LIGHT AND ELECTRONMICROSCOPIC EVALUATION OF THE TESTICULAR HISTOLOGY IN A CASE WITH AZOOSPERMIA PRIOR TO IVF USING EPIDIDYMAL SPERMA

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SUMMARY

The quantitative evaluation of the testicular biopsy is very common before the treatment of obstructive azoospermia or congenital absence of vas deferens. In the present study light and electronmicroscopic evaluation of the testicular histology in a case with obstructive azoospermia revealed the presence of adequate sperm counts and possible high numbers of motile spermatozoa in the epididymis. Thus a successful pregnancy was achieved using aspirated epididymal sperma for IVF, in our case. So, in addition to quantitative observations of the histology, the ultrastructural characteristics of the tubular germinal epithelium give more morphological data for IVF using epididymal sperm.

Key words : Electronmicroscopic evaluation., Epididymal sperma, Obstructive azoospermia, Reinke crystals, Testicular histology.

INTRODUCTION

In the cases with congenital absence of the vas deferens or chronic obstructive azoospermia the evaluation of the spermatogenesis in testis is going to be very important. Thus the occurence of the pregnacy with sperm aspiration from the epididymis is to be regarded a new treatment for such cases (1-4).

As mentioned by Silber et al. (5) the quantitative evaluation of the number of mature spermatids by testicular histology reflects the sperm production. These authors tried to compare the quantitative production of spermatozoa based on a testicular biopsy in patients. They used the technique reported for quantitative evaluation (5-8). They were interested in the total number of pachytene spermatocytes and the total number of mature spermatids per tubule to calculate the ratio of mature spermatids to pachytene spermatocytes,

In the literature we could not find any investigation on the ultrastructural evaluation of the testicular biopsy in cases with chronic obstructive azoospermia or in congenital absence of vas deferens.

The aim of the present study is to evaluate the light microscopic and electronmicroscopic structure of the testicular histology in a case with chronic obstructive azoospermia.

MATERIAL AND METHODS

In this case (35 years old) spermiogram revealed an azoospermia. In order to control the presence of a sperm production, testicular biopsy was performed to both of the testes.

For light microscopic investigations biopsy specimens were fixed in Bouin's solution, then dehydrated in ascending series of alcohol and embedded in paraffin. Sections prepared from paraffin blocks were stained with haematoxylin-eosin staining and PAS (periodic acid Schiff) reaction.

For electronmicroscopic investigation biopsy specimens cut into small pieces were fixed in phosphate buffered 3% glutaraldehyde for 2 hours at 4°C, then rinsed in buffer and postfixed in phosphate buffered 1 % osmium tetroxide solution for 1 hour at 4°C. Specimens dehydrated in ascending aceton series were embedded in Vestopal W. Thin sections stained with uranyl acetate and Reynold's lead citrate were examined under JEOL 100C electonmicroscope.

RESULTS

LIGHT MICROSCOPY :

In histological preparations all the stages of spermatogenesis and spermiohistogenesis were observed in the germinal epithelium of the seminiferous tubules in the testicular biopsy specimen (Fig. 1,2).

