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THE NORMAL, CRYPTORCHID AND RETRACTILE PREPUBERAL HUMAN TESTIS: A COMPARATIVE MORPHOMETRIC ULTRASTRUCTURAL STUDY OF 101 CASES

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Abstract

Fifty-two surgical biopsies from retractile testes of patients in pediatric age (3-14 years), of which 25 were treated with hormonal therapy (RT) and 27 did not undergo therapy before orchidopexy (RNT), were compared with the biopsies of 19 normal (N) and 30 cryptorchid or ectopic (E) testes. A light and electron microscopic morphologic and morphometric study was performed. For the quantitative investigation 4 parameters were selected: **a**) the mean tubular diameter (on 20 cross-sections); **b**) the mean spermatogonial number per tubular section; **c**) the mean nucleolar area of the Sertoli cells; and **d**) the mean thickness of the tubular basal lamina. The 101 biopsies were collected for statistical evaluation into four age groups: 3-6 years, 7-10, 11-13 without spermatogenesis and 10-14 with signs of early spermatogenesis.

In the RT category the mean tubular diameter and the mean spermatogonial number were similar to N in the first two age groups, but were significantly reduced in the RNT categories.

The morphometric study of the Sertoli cell nucleolar area confirms the delay of maturation observed in the categories of RT, RNT and E. In normal biopsies, the basal lamina shows a progressive reduction of the thickness, with the lowest values around puberty, while constantly higher values were found in the other categories, although this increase is not statistically significant.

Key Words: Retractile testes, cryptorchidism, light microscopy, ultrastructure, morphometry, hormonal therapy, orchidopexy.

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Introduction

Retractile testis is a particular form of undescended testis. Characteristically in this condition the testes are not found in the scrotum upon clinical examination. Although they can be manipulated into their normal position, after a variable period of time the testis is again withdrawn possibly because of the cremasteric reflex, still very active in children. This condition is generally considered to be spontaneously regressing and devoid of both pathological lesions and clinical consequences after puberty (Kirmisson, 1898; Browne, 1949; Caucci, 1950; Gross, 1953; Caucci and Caucci, 1959; Benson *et al.*, 1962; Caucci, 1966; Puri and Nixon, 1977; Hedinger, 1979; Schoorl, 1982). Alexandre (1977), in a study of 22 adults, first proposed that retractile testis is a "degraded" form of cryptorchidism, responsible for decreased fertility, and raised the question whether therapeutic intervention was necessary. Nistal and Paniagua (1984) described a mosaic pattern of histological lesions in the testes of 23 infertile young adults, in which the presence of bilateral retractile testes was the only apparent cause of infertility. Sperm cell count improvement was seen in 9 of the 15 patients examined after fixation of retractile testes. In the same period, a five years follow-up study reported a reduction of the testicular volume in 49 out of 100 children with monolateral retractile testes (Wyllie, 1984).

More recently Ito *et al.* (1986) described a reduction in testicular volume, mean tubular diameter and the number of spermatogonia in monolateral retractile testes, when compared with the contralateral organ, in a series of 28 prepuberal cases.

In a preliminary study of 20 prepuberal patients with retractile testes, we observed some ultrastructural lesions in the testes quite comparable with those described in cryptorchid subjects (Cinti *et al.*, 1987).

In the present study we have investigated the morphology and the morphometry both at light and electron microscopic level of 52 biopsies taken from retractile testes in a group aged between 3 and 14 years. The data were also compared with 19 normal cases and 30 cryptorchid biopsies, taken by the same surgeon and processed according to the same method.

Materials and Methods

Clinical data

In the period 1980-1990, 880 children with undescended testes were investigated in the Surgical Pediatric Division of the Salesi Hospital, Ancona, Italy. There were 195 (22.1%) cases of retractile testes. Among these 52 (27%) descended spontaneously into the scrotum after a variable period of time but all before the sixth year of age, 91 (46.6%) were cured by the hormonal therapy and 52 (26.7%) treated by orchidopexy. Among the 52 operated cases, 25 RT (RT = retractile with previous hormonal therapy) had previously undergone two cycles of hormonal therapy according to a protocol adopted by different authors (Benso, 1984; Rajfer *et al.*, 1986). These 25 RT cases underwent surgical therapy because the hormonal therapy failed to fix the testes in the intrascrotal position; furthermore the consistency of these testes was reduced compared to normal. Fifteen out of the 25 RT (60%) were monolateral and 10 (40%) bilateral. The remaining 27 RNT cases (RNT = retractile without hormonal therapy) were directly operated: in fact, these testes either were hypotrophic and reduced in consistency, or remained for long periods in an extrascrotal position. Twenty four out of 27 RNT (88.8%) were monolateral and 3 (11.2%) bilateral. The 30 cases of cryptorchid testes (E), of which 24 (80%) monolateral and 6 (20%) bilateral, refer to situations of different anatomical impairment of the normal descent into the scrotum at various levels, according to different classifications (Kirmisson, 1898; Browne, 1949; Caucci, 1950; Gross, 1953; Caucci and Caucci, 1959; Caucci, 1966; Puri and Nixon, 1977; Hedinger, 1979; Schoorl, 1982). The 19 normal cases (N) were taken during operations on spermatic cord cysts or inguinal hernias, to determine the histology of the seminiferous epithelium. All of these cases were in the range of 3-14 years of age.

Morphology

A total of 120 biopsies were studied (19 N; 36 E; 35 RT; and 30 RNT). Specimens were processed according to standard methods for ultrastructural investigation: rapid fixation in the operating room using a 2% glutaraldehyde in 0.1% cacodylate buffer pH 7.4 for two hours; 1 hour postfixation in 1% osmium tetroxide, dehydration in alcohols, and Epon-Araldite embedding. Semithin sections (2 μm thick; 3-8 for every sample, stained with toluidin blue) were observed under a light microscope. Ultrathin sections, mounted on 300 mesh nickel grids and counterstained with lead acetate, were observed under a Philips CM10 transmission electron microscope (TEM).

