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ELECTRON MICROSCOPY IN TESTICULAR BIOPSY*

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Clinically, male infertility has been classified mainly on the basis of gonadotropin levels, namely, hypogonadotropic, hypergonadotropic and normogonadotropic. However, a classification from the histopathological point of view, correlating with the clinico-biochemical classification, would be of great use to the physician, especially when one bears in mind the fact that 75% of the causes of male infertility are of testicular origin while the remaining 25% are pre- and post-testicular[1], the latter being more easily diagnosed and treated than the former.

Some of the main causes of male infertility of testicular origin are listed in Table I[2].

TABLE I

Testicular Causes of Male Infertility

1. Chromosomal disorders
   a) Klinefelter's syndrome
   b) XYY syndrome
2. Vanishing testis syndrome
3. Varicocele
4. Mumps orchitis
5. Cryptorchidism
6. Chemicals
7. Irradiation

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8. Idiopathic oligospermia
   a) Maturation arrest
   b) Hypospermatogenesis
9. Germinal cell aplazia (Sertoli's cells only)
10. Idiopathic testicular failure

In this study we examined testicular biopsies obtained from oligo- and azoospermic patients under light and electron microscopy to determine whether observing the detailed ultrastructure would lead to a more definite diagnosis and would be of help to the urologist in deciding on a course of treatment.

Material and Methods

The biopsies for this study were obtained from patients applying to the Urology Departments of the İstanbul and Cerrahpaşa Faculties of Medicine with the complaint of infertility. The patients were selected according to specific criteria: they had to be young, infertile, with oligo - and azoospermia, and without any clinical or hormonal findings suggesting a pre- or post-testicular cause for infertility. Of the 22 patients selected, 3 had left varicocele, 3 were cryptorchid, 1 had Klinefelter's syndrome, 2 had received irradiation to the testicular region, one for a period of one month and the other for 8 months.

Before the biopsies were taken, the serum levels of follicule-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (T) were estimated and spermiograms were obtained.

The testicular biopsies of the patients were performed bilaterally under general anesthesia. Biopsy material was about 1 mm^3 and was fixed with buffered osmic acid and then embedded in Vestopal plastic material. The sections were examined under Zeiss EM 9 2S light and 100 C Jeol electron microscopy.

Results

The histological examination was done for mean tubule diameter, level of spermatogenesis, germinal epithelium, fibrosis or hyalinization of the tubule wall, the number and quality of Leydig's cells and percentage of abnormal spermatids.

Idiopathic oligospermia patients constituted the majority of our study group. In these cases, the histological picture is either hypospermatogenesis or maturation arrest.

A Selection of the Cases

Case 2. An oligospermic patient (8×10^6), with a slightly elevated serum level of FSH but normal serum levels of LH and T. The examination of the biopsy material under light microscopy showed that the germinal cells in the tubules had not come to the spermatozoa stage. Peritubular tissue was minimally thickened. However, electron microscopy of the same section revealed spermatogoniums, Sertoli's cells and spermatocyts on the peritubular tissue towards the lumina (Figure 1).