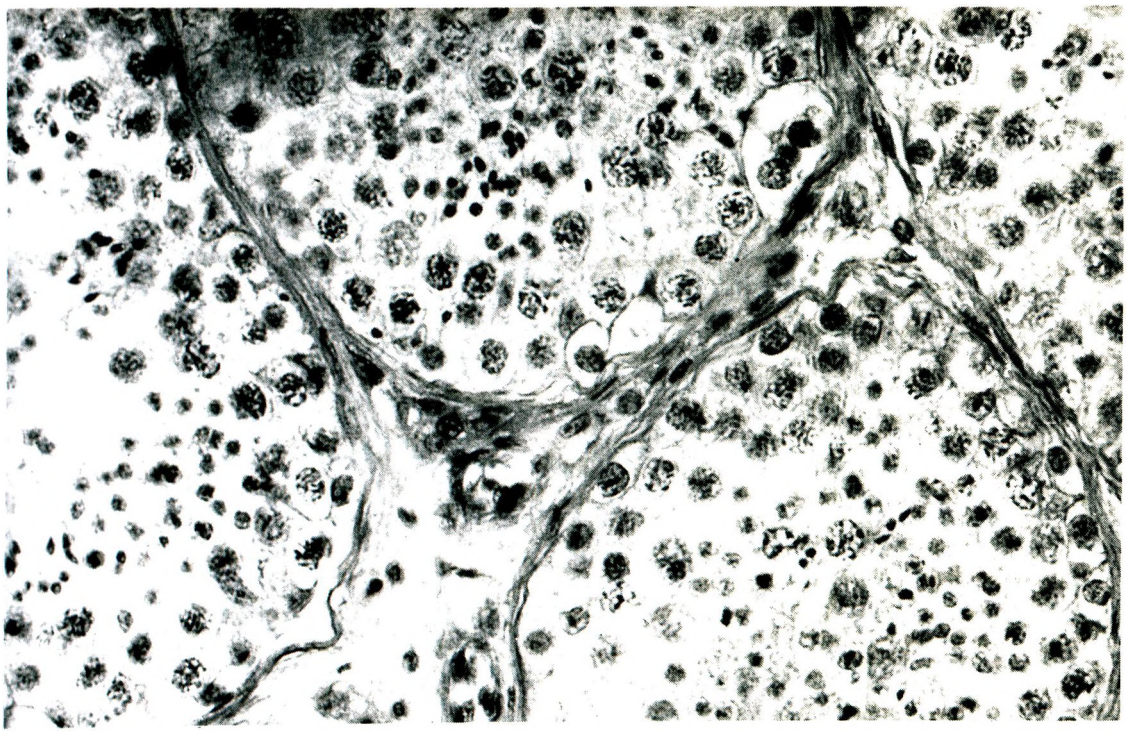

Fig. 1: Light microscopy of the seminiferous tubules from left testis biopsy specimen. In the germinal epithelium spermatogonia, spermatocyte I, II, spermatids and spermatozoa were seen. Peritubular tissue was normal. In the interstitial tissue Leydig cells were demonstrated. X: 210.

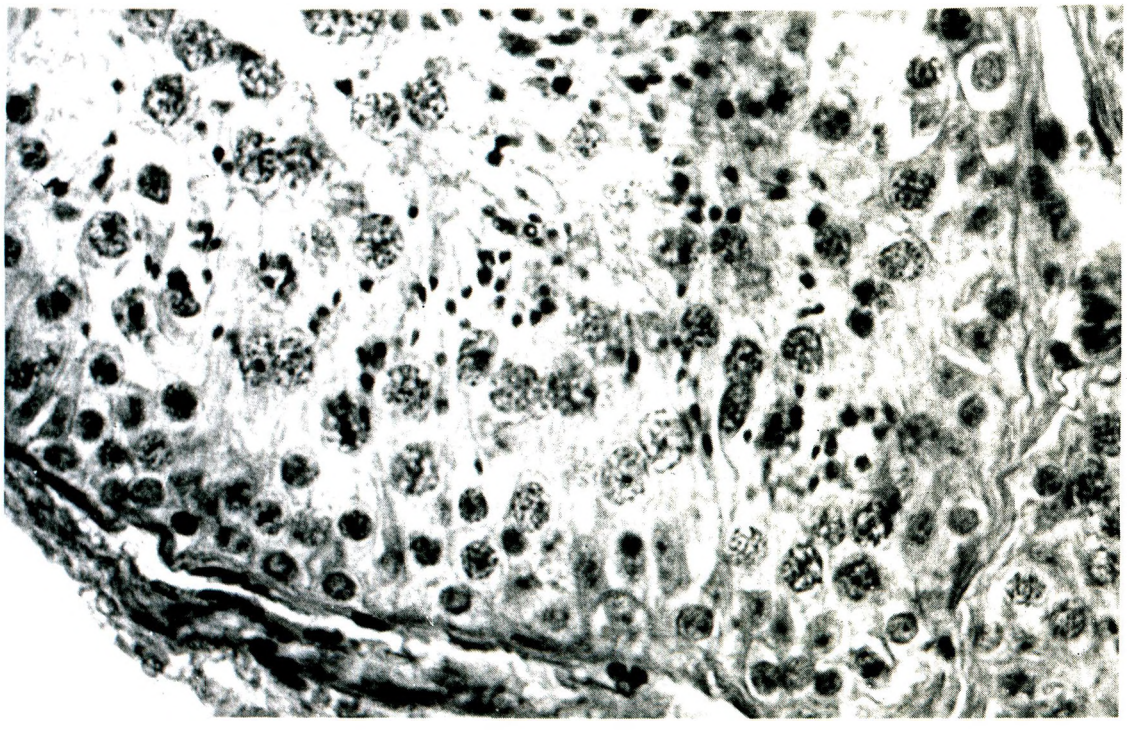

Fig. 2: Histologic preparation from the right testis biopsy. Similar appearance as in Figure 1 was noted in the light microscopic section. X: 330.