Morphometry

Of the 120 biopsies studied in total, we performed a quantitative study on a group of 101 biopsies (19 N; 30 E; 25 RT; and 27 RNT), as the morphological investigation (see **Results**) of the two biopsies taken in cases with bilateral retractile testes failed to show any significant differences. To draw a quantitative profile of the maturation of the prepuberal seminiferous tubuli (Nistal *et al.*, 1980; Gambacorta *et al.*, 1980), we measured four parameters: **a**) the mean tubular diameter; **b**) the mean number of spermatogonia per transverse section; **c**) the mean nucleolar area of the Sertoli cell; and **d**) the thickness of the tubular basal lamina.

a) **Mean tubular diameter** was evaluated on 20 transverse sections under a light microscope, using a 40X objective and a micrometric caliper (Ito *et al.*, 1986; Holstein *et al.*, 1988).

b) **Number of spermatogonia** was evaluated on the same sections used for the measurement of the tubular diameter. For the statistical analysis the mean number of gonia per section was calculated.

c) **Sertoli cell nucleolar area**. Ultramicrographs of 20 Sertoli cells with a nucleus showing a nucleolus, at a final magnification of 4,750X, were taken using the Philips CM10 TEM. Each nucleolar area was measured by an IBAS Kontron image analyzer. These data were collected "blindly" by the same examiner, without any previous knowledge neither of the age and the group nor of the results of the other parameters.

d) **Basal lamina thickness** was directly measured at a final magnification of 140,000X, using the distance function integrated in the Philips CM10 TEM computer program. Twenty measurements per case were collected by an operator and compared with a second set of measurements from a second examiner. The distance between the cellular membrane of the basal cells and the external part of the lamina densa of the basal lamina was calculated. Measurements were collected along the thinnest segments of every tubule and the mean value of 20 measurements used for statistical evaluation. The difference of the mean values between the two observers was less than 10 nm.

Statistical evaluation

The 101 testicular biopsies included in this morphometric study (see above) were subdivided into different age groups, in order to have a sufficient number of cases to submit to variance analysis. N and RT categories were grouped into four age groups: 3-6 years, 7-10 years, 11-13 years without signs of spermatogenesis, and over 10 years with signs of early spermatogenesis. All the RNT and E biopsies fell into the first three age groups, as they did not show signs of spermatogenesis. The mean value and the standard errors (SE) of the quantitative data of the four categories (N, RT, RNT, E) falling into the four age groups, were submitted to variance analysis.

Results

Morphology

Normal cases. The testicular biopsies taken upon surgery for sperm cord cysts or inguinal hernia, showed a normal spectrum of light and electron microscopic aspects.

The basal lamina in the 9 biopsies from patients aged between 3 and 6 years did not reveal irregular or thickened segments. The germ cells, mostly A pale and A dark with scattered B types, failed to show any ultrastructural alterations or regressive aspects. The Sertoli cells exhibited the immature pattern typical at this age: a round to oval nucleus with regular outline, a small sharply defined nucleolus, a cytoplasm containing a distinct Golgi apparatus, some rows of rough endoplasmic reticulum (RER), ribosomes and polyribosomes. Immature cells filled the interstitium between the tubules. A similar pattern was evident in the biopsies of the second age group. The three cases falling in the third age group (11-13 years) revealed a more irregular pattern: the tubular diameter was increased in some areas, with a uniformly thin basal lamina; the morphology of spermatogonia was similar to that observed in the younger age groups, while the Sertoli cell nuclear outline appeared more irregular with some small indentations, as well as enlargement of the nucleolar area. The Sertoli cytoplasm appeared richer in organelles and had a higher number of both smooth (SER) and rough endoplasmic reticulum and some small lipid vacuoles. The three biopsies from the fourth age group (11-14 years with spermatogenesis) showed very large tubuli with more numerous spermatogonia, often located also in a parabasal position and with more frequent mitotic figures. Spermatocytes with the different patterns of chromatin condensations were also present. The basal lamina was thin and regular. In these cases, the Sertoli cells showed a more irregular nucleus with indentations. The nucleolus exhibited a typical pattern for mature Sertoli cells: a granular electron dense compact mass from which thin and tortuous stacks arose; spheridia could also be observed. The cytoplasmic organelles were more prominent: both RER and SER were abundant, as well as lysosomes and lipid vacuoles. In the interstitium mature Leydig cells were recognizable.

Cryptorchid testes. At light microscopic level a reduction in both the number of gonia and the tubular diameter with an increased interstitial space was evident. This pattern was more marked in the older age groups. The Sertoli cell cytoplasm, specially in older patients showed abundant lipofuchsins. Binucleate and multinucleate spermatogonia, as well as giant germinal cells, were frequent. Especially in these last cells, electron microscopy revealed the presence of nuclear blebs. Sertoli cells with an immature pattern (roundish and regular, euchromatinic nuclei with a small and compact nucleolus, poorly differentiated cytoplasm) were frequent in testicular biopsies of 10 years old patients. Dilated cisternae of SER in the supranuclear cytoplasm, a reduced amount of phagolysosomes and lipofuchsins, as compared with the normal tissue in the same age groups, were observed. Spindle shaped fibroblast-like cells were present in the interstitium. Mature Leydig cells were rarely observed in the older age group. The basal lamina was focally or diffusely thickened, or laminated with frequent knobs. Again, the lesions were more evident and diffuse in older patients. No significant differences between the two testes in children with bilateral lesions were observed.

Retractile testes (Figures 1-4). The retractile testes shared many light and electron microscopic characteristics with the cryptorchid testes. Thus, binucleated and multinucleated gonia, with nuclear blebs were often found. The Sertoli cells showed signs of immaturity and damage: dilated cisternae of SER, lipofuchsin storage, regular, immature-type nuclei were still persistent in boys older than ten years. The basal lamina was often thickened with frequent knobs. Analogously to cryptorchid testes, no differences were found between the testes in subjects with bilateral retractile testes. In the interstitium, some mature Leydig cells were recognizable in the older age groups. They were more numerous in RT as compared with RNT testes.