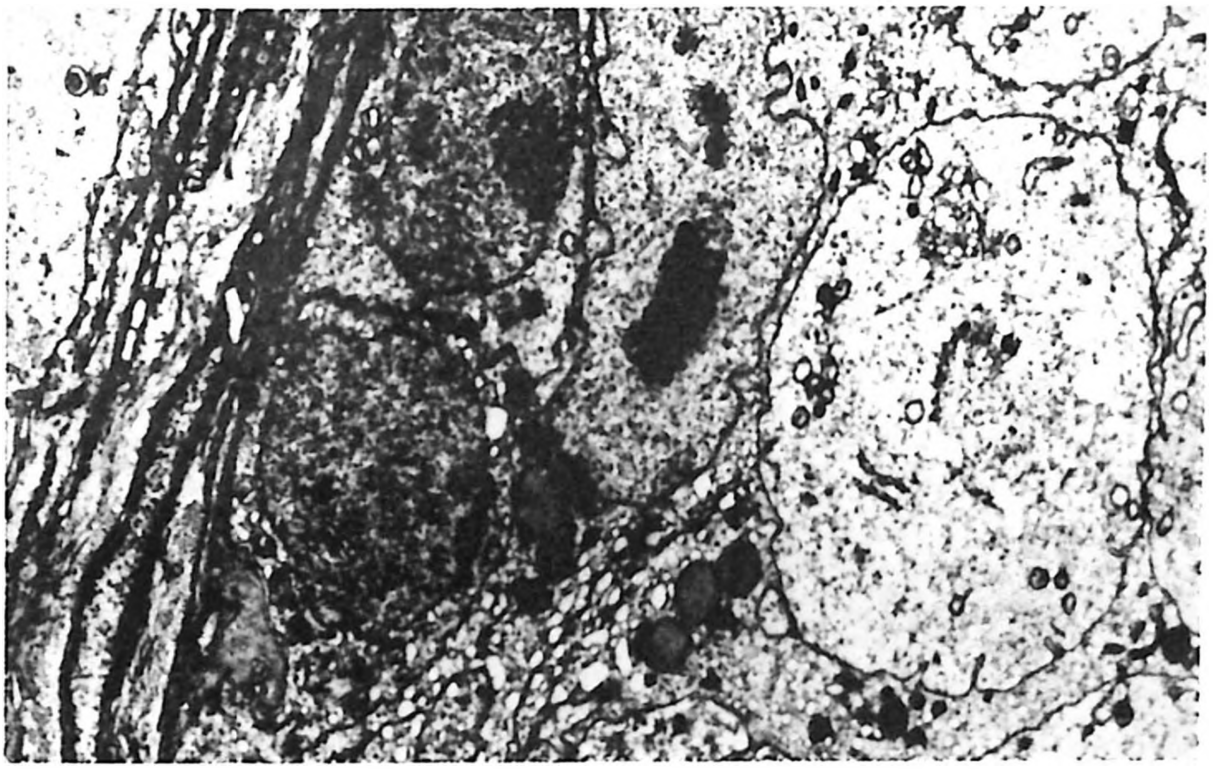

Figure 1.

On the peritubular tissue towards the lumina are seen spermatogoniums, Sertoli cells and spermatocytes. An oligospermic patient with serum level of FSH elevated.

The value of electron microscopy in this case was that it showed detailed differentiation stages of spermatogenesis, thus giving us hope for therapy.

Case 4. Patient with oligospermia (10×10^6), with serum levels of FSH, LH and T within normal limits. A study under light microscopy revealed spermatogenesis up to the spermatid level, with thickened lamina propria.

Maturation arrest is the most prevalent testicular cause of infertility revealed in biopsies from these patients. Maturation may stop at any phase, differing from patient to patient. There are no abnormalities in the Sertoli's cells, tunica propria of the seminiferous tubules or Leydig's cells[3].

Figure 2 is the view under electron microscopy of the same biopsy. A differentiation disorder is seen. Further study of the biopsy material showed binucleid spermatids.

In such cases, a detailed study of the biopsy material is necessary, because especially when there is moderate oligospermia and the serum hormone levels are normal with an arrest at the spermatid stage, outcome of therapy depends mostly on the ratio of abnormal forms of sperms. In severe cases, when the arrest is at the primary spermatocyte level, the serum FSH is elevated while LH and T are normal[1].