ELECTRONMICROSCOPY :

Specimens taken from the right testis showed the different stages of spermiogenesis in seminiferous tubules. The Sertoli cells in the germinal epithelium of tubules had lobated nuclei and possessed increased number of lipid inclusions in their cytoplasm (Fig. 3). Granular endoplasmic reticulum masses and cisternal dilation were also seen. Rarely in some tubule, Sertoli cells with flattened, pycnotic, basal nuclei and high content of lipid were noted. Spermatogonia found in the basal layer of the germinal epithelium and the spermatocytes lined toward the lumen were observed with their typical structures (Fig. 4). Numerous spermatids and spermatozoa structures at different stages were seen (Fig. 4,5,6). Among them, formation of head and tail as well as centriole localization and flagellar development were demonstrated (Fig. 5). Meanwhile numerous sections of tails were examined.

The lamina propria (peritubular tissue) surrounding externally the tubules mostly were normal. In some tubules there was a thickening of the peritubular tissue but no foldings of the basal lamina.

Within the interstitial connective tissue blood vessels sections and Leydig cells were present. Some Leydig cells were possessing two nuclei. Smooth endoplasmic vesicles, lipid inclusions and Reinke crystals were noted in their cytoplasm (Fig. 7).

Specimens taken from the left testis were similar to the right testis with light microscopic and ultrastructural appearances.