Morphometry

Tubular diameter (Figure 5). The normal testis shows a constant increase of the values of the mean tubular diameters, from 50 μm in the first age group to 130 μm in testes with spermatogenesis. The three other categories (E, RT, RNT) show smaller values as compared with N, with the exception of the 11-13 years age group which shows quite similar data. The statistical evaluation reveals a significant difference in the 7-10 years age group between N and E, N and RNT respectively ($p < 0.05$). Only N and RT showed the spermatogenesis in the 11-14 years age group, but the mean diameter of RT in this age group was significantly smaller when compared with controls ($p < 0.05$).

Mean spermatogonial number (Figure 6). Normal tissue biopsies show only a light quantitative increase of spermatogonia in the first three age groups, with counts rising sharply with the onset of spermatogenesis. The other categories (RT, RNT, E) show smaller mean values when compared with controls. Also, the age-related quantitative increase is less evident in the three pathological categories. The differences between N and E in the first ($p < 0.001$), second ($p < 0.001$) and third ($p < 0.05$) age groups were significant. Furthermore, significance was shown for the differences between N and RNT in the first two age groups ($p < 0.01$) and between N and RT in the group with spermatogenesis ($p < 0.01$).

Mean nucleolar area of Sertoli cells (Figure 7). A sharp increase of the nucleolar area is evident in normal testes with the onset of spermatogenesis. The three pathological categories show smaller values as compared to normal tissue in all the age groups. Significant differences exist in the 3-6 age group between N and E ($p < 0.001$) and between N and NRT ($p < 0.05$); in the 7-10 age group between N and E ($p < 0.05$), between N and RT ($P < 0.01$) and between N and NRT ($p < 0.01$). A similar result was evident in the third age group, while no significative differences were found in the age group with spermatogenesis.