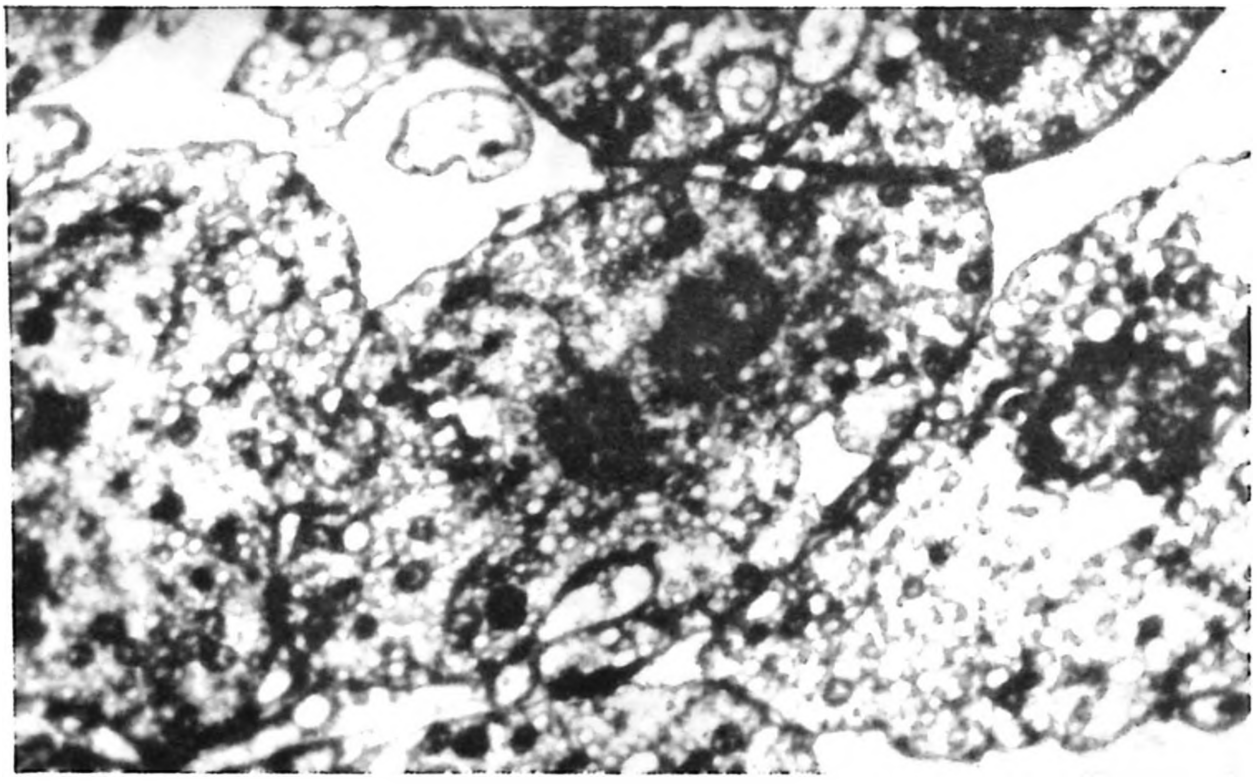

Figure 2.

A case of maturation arrest. Oligospermic patient with normal serum hormone levels. Differentiation disorder of spermatids is seen.

Case 8. The patient had received irradiation to the testicular region for a period of one month. In the second week of irradiation he was oligospermic (12×10^6), with normal serum levels of FSH, LH and T. Light microscopy evaluation of the biopsy material at this stage revealed extreme vacuolization between germ cells. Some germ cells were sloughed in the lumina.

Continuous irradiation causes progressive changes. Germ cells undergo degeneration and are eventually lost[1].

The electron microscopy study of the testicular biopsy of the same patient at the end of one month of irradiation is seen in Figure 3. Here, in addition to the vacuoli-

zation of the germ cells, spermatocytes and immature germ cells sloughed in the lumina are observed. The diameter of the tubules has become smaller, and their tunica propria is thickened. The patient was oligospermic (9×10^6) and the serum level of FSH was elevated at this stage.