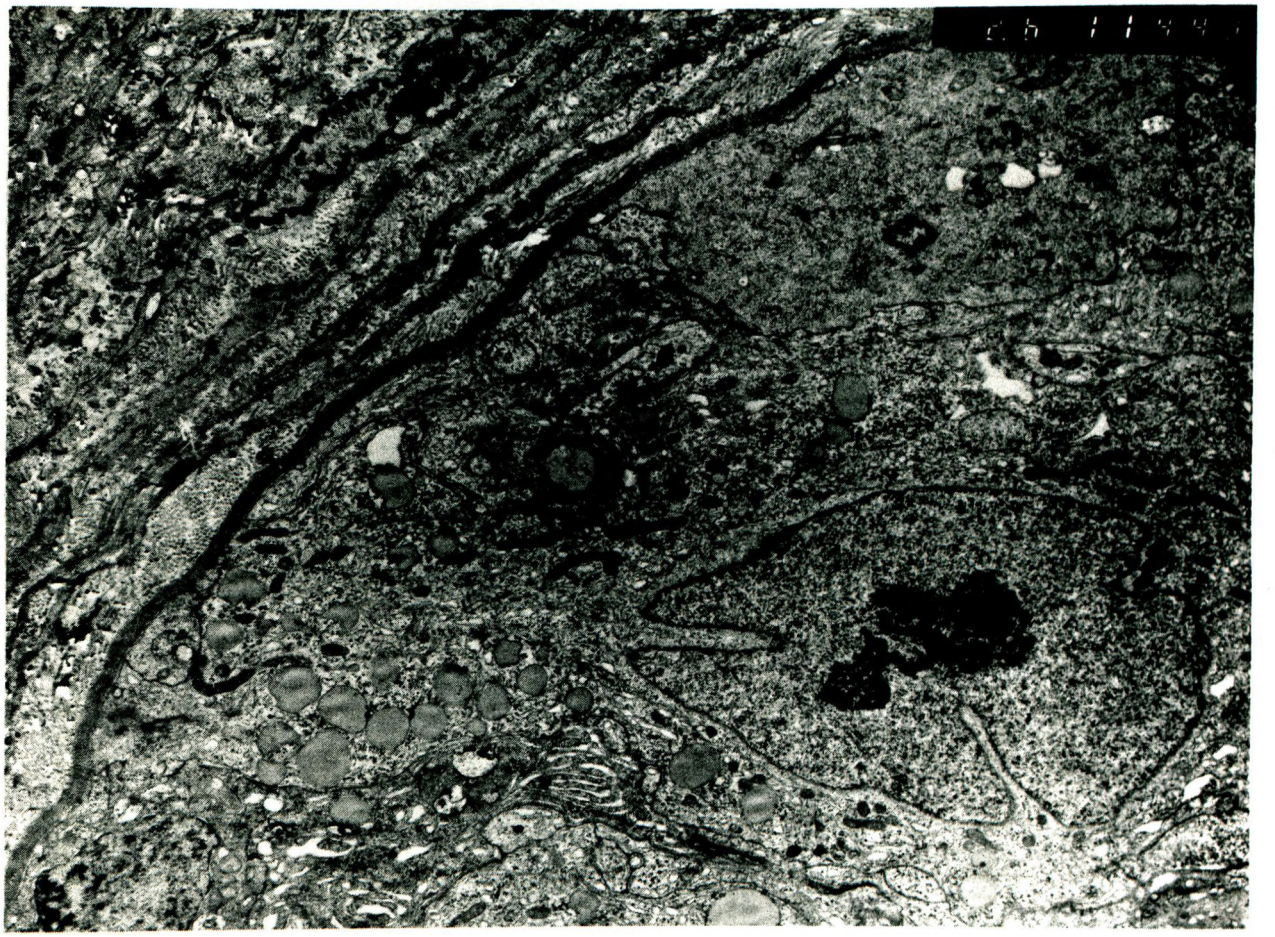

Fig. 3: Electronmicrograph of seminiferous tubule from the left testis biopsy. Sertoli cell lying on the basal lamina with typical nucleus and increased number of lipid inclusions were seen. X: 7.400.

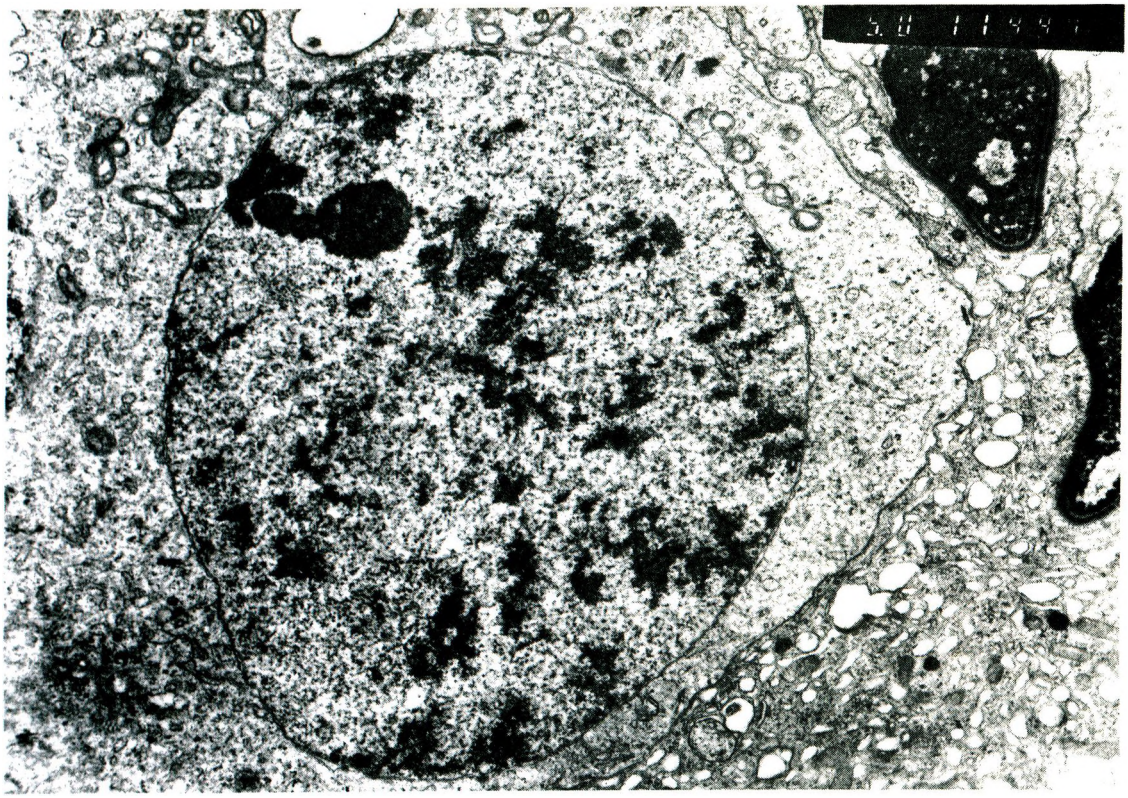

Fig. 4: Electronmicrograph of seminiferous tubule.
Spermatocyt and spermatozoa formation were observed. X: 7.600.