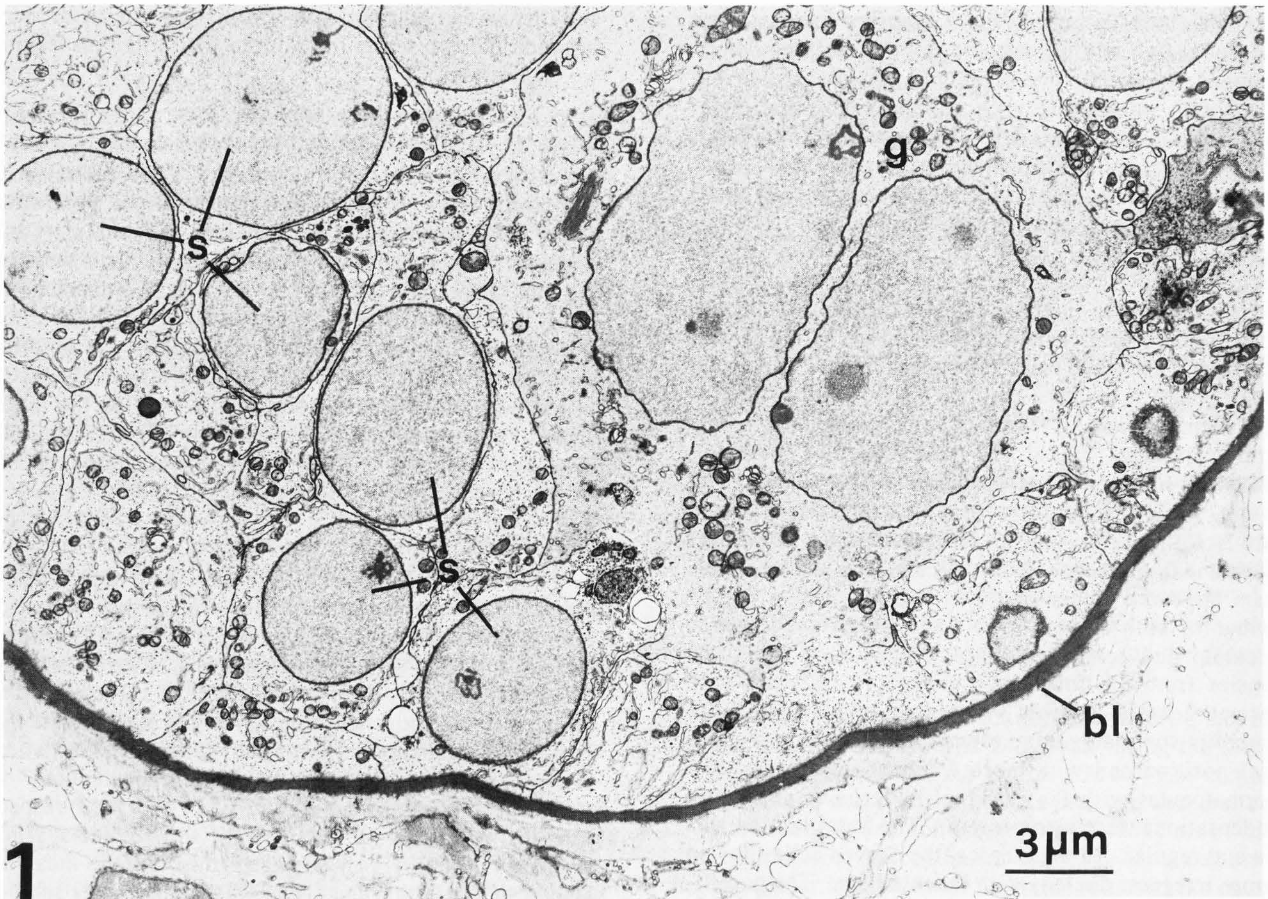


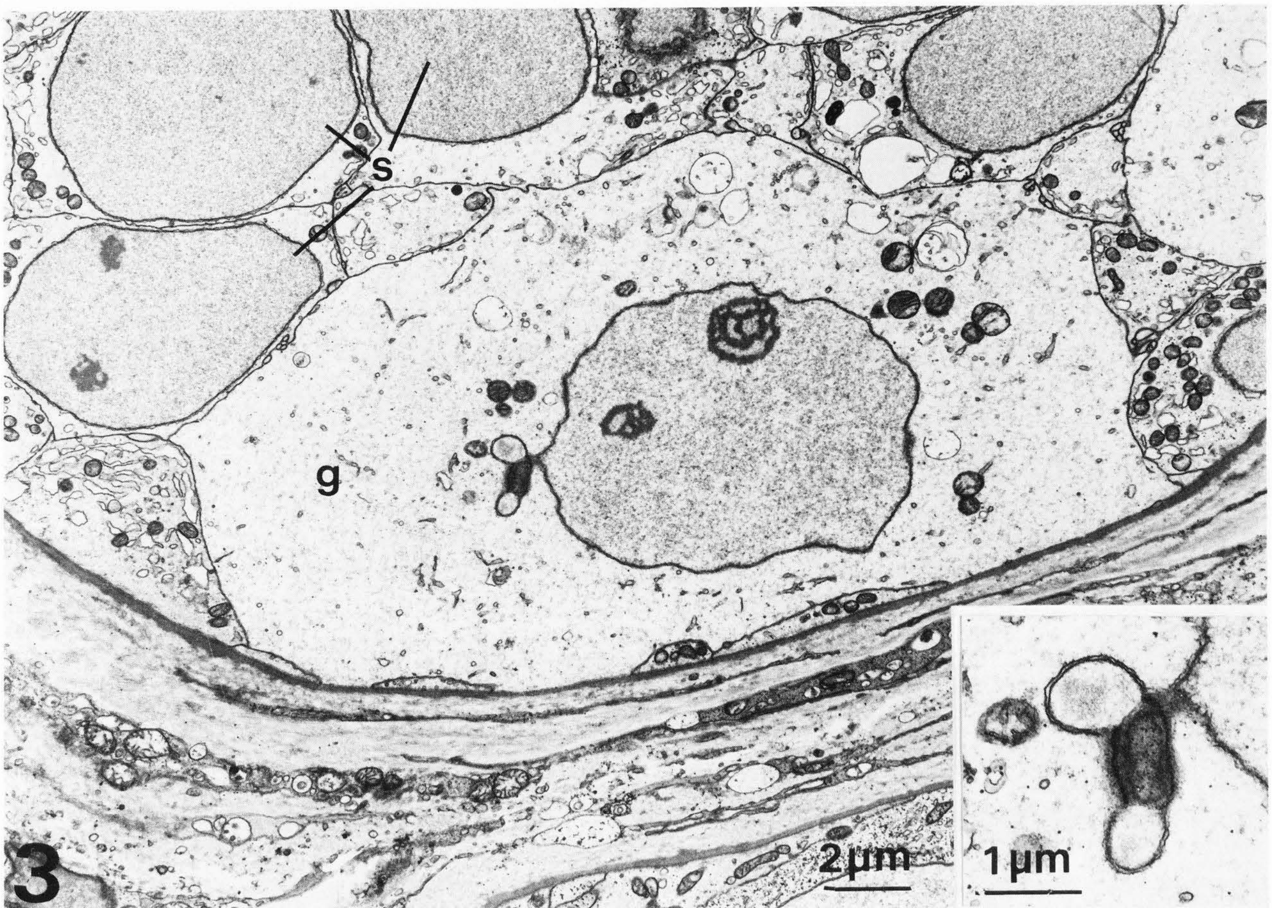
Figure 1. RNT, 6 years old. The basal lamina (bl) is diffusely thickened. The Sertoli cells (s) are poorly differentiated, with roundish nuclei and small nucleoli. The spermatogonium (g) is binucleated.

Figure 2. RNT, 6 years old. Three-nucleated spermatogonium. bl: basal lamina.

Basal lamina thickness (Figure 8). Normal testes showed a quite constant reduction of the mean values of the basal lamina thickness, ranging from a value of 180 nm in the first age group to 90-100 nm with the beginning of spermatogenesis. The thickness in the other three categories (RT, RNT, E) was similar or slightly higher as compared with normal, in the 3-6 years age groups. This value is constant in the older age groups of RT, RNT and E categories, whereas normal testes show a reduction of the thickness. In the oldest boys with spermatogenesis, the thickness we measured in RT biopsies was significantly higher when compared with normal tissue ($p < 0.01$).

Discussion

It is generally agreed that tubular lesions with onset in early infancy are found in the true undescended testis (Hadziselimovic *et al.*, 1975; Schoorl, 1982). These lesions can be responsible for a gradual reduction, or even a complete loss of the germinal epithelium with hyperplasia and immaturity of the Sertoli cells. Ultimately a progressive thickening of the lamina propria, leading to end stage testicular hyalinosis may result in a high risk of infertility in adult life (Mengel *et al.*, 1974; Hadziselimovic *et al.*, 1975; Gambacorta *et al.*, 1980;