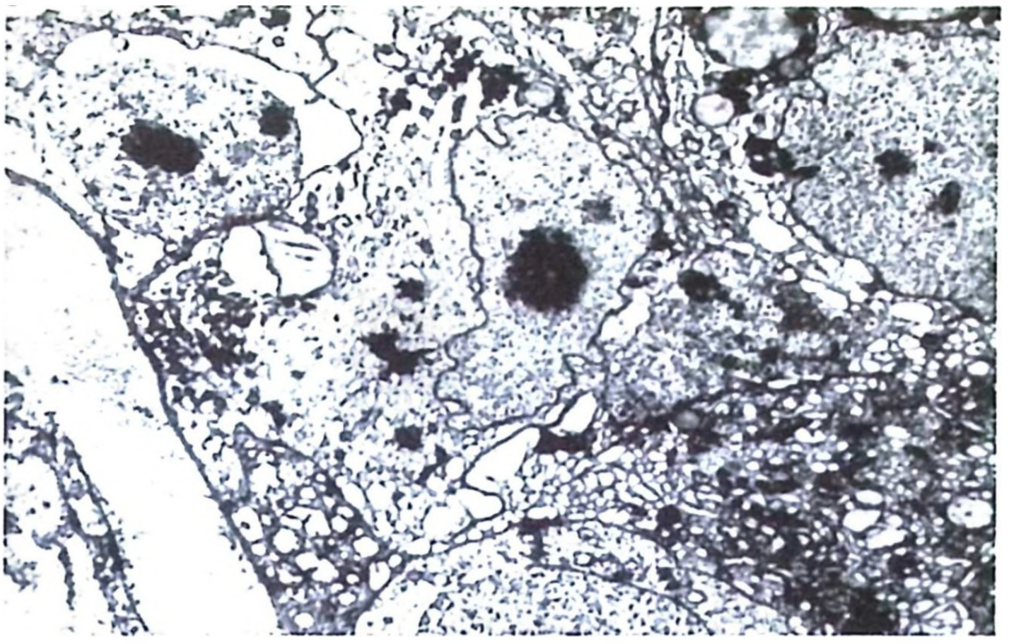

Figure 3.

Electron microscopic study of the patient who received 1 month of irradiation in the testicular region. Extreme vacuolization between germ cells is seen. Spermatocytes and immature germ cells are sloughed in the lumen. The diameter of the tubules has become smaller and their tunica propria is thickened.

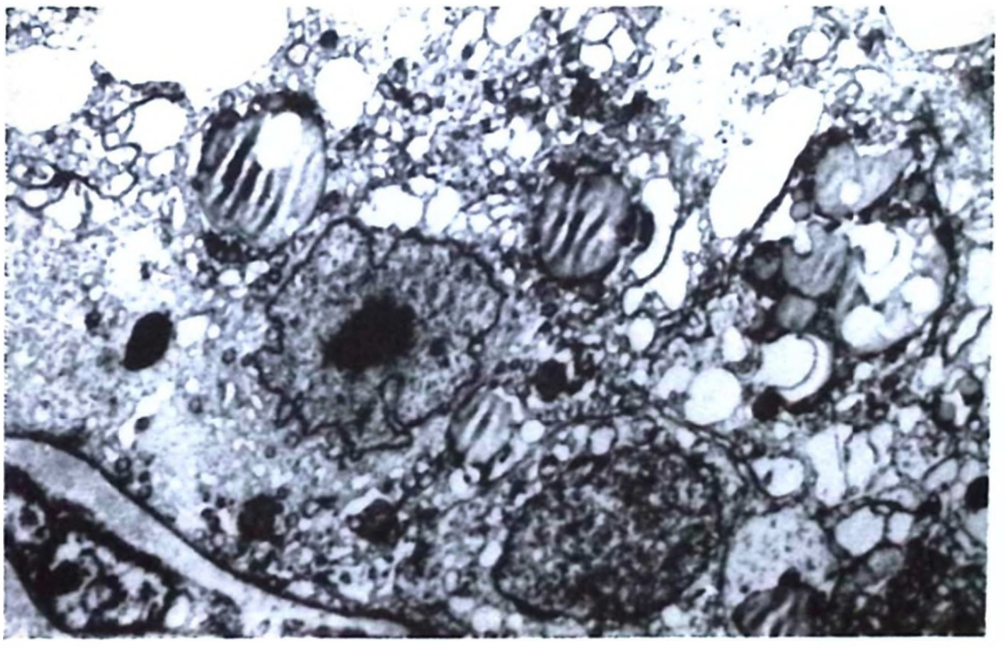

Figure 4.

Azoospermic patient with serum level of FSH elevated. Only Sertoli cells are seen. Germinal cells are vacuolized.

Case 10. A patient with azoospermia. Serum level of FSH elevated. Azoospermia with an elevated serum level of FSH is almost impossible to cure. In these patients light microscopy of the biopsy material reveals very few germ cells. A study under electron microscopy in this case (Figure 4) shows mostly Sertoli's cells, the small number of germ cells are vacuolized, thus leaving no hope for therapy.

Case 16. A left cryptorchid patient. Oligoazoospermia, serum level of FSH elevated, LH and T normal.