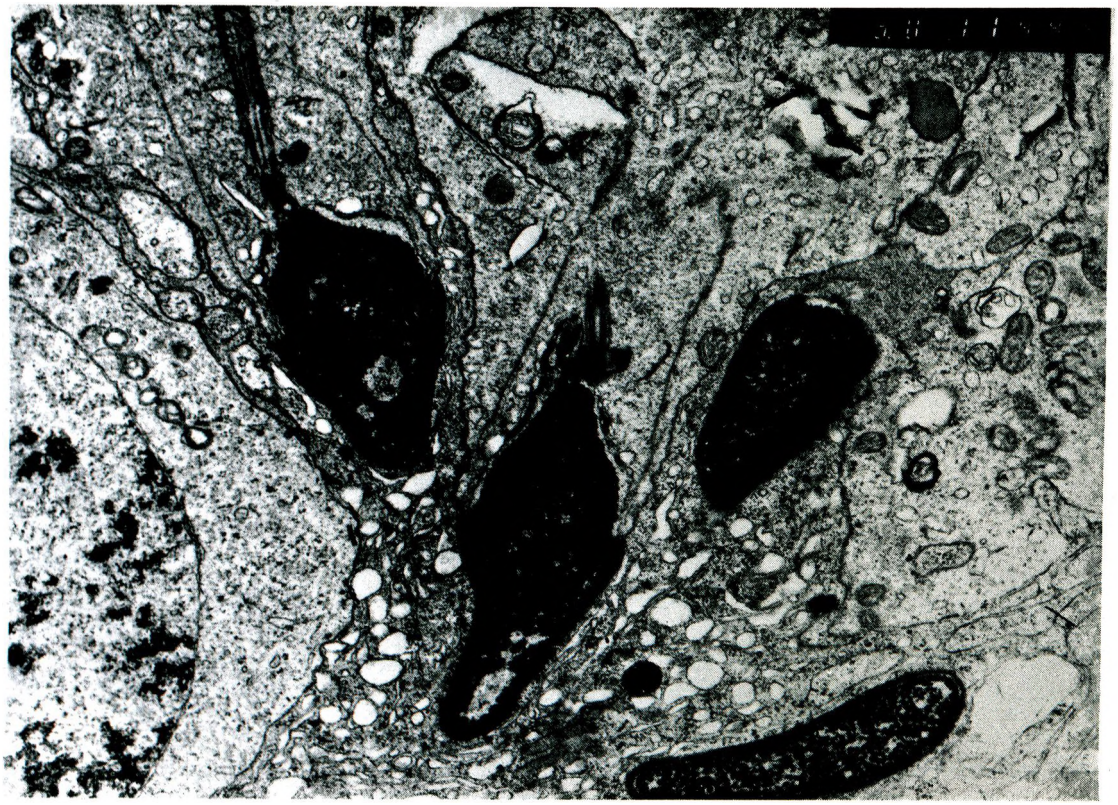

Fig. 5: The characteristic ultrastructural morphology of the mature spermatozoa were noted. X: 7.600.