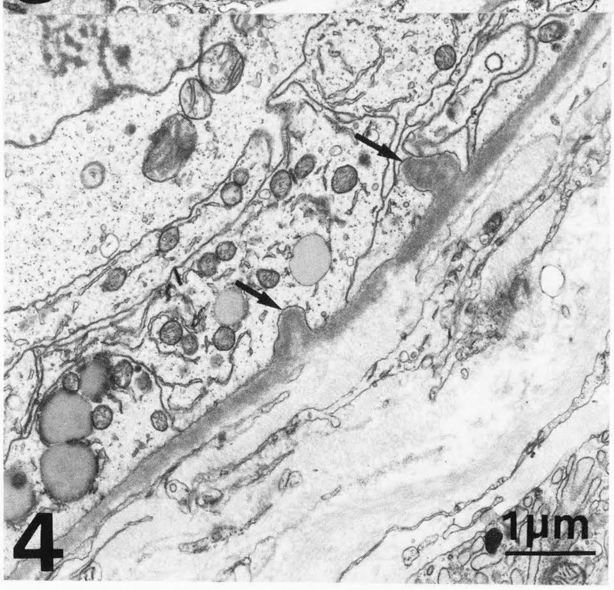
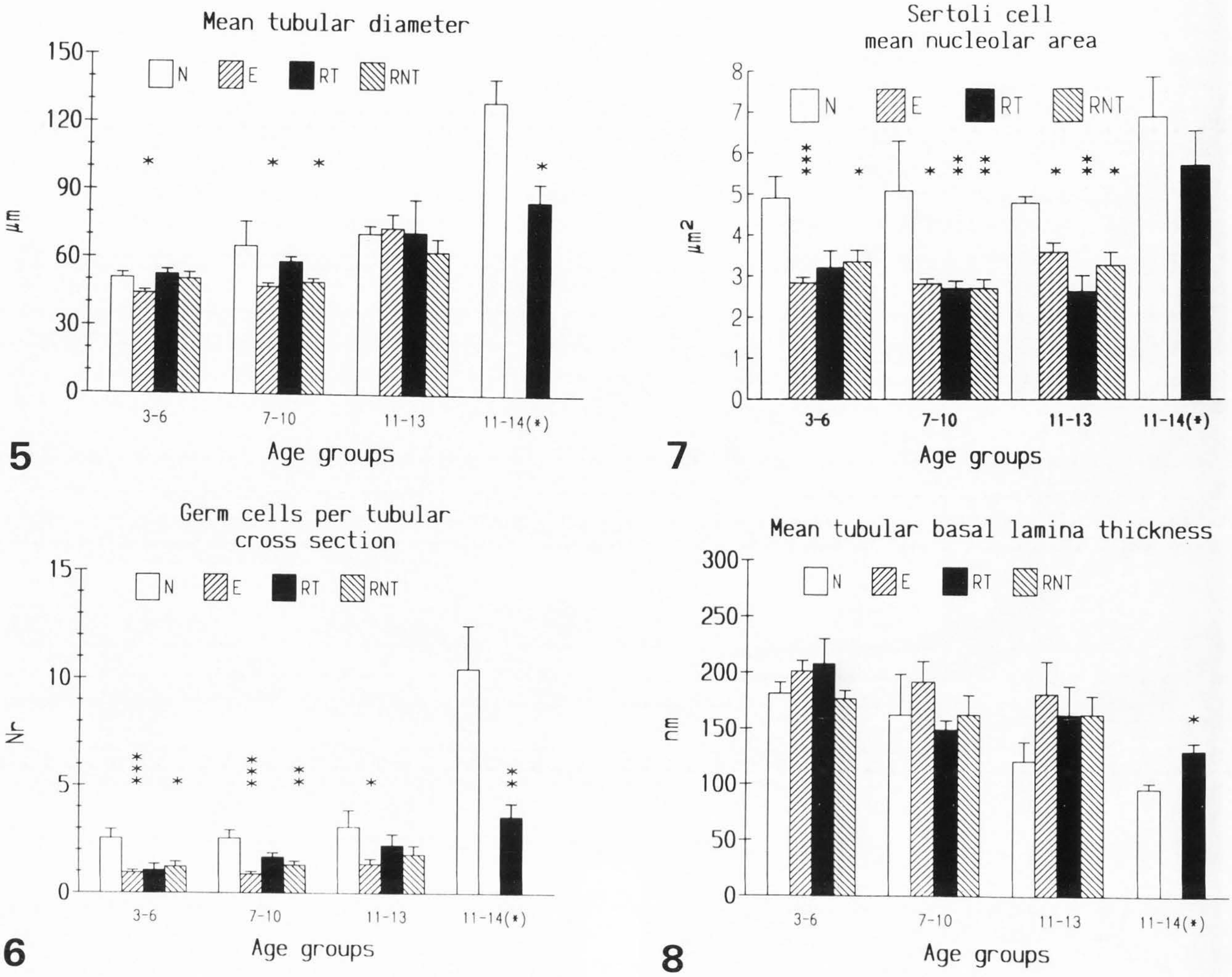


Figure 3. RNT, 10 years old. The Sertoli cells (s) are poorly differentiated. The spermatogonium (g) shows nuclear blebs (shown at higher magnification in inset). **Figure 4**. RNT, 13 years old. The thickened basal lamina shows numerous knobs (arrows).

Nistal *et al.*, 1980, 1982, 1985; Thorup *et al.*, 1984; Söderström, 1986). Although the pathogenesis of these lesions is still debated (Salle *et al.*, 1968; Farrington, 1969; Hedinger, 1971; Atkinson, 1973; Jones *et al.*, 1977; Hadziselimovic, 1982) a general consensus exists about the need of an early surgical therapy with the aim to restore the undescended testis to its natural, scrotal, site (Hadziselimovic *et al.*, 1975; Editorial, 1986).

The retractile testis in its classical definition (Kirmisson, 1898; Browne, 1949; Caucci, 1950, 1966, 1979; Scorer and Farrington, 1971; Schoorl, 1982) was described as a benign condition, in which therapy was not deemed to be necessary because of spontaneous descent at puberty. Over the last 15 years, however, pathological analysis of retractile testes has revealed some alterations comparable to those found in cryptorchid lesions. Such lesions might be responsible for the infertility of some adult males with a history of retractile testes (Alexandre, 1977; Wyllie, 1984; Nistal and Paniagua, 1984; Ito *et al.*, 1986; Caucci, 1987; Cinti *et al.*, 1987).

