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Testicular focal atrophy – tubular blockade as partial degeneration of the tubule

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Abstract This study investigated whether focal atrophy is a degenerative process of a whole tubule or tubular blockade as partial intratesticular degeneration. Serial section analyses of testicular tissue from 19 men with different andrologic diseases were examined. From every fifth section of any series, defined areas were viewed under a light microscope, and tubule sections were drawn. Three-dimensional reconstructions were made from these materials. Reconstructions of the seminiferous tubules showed tubular blockade as partial degeneration of the tubules. Transition from an intact portion to a blockade was accompanied by an increase in the thickness of the lamina propria. The blockade was a cell cord that contained Sertoli cells and, at most, spermatogonia, or it was a completely atrophied tubule, a so-called tubular scar. In a given tubule, defined areas of atrophy and areas of spermatogenic activity were both found. Occurrence of tubular blockade increased with age. Serial section analyses of testicular tissue showed tubular blockade as the partial degeneration of seminiferous tubules. In focal atrophy, a given tubule can have defined areas of atrophy and areas of spermatogenic activity.

Key words Testis · Tubule · Oligozoospermia · Testicular focal atrophy · seminiferous tubular blockade as partial tubular degeneration

Introduction

One of the main reasons for reduced male fertility is a low number of spermatozoa (oligospermia) in the ejaculate [14, 21]. This can be traced to either impaired spermatogenesis or an outflow obstruction of the spermatozoa. If the seminal path is obstructed, one might find single or multifocal seminiferous tubular blockade in the testis [1]. The degree of obstruction can affect the sperm count in the ejaculate [3, 17]. The observed clinical picture of focal degeneration is seen in histologic sections that show tubular blockages next to intact tubules [6, 10, 17, 20]. About 40–60% of fertility impairment cases are the result of focal atrophy of testicular tissue [1, 14, 17]. Slight histologic changes in testicular biopsies do not correlate with infertility impairment.

We investigated intratesticular blockades in histologic sections and described the process of degeneration along the course of one tubule.

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Materials and methods

Twelve men underwent unilateral or bilateral orchiectomy because of testicular tumor ($n = 4$), trauma ($n = 2$), or prostatic carcinoma ($n = 6$). Seven men underwent testicular biopsy because of infertility ($n = 4$) or testicular tumor ($n = 3$). The mean age of the 19 patients was 47.9 years (range, 20–79 years). The patients who underwent bilateral orchiectomy for treatment of prostatic carcinoma had no previous hormonal therapy.

Orchiectomies

Serial sections of whole testes were made. Specimens for paraffin histology were fixed in Bouin's solution and processed routinely with paraffin embedding. From each testis, 300–600 sections, 8 μm thick, were prepared and stained with hematoxylin-eosin [2]. Only every fifth section of any series was analyzed. A defined area (one lobule) is easily identified in different sections. Vessels or the lobular boundaries were helpful for orientation. In the analyzed sections, underlying areas measuring 4 × 4 mm were viewed under a light microscope (×180), and tubular sections were drawn using a drawing tube on greaseproof paper. By laying one drawing on top of another, we could follow the course of a tubule. One tubule with a diameter of 80—220 μm in steps of 32 μm was easy to follow. Few changes occur in such short steps. We made three-dimensional reconstructions of single tubules using foam rubber.

From these tubule reconstructions, we determined the status of spermatogenesis using Holstein's [7, 8] scores. Using a special graduated microscope ocular, we measured tubular diameter and the thickness of the lamina propria in micrometers.

Testicular biopsies

Semi-thin serial sections of testicular tissue were examined. The specimens were fixed in phosphate-buffered 5.5% glutaric aldehyde. Counterfixation was carried outwith 1% osmium acid for 2 h. Following dehydration with alcohol, the samples were embedded in glycid ether. Serial sections of the whole biopsy (1.5 μm thick) were made and stained with toluidine blue pyronin [9]. Every fifth section of any series was analyzed, resulting in intervals of 6.0 μm from section to section. As with the orchiectomy specimens, three-dimensional reconstructions of the tubules were made.

Teased-out preparations from whole testes

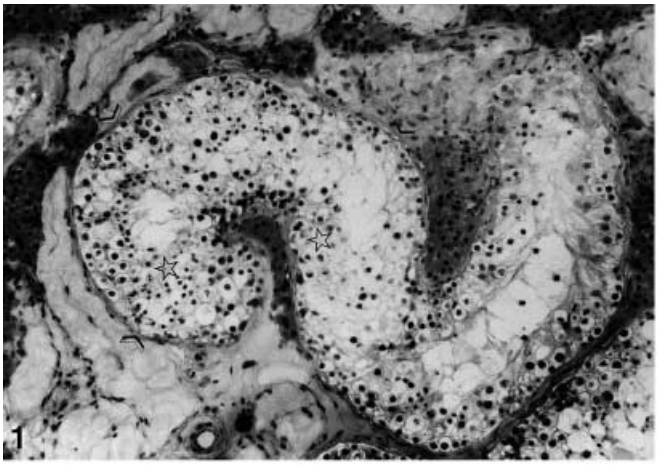
Large biopsies were taken from three of the four fresh and unfixed testes from the orchiectomies. From these biopsies, individual convoluted seminiferous tubules were teased out after 2 h of partial digestion in 0.2% collagenase at 37 °C. Further processing was carried out using the same procedure as the semithin histology of the testicular biopsies.