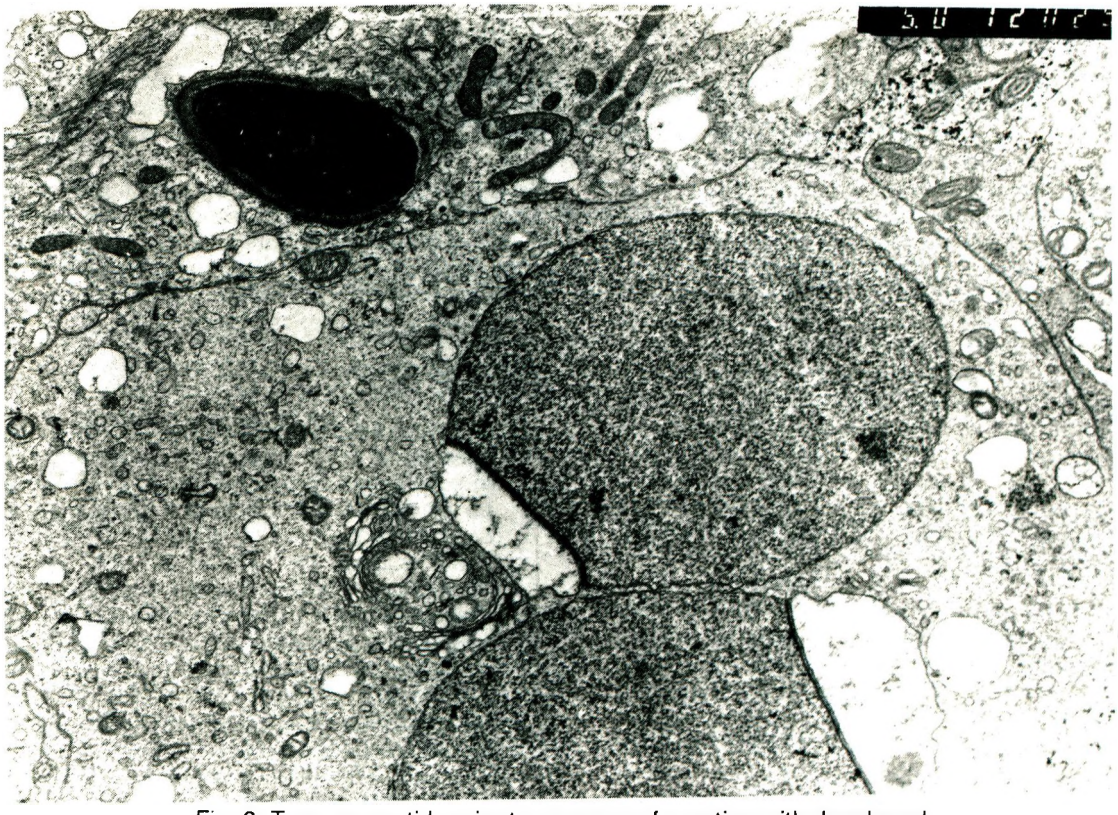

Fig. 6: Two spermatids prior to acrosome formation with developed Golgi complex and a head formation were demonstrated. X: 7.600.