In cryptorchidism, we see small, immature tubules with varying amounts of spermatogenesis, depending partly on the severity of intrinsic germ cell hypoplasia, and partly on how long the testis has remained in the undescended position.

Light microscopy examination of the biopsy showed few seminiferous tubules with reduced diameter. In the lumina a peripheral ring of Sertoli's cells and occasional spermatogonia were seen. There was little evidence of spermatogenesis.

A further evaluation under electron microscopy (Figure 5) revealed the tubule diameter to be reduced and the wall thickened. No germ cells were seen in the tubules; they were lined with Sertoli's cells only. The nuclei of the Sertoli's cells were lobulated, typical for cryptorchidism, and their cytoplasm contained large numbers of fat vacuoles.

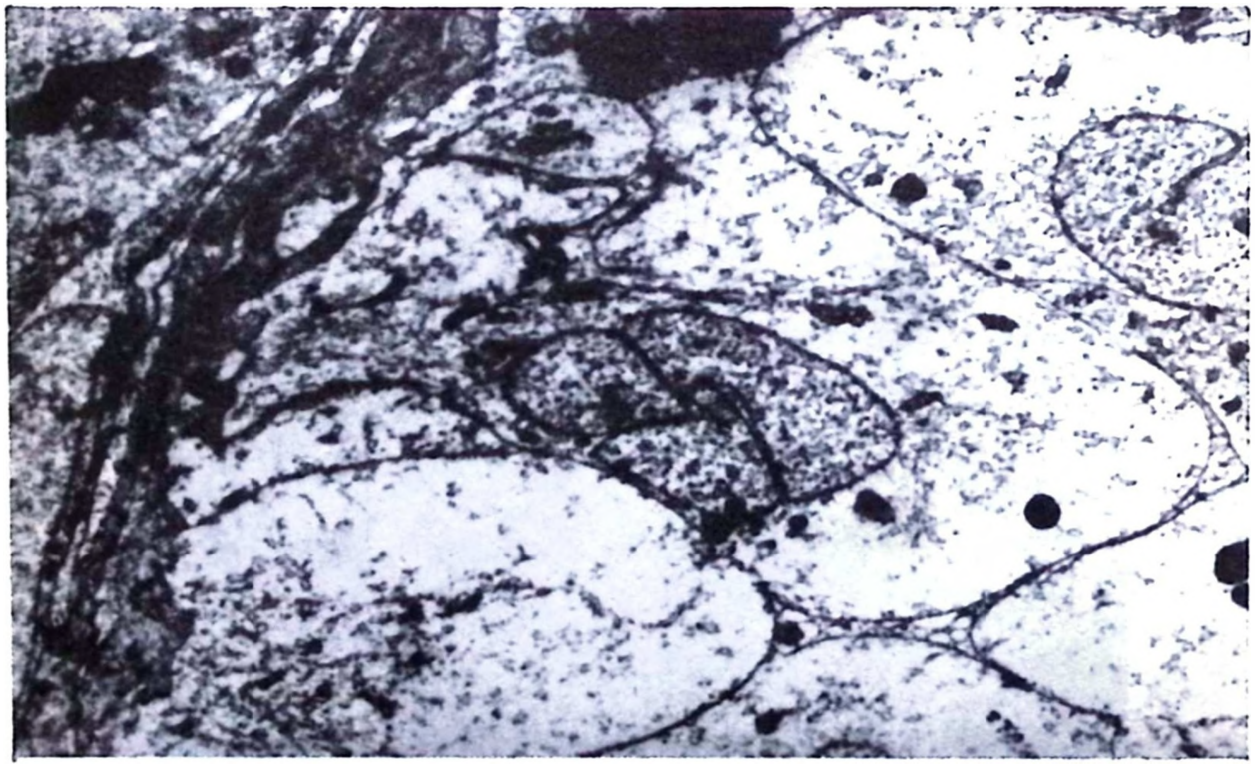

Figure 5.

Cryptorchid patient. Tubule diameter reduced, wall thickened. There are no germ cells in the tubules, only Sertoli cells are seen. The nuclei of the Sertoli cells are lobulated, typical for cryptorchidism.