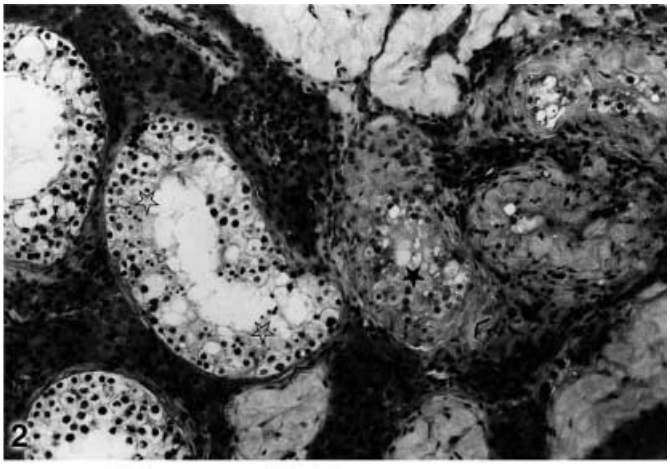
Results

Semen parameters were available for the seven men who underwent testicular biopsy. One showed normal parameters, four oligozoospermia, and two teratozoospermia.

The quantitative spermatogenesis score of the serial sections was confusing; no strong correlation between tubular diameter and spermatogenesis was seen.

The serial sections allowed us to track a tubule in the three-dimensional model. The reconstructions showed individual tubules to be very convoluted. They also showed partial degeneration of the tubule, so-called tubular blockade. In a given tubule, defined areas of atrophy are interspersed with areas of spermatogenic activity (Figs. 1, 2). Atrophic tubules (blockades) were seen next to tubules with a diameter of 180–240 μm. Seminiferous tubules averaging up to 240 μm in diameter – with germinal epithelium of 6070 μm – permitted easy identification of all germinal cell generations. Spermatids were more frequently reduced in number and were often malformed. Sections of narrow tubules with diameters of less than 180 μm were regularly several centimeters long. In blockades, the germinal epithelium was fused to one cell cord with unrecognizable cell boundaries (Fig. 3). Blockades usually contained Sertoli cells and, at most, spermatogonia. Once a tubule was completely atrophied, simple scar tissue was left behind. The blockades were not restricted to a single lobulus testis, which normally contains two or three tubules, but were irregularly distributed throughout the whole testis. Transitions between spermatogenically





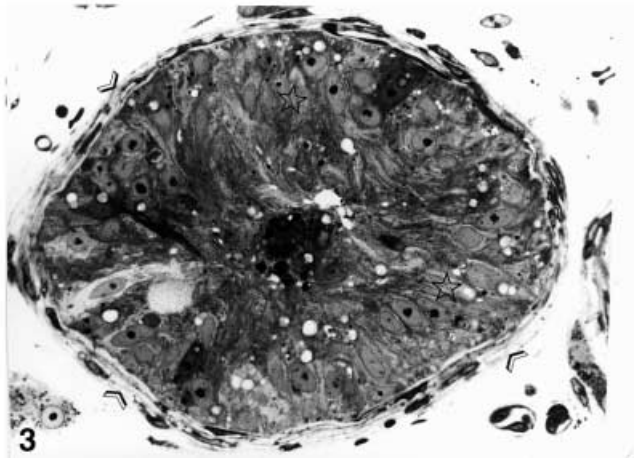


Fig. 1 Sections of one tubule in 300-μm intervals with typical transition to blockade (*star*). Lamina propria (*arrowhead*), germinal epithelium (*open star*). Paraffin, ×220

Fig. 2 Sections of one tubule in 300-μm intervals with typical transition to blockade (*star*). Lamina propria (*arrowhead*), germinal epithelium (*open star*). Paraffin, ×220

Fig. 3 Section of blockade. Germinal epithelium is fused to one cell cord with unrecognizable cell boundaries. Lamina propria (*arrowhead*), germinal epithelium (*open star*). Semi-thin section, ×550

active tubular sections and blockades were more frequent in high-grade atrophy.