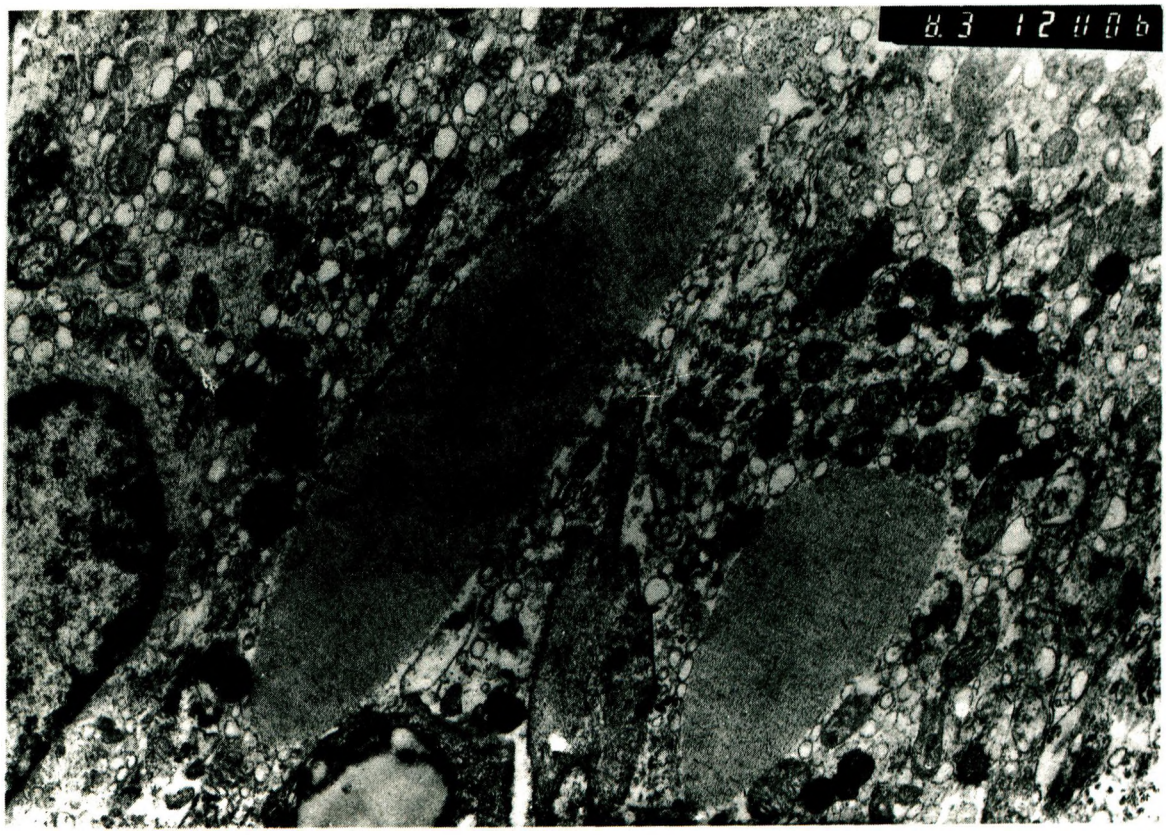

Fig. 7: The presence of two Reinke crystals in the cytoplasm of a Leydig cell in testicular biopsy specimen. X: 23.000.

DISCUSSION

As previously reported there are quantitative investigations on the testis tubules to find out the number of spermatozoa expected in semen in non-obstructed patients (8,9). According to Silber et al. (1) this method gives some prediction of the quantity of spermatozoa retrievable from the epididymis for in vitro fertilization in men with congenital absence of the vas deferens. As previously described for IVF there are other cases that spermatozoa recovered from the obstructed vas deferens (2,10). Osborn et al (11) reported a successful pregnancy occured after intratubal insemination with eqididymal spermatozoa recovered from a patient with obstructive azoospermia. Also Jequier et al (3) reported a pregnancy achieved after intra Fallopian embryo transfer using epididymal sperma in idiopathic obstructive azoospermia.

Silber (9) using technically successful anastomosis along corpus epididymis in patients with obstructive azoospermia showed that "sperm do not necessarily have to traverse the entire corpus or cauda epididymis in the human to achieve fertilizing capacity". Even there are such clinical techniques to succeed a pregnancy in cases with obstructive azoospermia or congenital absence of vas deferens we couldn't find studies made on the testicular fine structure in such cases.

In our case, as seen in light microscopic and electronmicroscopic micrographs, there are all cellular elements of the germinal epithelium in the seminiferous tubules: spermatonogonia, spermatocyt I and II, spermatids and serial steps of spermiohistogenesis to become spermatozoa including Sertoli cells (Fig. 1,2,3,4,5,6).

The ultrastructure of these cells were similar to those cells which were demonstrated in the testicular tubules in the unobstructed human. Lipid inclusions were increased in the cytoplasm of the Sertoli cells. Rough surfaced endoplasmic reticulum also were increased. Ultrastructure of the spermatocytes and mature spermatids reflects spermatogenesis and spermatozoa production in the seminiferous tubules. Electronmicrographs revealed the differentiation of the motile spermatozoa from mature spermatids with their acrosome and other internal fine structure of head, midpiece and tail (Fig. 5).

Peritubular tissue was slightly thickened in some seminiferous tubules (Fig.3). In the interstitial tissue, Leydig cells showed an increase in their secretory production. Additionally the occurence of Reinke crystals (Fig.7) in the cytoplasm of these Leydig cells is an obvious evidence of effected release function.

In concluison, our ultrastructural observations revealed the adequate sperm count and the possible presence of high numbers of motile spermatozoa in epididymis. Also, internal structure of the germinal cells and maturation characteristics reflected the fertilization capacity of the spermatozoa. Thus, in our case, a successful pregnancy was achieved using aspirated epididymal sperm for in vitro fertilization.

So it is possible to suggest that electronmicroscopic evaluation of the testicular histology gives more detail to reveal the sperm capacity for the procedures of in vitro fertilization, intra Fallopian insemination or embryo transfer in cases with azoospermia or congenital absence of vas deferens.

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