On the basis of a large number of cases, our present data confirm the qualitative and quantitative alterations observed clinically or under the light microscope (Alexandre, 1977; Wyllie, 1984; Nistal and Paniagua, 1984; Ito *et al.*, 1986; Caucci, 1987; Cinti *et al.*, 1987). In addition, we have performed a comparative analysis



Figures 5-8. Comparison of the morphometrical data obtained from the light microscopic (**Figures 5, 6**) and the electron microscopic (**Figures 7, 8**) investigation of the testicular biopsies. N: normal testes; E: cryptorchid (or ectopic) testes; RT: retractile testes treated with hormonal therapy prior to orchidopexy; RNT: retractile testes not treated with hormonal therapy prior to orchidopexy.
(*)= subjects with signs of spermatogenesis. * = $p < 0.05$; ** = $p < 0.01$; and *** = $p < 0.001$.

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among normal, retractile and cryptorchid testes at different prepuberal stages. The results from this comparative study suggest that hormonal therapy should be recommended, especially during the first 10 years of life. In fact, the mean tubular diameter and the mean spermatogonial number of our RT cases, though reduced, are not significantly different from normal. Nevertheless, we cannot be sure that the better morphological conditions of the RT testes when compared to RNT, is due to the therapy. In fact, the RNT cases were directly operated because their clinical characteristics, i.e., hypotrophy and reduced consistency of testes, indicated the need for an immediate surgical approach.

Furthermore, it should be mentioned that RT biopsies in the group with spermatogenesis (10-14 years) show a significative reduction, both of the mean tubular diameter and of the number of gonia, despite the hormonal therapy. Our cases show a better morphology in the retractile testes treated with therapy, but their values never exceed those in the normal cases, demonstrating the absence of undesired hormonal side effects, at least on the gonadic tissue.

The morphometric investigation we performed at the ultrastructural level showed a significant delay of the maturation of the Sertoli cells in the categories of retractile and cryptorchid testes, as stressed by the degree of nucleolar development. This morphometric parameter correlates with the globally immature pattern we observed at the ultrastructural level in the qualitative study. However, no significative difference was found in the group with spermatogenesis. Other authors observed an immaturity of Sertoli cells in pediatric cryptorchid testes, by studying other parameters such as the period of maturation of the occludentes junctions (Läckgren and Plöen, 1984a). The same authors also studied the effect of therapy with human chorionic gonadotrophin (hCG) on the morphology of both undescended and contralateral testis in the prepuberal period (Läckgren and Plöen, 1984b). These authors observed an increase (however temporary) of the blood levels of testosterone.

Quantitative morphology has not been performed on tubular basal lamina in normal prepuberal testes. Data from adult samples showed a wide range of variation (Holstein *et al.*, 1988). Our data indicated a progressive reduction of the thickness, with the lowest value (90-100 nm) corresponding to the cases with early spermatogenesis. The thickening of the basal lamina and the hyalinosis of the lamina propria is a well recognized observation both in the prepuberal and in adult life. This lesion progresses with the progressive increase of the damage (Cotelli *et al.*, 1979; Hedinger, 1982; Nistal *et al.*, 1985; Holstein *et al.*, 1988). In our cryptorchid series we found constantly higher values of basal lamina thickness after the first years of life, with the highest levels at puberty. Retractile testes show a more irregular trend: in fact we found cases with thick, as well as cases with normal basal lamina, in the same age groups. This heterogeneity confirms results obtained earlier (Cotelli *et al.*, 1979) and explains the absence of statistical significance, although the mean values are constantly higher than normal. Only in the group with spermatogenesis, we found a significant difference, with retractile testes showing higher values than normal.

In conclusion, the retractile testes show important alterations at the level of tubular diameter, the germinal epithelium and the basal lamina. The quantitative morphometric investigation delineates a definite category, the retractile testis, which, with the exception of the Sertoli nucleolar area, is significantly different from normal.

In our opinion, the present work confirms earlier suggestions (Wyllie, 1984; Nistal and Paniagua, 1984; Ito *et al.*, 1986) that the "pendular" movement of the testis, whichever the cause, is responsible for morphological damage, that may develop during infancy. Damage is increasingly more evident in older patients and the morphological pattern in some cases of retractile testes is quite similar, both qualitatively and quantitatively, to that observed in cryptorchid testes. Therefore, considering that there is a general consensus about the opportunity to operate on cryptorchid patients at an early age (Hadziselimovic *et al.*, 1975) and that adult oligospermic patients with retractile testes obtained an improvement of the sperm cell count after orchidopexy (Nistal and Paniagua, 1984), we conclude that our data support the decision to submit to a hormonal therapy, and subsequently to surgery those patients with retractile testes lacking a spontaneous descent.

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Discussion with Reviewers

L. Plöen: Please discuss the maturation of Sertoli cells in cryptorchid testes; this has been studied also by others, who have used the development of the inter-Sertoli cell occluding junctions as a sign of maturation (Läckgren and Plöen, 1984b). Based on the observed changes in retractile testes, the authors conclude that this condition should be treated, and they suggest hormonal therapy as the first choice. This conclusion seems relevant, considering the high risk of injuries to the testis or the testicular vessels when operating very young boys. Could they discuss their finding from a physiological point of view, mentioning differences between hormone treated and not treated testes, which is of interest since a premature testicular development would imply that hormone therapy has severe side effects.

Authors: We agree that the development of the inter-Sertoli occluding junctions could be used to study the degree of maturation of these cells, but we preferred those parameters that in our opinion are the best to get the morphological differences of the Sertoli cells during their development in the prepuberal period. Furthermore, our parameters are easily investigated also in specimens processed by standard methods.

Finally, it should be considered that mature occludent junctions appear in a late stage of growth, in the oldest children of the stage II of Tanner, while most of our cases fall in the prepuberal age.

Our figures and **Results** show the differences of the qualitative and quantitative data between not treated and treated testes. Furthermore, the comparison with a control group, considered as normal, is shown. Our results demonstrate that the cases with retractile testes treated with hormonal therapy show an improvement of the parameters studied. These parameters approach to, but do not exceed those of the normal cases, so that the therapy does not seem to cause any undesired side effect of maturation, as suspected by the reviewer.