In this patient, aged 20, both factors contributing to the degeneration have produced a "Sertoli Cell Only" syndrome. It is interesting to note that the right testis, which was in the scrotum, was somewhat decreased in size upon physical examination.

Case 19. An oligospermic patient with normal serum hormone levels. The light microscopy study of the biopsy material in this case showed immature germ cells sloughed in the lumina. The nuclei of these cells had aggregated together to form a group. Germinal cells were present in the tubules. Leydig's cell hyperplasia was seen in the peritubular tissue. Electron microscopy study of the same section revealed vacuolized germinal epithelium and degenerated germ cells (Figure 6).

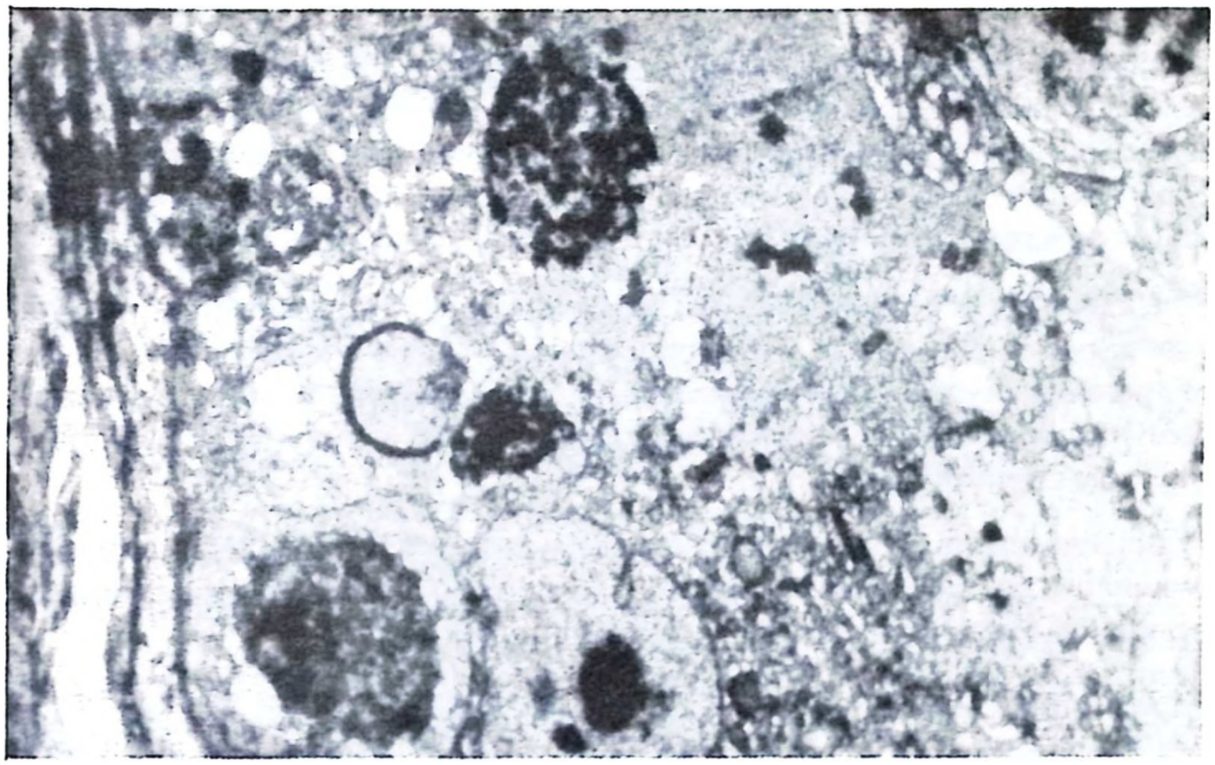

Figure 6.
A case of latent varicocele. Degenerated germinal cells are seen. Germinal epithelium is vacuolized.

This finding, which resembles hypospermatogenesis, led us to do a spermatic venography, which revealed a latent varicocele, confirmed by operation.

Discussion

The value of histopathological findings in testicular biopsies of infertile patients has been reported by many authors[4]. Some authors, however, have tried to simplify the pathological classification in order to have easy correlation with the clinical and

laboratory data[5]. We have reviewed our experience of testicular biopsies under electron microscopy in order to determine whether a prognosis for the infertile male patient can be developed in view of this new approach.