In normal tubules, the lamina propria was 8–12 μm thick. In narrow tubules, the thickness increased to 20 μm. The transitions were accompanied by an irregular increase in thickness of the lamina propria to 15–25 μm. In completely atrophied tubules, which amount to scar tissue, the lamina propria was up to 40 μm thick.

On the basis of our histologic measurements of whole testes, we examined testicular biopsies. In semithin sections, blockade was easy to identify. The scanty tissue of testicular biopsies contained only few sections of the tubule (12–25); it did not show transitions from normally structured tubule to blockade. The occurrence of tubular blockade seemed to increase with age (Fig. 4).

Teased-out tubules were too fragile to prepare long-enough sections for finding transitions to blockade; it seems that they are not suitable for finding transitions.

Discussion

To our knowledge, this is the first study to show in a three-dimensional model the transition from normal tubular structure to tubular blockade. A defined tubule was easy to identify in the different sections. Vessels or the lobular boundaries proved helpful for orientation. Reconstructions of these areas were made using foam rubber. Because the model only showed the circumferences, it is not possible to point out specific details.

In the histologic serial preparations, groups of atrophied tubules were seen next to groups of intact tubules. This histologic picture is described as mixed or focal atrophy [6, 10]. The three-dimensional reconstructions of the seminiferous tubules using serial sections showed that in focal atrophy the degeneration did not occur in the complete tubule, but was restricted to shorter or longer stretches of the tubule. In the transitions between normal tubule and blockade, an increasing depopulation of complete germinal cell generations could be seen in association with a typical increase in the thickness of the lamina propria [3, 4, 16]. Such degenerative processes are accompanied by intensive phagocytosis of Sertoli cells and by the immigration of macrophages [3, 15]. The morphologic substrate of the blockade is a cell cord containing Sertoli cells and, occasionally, spermatogonia, which later becomes a tubular scar [18].

The blockades were irregularly distributed throughout the whole testis. The tubular blockade is presumably caused by local disturbance of the microvascular architecture in the testicular interstitium, as was recently shown [5, 16].

Yamamoto et al. [20], using a light and scanning electron microscope, showed short stretches of blockade of about 50 μm in patients with idiopathic infertility. They also described short-run blockades interrupted by short-run tubular sections that had spermatogenesis. They did not find an increase in the thickness of the lamina propria in areas of blockade. Our results contradict those of Yamamoto et al., showing stretches of blockade up to several centimeters long. It seems doubtful that the tubular sections described earlier were blockades.

In 1934, Johnson [11] found individual tubules to be about 30–70 cm long and described atrophied tubular sections up to 10 cm long isolated in macerated preparations. Our biopsies contained only 25 tubule sections. Thus, the chance of finding transitions between normal tubule and blockade in a biopsy is unlikely. Either a normal tubular section or an atrophied one is present.

Only few semen analysis results were available in our study. A slight correlation was found between semen parameter and histology results. Our study primarily observed a degeneration phenomenon. If a testicular biopsy is carried out and shows high-grade atrophy, counseling should be offered with regard to the option of testicular sperm extraction (TESE) for sperm use with intracytoplasmic sperm injection (ICSI).

Men with severe infertility as a result of intratubular blockade are potentially capable of fathering children by means of ICSI. Even men with azoospermia, who were previously considered infertile and untreatable, may now have spermatozoa harvested from a testicular biopsy [12].

Conclusion

Serial section analysis of testicular tissue showed tubular blockade to be a partial degeneration of seminiferous tubules. A given tubule can have defined areas of atrophy and areas of spermatogenic activity; the presence of

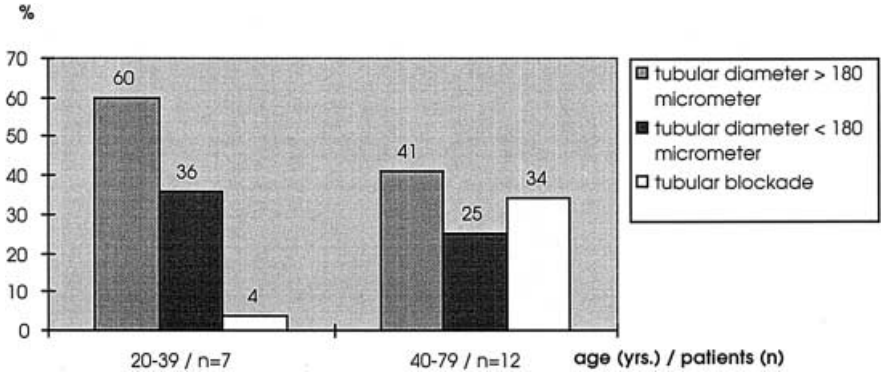


Fig. 4 Mean tubular diameter in different age groups

a blockade would hinder the outflow of spermatozoa. Testicular biopsies and teased-out preparations, which we used for our study, are not suitable for finding tubular blockade.

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