We did not discuss our data from a physiological point of view because there is a wide consensus in the literature on the correspondence between the morphology and the functional state of the testis.

L. Plöen: About the observation of "bi- and multinucleated spermatogonia and giant germinal cells", what type of germ cells were the latter?
Authors: The cell that we indicated in the text as "germ cell" is a spermatogonium; "germ cell" was used just to avoid using repeatedly the word "spermatogonium".

L. Plöen: Do you think that there is always an inverse relation between tubular diameter and thickness of the basal lamina implying that no additional synthesis is required or is it perhaps kept to a certain amount meaning it would be thinner if the diameter increased further?
Authors: According to the quantitative data of our investigation, an inverse relationship seems to exist between the tubular diameter and the basal lamina thickness. In our opinion, it fits with the concept that a reduced thickness of the basal lamina could facilitate the exchanges between the blood flow and the seminiferous tubuli, exchanges that could be intensified with the onset of the puberty. A further reduction of thickness, as compared with the future development of the tubuli, can be suggested but not demonstrated by our work.

L. Plöen: Which was the most developed germ cell type you observed, and are germ cells other than spermatogonia included in your counts as indicated in Fig. 6?
Authors: The most developed germ cell that we observed in the group with spermatogenesis are the spermatocytes with the various patterns of condensation of the nuclear chromatine. The number of germ cells indicated in Fig. 6 refers to spermatogonia only, in all the four groups.

B. Afzelius: You mention that testis maturation will be delayed in patients with retractile testes and that they become oligospermic. Will such patients upon maturation produce spermatozoa with a normal appearance? Is the condition compatible with fertility, even if not treated?
Authors: The statement that patients with the retractile testes have a higher risk to become oligozoospermic has been made by other authors (Alexandre 1977; Nistal and Paniagua, 1984; Wyllie, 1984; Ito *et al.*, 1986) and only reported in our paper. In any case, our data support that statement because there are many similarities between retractile and undescended testes, and it is well established that the latter condition causes oligozoospermia in the adult. We are collecting data on the seminal fluid of the young adults operated for retractile testis and we will publish the results of this study as soon as possible.

B. Afzelius: You have found that there is an inverse relationship between the diameter of the seminiferous tubules and the thickness of its basement membrane. In other words, there exists here a case of negative allometry. Such cases are rare, as pointed out by Jack Cummins (Proc. 4th Internatl. Symp. Spermatol. Seillac, 1982; J. André, editor). Do you think that the basement membrane stretches when the tubules grow thicker? Or do you have some other explanation?
Authors: We think that this is due to a metabolic activity of the cells producing the basal lamina, rather than to the stretching of the basal lamina, as a consequence of the growth of the tubuli, but this is only speculation, and the question is still open.

G.M. Roomans: Did I understand correctly that only micrographs of cells showing a nucleolus were used? Does this not give an overestimation of the nucleolar volume?
Authors: The decision to measure only the nucleoli in the Sertoli cells showing the nucleolus certainly creates an overestimation of the nucleolar area among the cells in the various patients, but such an overestimation is present in all the subjects of all the age groups and our aim was only to compare different groups, not to measure the absolute nucleolar area of the Sertoli cell. In our opinion, a confirmation that this approach is reliable, comes from the observation that in the normal group the Sertoli cells increase their nucleolar area at puberty. This data is in agreement with the qualitative pattern of a clear maturation of the cell. Furthermore, the Sertoli cells of the ectopic and the retractile testis show a significant reduction of this area and this is in agreement with the qualitative aspect of a reduced maturation of this cell.

J.R. Poley: What were the criteria for inclusion into the study group?
Authors: Considering what we described in the **Clinical data**, and in **Materials and Methods** sections, we confirm that, in our study, we included all the surgically treated patients.

J.R. Poley: Why were patients not included, whose testicles descended well after hormonal therapy?
Authors: We chose to avoid the testicular biopsy in the patients with retractile testis successfully treated by hormonal therapy for ethical reasons.

J.R. Poley: Where patients randomized?
Authors: The patients were not randomized, but we included all the patients treated by orchidopexy.

J.R. Poley: The authors state that morphometric and morphologic data apparently supported the decision to proceed with to hormonal therapy and eventually to orchidopexy: did the ultrastructural and morphometric data allow a timed decision as to when to proceed with what therapy? Did biopsies, which were obtained from hormonally treated patients give any clues as to an anticipated failure rate?

Authors: In our opinion, the ultrastructural and morphometrical results we obtained, support our decision to submit to hormonal, and eventually surgical therapy, the patients which failed to show a spontaneous descent of the retractile testis. As a matter of fact, the treated group shows, in general, better qualitative and quantitative results as compared with retractile not treated group. Nevertheless, we cannot be sure that the better morphological conditions of the RT testes when compared to RNT, is due to the therapy. In fact the RNT cases were directly operated because their clinical characteristics, i.e., hypotrophy and reduced consistency of testes, indicated the need for an immediate surgical approach.

About "when" to start therapy, we follow the criterium to submit to hormonal therapy the patients with a clinically reduced consistency of their testes and those with a prolonged permanence of the gonad in a higher, extrascrotal position,. On the other hand, we decided to operate after two cycles of hormones those testes still affected by the pendular movement which are clinically hypotrophic and with reduced consistency. Our data demonstrate that the lesions in the retractile are comparable with those in the undescended testis. This similarity supports the hypothesis that retractile testes, if untreated, might become oligospermic in adult life. About this last point, in our experience the more precocious the therapy, the better the results.

J.R. Poley: Do your data support the conclusion that all "retractile" testes should undergo orchidopexy right away, rather than wait for hormones to work, avoiding delays for testicular structures to mature more quickly?