It is generally accepted that elevated FSH levels in azoospermic individuals indicate severe germinal epithelial damage, and there is no therapeutic regimen that can help this situation[6]. Detailed study of case 10 in our series revealed the same result, in addition to the finding that germinal cells were vacuolized.

The idiopathic oligospermic group, which is the majority group, is of special interest. We believe that this group will decline in the years to come, because as our knowledge is expanding, we will be able to fit these patients into other categories, as with our case 19.

Of clinical importance are the different presentations of the latent varicocele group, where the patient may present with changing clinical findings[7] or with various histopathological findings[8,9]. It is our belief that any patient with moderate oligospermia, normal serum hormone levels and a histopathological finding of degenerated germ cells needs further evaluation.

In the idiopathic oligospermic group, the determination of the stage of maturation arrest is important, because a T rebound therapy may be effective if the arrest is at the spermatid level[10]. However, in these cases, the ratio of abnormal forms of spermatids must be checked also, because if the ratio is more than 25%, then the prognosis is not very promising.

Cryptorchidism is another enigma. We believe that for any case of cryptorchidism, a peroperative biopsy would not only influence the operation, but also if the patient is having orchiopexy, would give an idea of his future fertility.

Although newer diagnostic methods and new approaches for the infertile male patient are being developed, we believe that oligo- and azoospermia resulting from testicular causes have little chance for therapy. However, via these methods for diagnosis it is possible to tell the patient frankly that he has no change of fathering a child; to inform him of other ways of acquiring a baby is also a way of helping him, and saves him from going from one doctor to another, and thus is one of the tasks of the urologist.

Summary

Alongside laboratory techniques for evaluating ejaculate, testicular biopsies are rapidly gaining in importance in cases of infertility. Here we describe a series of testis biopsies carried out on patients who complained of infertility, and the evaluation of them under light and electron microscopy. Relevant literature is reviewed and compared.

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DISCUSSION

Hargreave: First I would like to say how very much I enjoyed Doctor Besim's presentation and how I would absolutely agree that, if you can localize these testicles in some way, that that will save a lot of patients having extensive surgery because you know immediately what to go for; now you are doing venograms for varicoceles in some cases I imagine.

Besim: I have only three such cases.

Hargreave: The only reason I'm asking about this is because we actually stopped using venography for looking for intra-abdominal testes in favour of testicular arteriography and we now do an arteriogram and we find that this gives us the edge on venography. Although it is easier to do venography, and it is a good localization technique, I just worry in the occasional case, whether it may fail you.

Tellaloğlu: Is CT scanning less unpleasant for the patient?

Hargreave: CT scanning in small testes is not necessarily going to pick them up.

Whitaker: As regards the anatomy, as I understand it, as described by some people who really have done a lot of work on the embryology of the undescended testis, the testis is never, at any stage embryologically, above the iliac vessels. Now I was interested to see some of yours, because they looked some of them, as it they might have been a little bit higher than that, but at least in theory there should never be a testicle above the vessels; the testicle is always in the groin, the kidney of course ascends, but the testicle stays down there; the body strengthens but the testicle is never anything but in the groin. Therefore, fancy though these techniques are for isolating it, as you're going to explore anyway then you're going to find it presumably below the vessels. The thing that we need to know is, in the unilateral case, what your degree of confidence is when you say that there is or there isn't one. For this will allow us to say we won't explore in this one or may be we're not sure so we'd better explore anyway. So if you're going to explore anyway you're going to look the whole of the distance between the vessels and the groin and no further, and then say there isn't one. I'd like your comments on this.

Hargreave: What do you do, Bob?

Whitaker: Well, I just explore; if I need to I do extend the incision of the lateral margin of the ring a bit, so I can get a bit further, but I never go above the vessels.

Chisholm: I would ask, why doesn't he use a nephroscope?

Blandy: I was going to make exactly the same point. If you trust one, you can always get a gynecologist to put it in for you and have a look.

Whitaker: They're often a tiny little streak of testicle which I doubt if you can see in a nephroscope.

