635 (1969)
5. Bay, V.: Diagnose und Therapie der Deszensusstörungen des Hodens. Pädiatr. Prax. 9, 215 (1970)
6. Gross, E., Jewett, C.: Surgical Experiences from 1 222 operations for undescended testis. J. Amer. med. Ass. 160, 634 (1956)
7. Hecker, W. Ch., Hienz, H., Mengel, W.: Frühbehandlung des Maldeszensus testis. Eine Konsequenz neuer bioptischer Untersuchungen. Dtsch. med. Wschr. 97, 1325 (1972)
8. Hennig, A.: Kritische Betrachtungen zur Volumen- und Oberflächenmessung in der Mikroskopie. Zeiss-Werkz. 30, 78 (1958)
9. Hennig, A.: Fehlerbetrachtungen zur Volumenbestimmung an der Integration ebener Schnitte. In: Quantitative Methoden in der Morphologie. Hrsg. Weibel, E. R., Elias, H. Berlin Heidelberg, New York: Springer 1967
10. Ichikawa, T.: cited by Gasser, G.: Kryptorchismus. Urologe 2, 316 (1963)
11. Karcher, G.: Die Fertilität des behandelten pathologischen Hodenhochstandes (Bauch- und Leistenhoden) unter besonderer Berücksichtigung des Zeitpunktes der hormonellen bzw. operativen Therapie. Langenbecks Arch. Chir. 317, 288 (1967)
12. Knorr, D.: Die Hormontherapie der Retentio testis. Arch. klin. exp. Derm. 227, 707 (1966)
13. Knorr, D.: Diagnose und Therapie der Deszen-

susstörungen des Hodens. Pädiatr. Prax. 9, 299 (1970)
14. Knorr, D.: Testosteronproduktion. Dtsch. med. Wschr. 98, 1087 (1973)
15. Louwerens, B.: Einige Bemerkungen zur Phermakologie der Gonadotropen Hormone. Acta endocrinol. (Suppl.) 148, 46 (1970)
16. Maddock, W.W., Nelson, W.O.: The effects of chorionic gonadotropin in adult men: Increased estrogen and 17-ketosteroid excretion, gynecomastia, Leydig-cell stimulation and seminiferous tubule damage. J. clin. endocrinol. Metab. 12, 985 (1952)
17. Maier, W., Spann, W.: Die Bedeutung der rechtzeitigen Behandlung des Hodenhochstandes für die Fertilität. Dtsch. med. Wschr. 87, 1697 (1962)
18. Mönks, B.-R.: Ergebnisse von Tubulusmessungen an Hoden puberaler Hunde unter verschiedenen Bedingungen des experimentellen unilateralen Kryptorchismus. Diss. Bonn 1975 (in Vorbereitung)
19. Nelson, W.O.: Interpretation of testicular biopsy. J. Amer. med. Ass. 151, 449 (1953)
20. Nowakowski, H.: Diagnose und Therapie der Hodenfunktionsstörungen im Knaben- und Mannesalter. Med. Klinik 47, 1995 (1955)
21. Pröscholdt, M.: Spermatographische Nachuntersuchungen nach HCG-behandeltem Hodenhochstand. Diss. München 1972
22. Seguy, E.: Ectopie testiculaire et stérilité masculine. These Paris 1961
23. Shapiro, B.: Können wir die verzögerte Pubertätsentwicklung durch Hormontherapie fördern? Med. Welt 45, 1637 (1929)
24. Shapiro, B.: Ist Kryptorchismus chirurgisch oder hormonell zu behandeln? Dtsch. med. Wschr. 57, 718 (1931)
25. Shirai, M., Matsushita, S., Ichijo, S., Takeuchi, M.: Histological changes of the scrotal testis in unilateral cryptorchidism. Tohoku J. exp. Med. 90, 363 (1966)
26. Sitte, H.: Morphometrische Untersuchungen an Zellen. In: Quantitative Methoden in der Morphologie. Wrsg. Weibel, E.R., Elias, H.. Berlin, Heidelberg, New York: Springer 1967
27. Tittert, D.: Quantitativ-histomorphometrische Untersuchungen mittels Punktzählverfahren am orthotopen Hoden des Hundes bei experimentellem unilateralem Kryptorchismus mit und ohne HCG-Behandlung. Diss. Bonn 1976 (in Vorbereitung)
28. Weißbach, L., Müller, R.: Histometrische Untersuchungen am puberalen Hundehoden bei experimentellem unilateralem Kryptorchismus. Z. Kinderchirurgie (im Druck)
29. Weißbach, L.: Histomorphometrische, autoradiographische und enzymhistochemische Untersuchungen am puberalen und postpuberalen Hundehoden unter den Bedingungen des experimentellen unilateralen Kryptorchismus mit und ohne HCG-Applikation. Med. habil. Bonn 1975

Dr. B. Ibach
Urolog. Univ.-Klinik
D-5300 Bonn-Venusberg
Federal Republic of Germany