Authors: Our data do not allow us to state if it is necessary to submit directly to orchidopexy all the retractile testes because, as stated in the text paper, under **Materials and Methods** there was a different clinical condition at the onset, between the children submitted directly to orchidopexy and those operated after a hormonal trial, the former being more damaged than the latter. However, these data show significant differences between normal and retractile, both treated and untreated, testes. Furthermore, taken into account that: **a**) the pattern of the lesions of retractile is similar to that of undescended testes; **b**) the undescended testes operated early in the first infancy have a better clinical outcome as compared with children operated in a more advanced age (Hadziselimovic *et al.* 1975); and **c**) the clinical data presented in **Materials and Methods**, our experience suggests that about 25% of retractile testes must be operated.

The group of RT shows a better general pattern as compared with the RNT group, but, on the other hand, also the clinical conditions at the onset were better. Nevertheless, it should be born in mind that also RT show significant differences in the group with spermatogenesis, as compared with normal testes. The correspondence between the degree of the morphological lesions and the functional impairment is well established (Hedinger, 1979, text reference). By analogy, a similar correspondence could be hypothesized for the retractile testes.

J.R. Poley: If ANOVA (analysis of variance) was used, where are the F values? The P values may not give appropriate discriminatory values.

Authors: We used the following method: for every age group we performed the ANOVA (Table 1 shows the F values).

After verifying that one or more groups were significantly different, as we wanted to compare each group with the normal one, we chose Student's t-test for this comparison, following the indication of various authors (Hermitage and Berry, 1991). It should be mentioned that various papers dealing with the morphology and morphometry on this argument, indicate the significance of the differences with only the P, without indicating the test used (Ito *et al.*, 1986).

J.R. Poley: The division of groups by ages is somewhat arbitrary and when Tanner stages (testicular size) are included, the data may look quite different as far as age groups are concerned.

Authors: We did not take into account the Tanner stages because most of our cases fall in a prepuberal age so that they are almost all comprised in the 1st Tanner's stage. We compared the retractile with the undescended testes and for this category, there are different studies dividing the patients into pre-school and school age groups (Bierich 1979; Chilvers *et al.* 1986). Therefore, we divided our cases in a pre-school group, a school group, a third group corresponding to the age around puberty without signs of spermatogenesis, and finally, a fourth group of the same age but with signs of spermatogenesis.

J.R. Poley: How exactly does your present work confirm that the pendular movement of the testes cause the morphologic damage described? Can you be more specific?

Authors: We can only hypothesize that different factors can participate to create the lesions in these testes and not necessarily that they are exclusively referred to the pendular mechanism. For example, a possible mechanism is the length of time that the testicle remains in the extrascrotal position, and therefore, an increase of the temperature or a venous stasis would occur, which could favor the permanence of toxic substances at this level. However, eventual disgenetic factors could contemporarily create both an unsuccessful fixation and a reduced development.

Table 1. Morphometry of testicular tissue.

Mean tubular diameter

Age group: 3-6 years, F = 2.97

Group	*n*	*Mean*	*SD*
N	9	50.9	6.64
E	12	44.16	4.90
RT	2	52.45	3.21
RNT	7	50.43	7.54

Age group: 7-10 years, F = 5.68

Group	*n*	*Mean*	*SD*
N	4	65.07	21.71
E	9	47.16	4.72
RT	14	58.22	8.56
RNT	12	49.17	6.04

Age group: 11-13 years, F = 0.46

Group	*n*	*Mean*	*SD*
N	3	70.68	6.26
E	9	73.28	18.56
RT	4	71.24	29.19
RNT	8	62.76	16.60

Age group: 11-14 years, F = 11.21

Group	*n*	*Mean*	*SD*
N	3	129	17.69
RT	5	85.13	18.07

Germ cells per tubular cross-section

Age group: 3-6 years, F = 7.33

Group	*n*	*Mean*	*SD*
N	9	2.55	1.26
E	12	0.95	0.38
RT	2	1.05	0.42
RNT	7	1.21	0.66

Age group: 7-10 years, F = 6.61

Group	*n*	*Mean*	*SD*
N	4	2.55	0.75
E	9	0.87	0.36
RT	14	1.66	0.78
RNT	12	1.30	0.66

Age group: 11-13 years, F = 2.41

Group	*n*	*Mean*	*SD*
N	3	3.07	1.36
E	9	1.35	0.72
RT	4	2.24	1.03
RNT	8	1.81	0.72

Age group: 11-14 years, F = 16.66

Group	*n*	*Mean*	*SD*
N	3	10.43	3.47
RT	5	3.56	1.40

Sertoli cells mean nucleolar area

Age group: 3-6 years, F = 7.75

Group	*n*	*Mean*	*SD*
N	9	4.89	1.59
E	12	2.82	0.48
RT	2	3.19	0.59
RNT	7	3.34	0.74

Age group: 7-10 years, F = 7.35

Group	*n*	*Mean*	*SD*
N	4	5.06	2.43
E	9	2.80	0.35
RT	14	2.68	0.71
RNT	12	2.69	0.75

Age group: 11-13 years, F = 4.99

Group	*n*	*Mean*	*SD*
N	3	4.77	0.28
E	9	3.57	0.66
RT	4	2.60	0.79
RNT	8	3.25	0.91

Age group: 11-14 years, F = 0.8

Group	*n*	*Mean*	*SD*
N	3	6.86	1.69
RT	5	5.67	1.89

Mean tubular basal lamina thickness

Age group: 3-6 years, F = 1.56

Group	*n*	*Mean*	*SD*
N	9	180.96	30.23
E	12	200.83	32.91
RT	2	207.50	31.82
RNT	7	176.29	19.60

Age group: 7-10 years, F = 1.27

Group	*n*	*Mean*	*SD*
N	4	162.06	72.56
E	9	191.33	55.08
RT	14	148.79	31.48
RNT	12	162.43	59.52

Age group: 11-13 years, F = 0.63

Group	*n*	*Mean*	*SD*
N	3	121.19	29.88
E	9	180.89	87.21
RT	4	162.25	51.34
RNT	8	162.75	46.63

Age group: 11-14 years, F = 10.98

Group	*n*	*Mean*	*SD*
N	3	95.79	9.02
RT	5	130.20	16.21

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