Blandy: I was going to make the same point. The only ones I've ever found when I couldn't find them in the groin or poking round the corner, have been streaks; and they don't look like testicles and I'm sure one would miss them on a CT scan.

Hargreave: Well, I can certainly recommend arteriography because, though its harder to do, you can in fact see the streaks.

Besim: Angiography is not easy to perform, it is a very thin artery.

Hargreave: It's a very thin artery and on the right hand side if you've got two renal arteries it often comes off the lower renal artery, and you need in fact, to do an aortogram just to see where the renal arteries are when doing this.

Kırkalı: I would like to ask your opinion on taking the testicles of patients who have carcinoma *in situ* in their testis.

Hargreave: There are one or two comments on this. The incidence of subsequent malignancy from John Pryor's series of carcinoma *in situ* has been 100%; they have all gone on to develop tumors. And I think if you've got that sort of statistic facing

you in the literature, and you find carcinoma *in situ,* then I would prefer to take the testicle or testicles from that patient and give him androgen replacement therapy rather than him subsequently have to have combination chemotherapy therapy or what have you.

Blandy: I've been very disturbed by this issue. And I have to ask myself would those testicles have gone on to become malignant if they hadn't been biopsied. Tim Oliver has been looking at the testicular achievements that he's collected jointly between Barts and the Institute of the London, and there's an uncomfortably high proportion of people who report an incidence such as a biopsy or some operation on their testicles, and it's too high to be mere chance. To follow the argument that you should treat these *in situ* tumors by chopping it off, seems to me to be quite absurd when you now have a 100% cure rate, with known available methods of treatment. Surely the thing to do is to observe the testicle.

Hargreave: Well, I think that sets quite a difficult problem because how frequently do you observe these testicles and we've certainly had one that got away from us; we were observing a particular patient, at roughly six monthly intervals but the question is, how do you observe them because actually when we spotted his tumor he'd got glands, and subsequently when we did CT scanning on his chest he had in fact got early lesions there; in fact we'd missed it.

Blandy: Yes, that's terrifying.

Hargreave: I think this problem is really a very difficult one.

Blandy: There are approximately 500 new testicular tumors per annum in the British Isles, that sort of order of magnitude, and how many undescended testicles?

Whitaker: About 3 thousand per million of population, I should say. I can give you the risk. We have tried to work out what the risk is for an adult with an undescended testis. For that's what really matters. You're not interested in what the incidence of tumors in a population is you want to know what the chances are for a chap who is left with a testicular tumor. We've done it on this basis, that in fact the incidence of undescent in the adult population is 0.3%, and the incidence of undescent in a series of testicular tumors, is 10%. If you've got a thousand testicular tumors 10% of them will occur in people with cryptorchidism; the other factor you need to know is the incidence of testicular tumors in the country, and for the United Kingdom that's roughly 200.000. So you take a million population, a million people and there will be 20 testicular tumors per population per year. So there are your 20 people with testicular tumors and you know that the incidence of undescent is 0.3, and 10% of those, will be in undescended testes and so therefore that's 2 per million and those 2 have to occur in the 3.000 people in the population with undescent, therefore that will be one testicular tumor per 1.500 population, of 1.500 people with undescended testes per year in a million population. So that doesn't sound very often per year but

if you work it out on a life time basis these people with undescended testes, adults, have a one in 30 chance; it just happens to have been worked out statistically on a life time basis. In summary an adult with undescended testes which is untreated has a one in 30 chance of getting a tumor in the course of his life.

Hargreave: Bob, what do you mean by "undescended testicles?"

Whitaker: Well, that's of course begging the question, because this 10% here, unfortunately includes people who have undescended testicles it's the tumors in the undescended testis, it may actually be the opposite one.

Hargreave: Do you make a distinction between ectopic testes and undescended testes in these figures?

Whitaker: Well, I personally would, but they don't in the sense that these are figures which have come from army studies of 40 years ago, and these are the only figures available.

Hargreave: If the risk applied only to true undescent as opposed to ectopics and undescent you might be talking about a very considerably increased risk.

Blandy: Yes, but there's no evidence to suggest that that is the case and 10% of those 10% gain in the "good" testis.