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Diverticula of Human Seminiferous Tubules in the Normal and Pathologic Testis

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The occurrence of diverticula in human seminiferous tubules was investigated in the adult human testis in normal as well as physiologic (aging) and pathologic (germ cell depletion, obstruction of male excretory ducts, varicocele and systemic arteriosclerosis) conditions. Diverticula, which are evaginations of the seminiferous epithelium surrounded by a thin tunica propria, were present in all groups studied. The number of diverticula per mm² testis was higher in the testis with obstruction than in those without obstruction at each age considered. The number of diverticula increased with age in both the obstructed and nonobstructed testis. No changes in the number of diverticula per mm² testis were found in relation to systemic arteriosclerosis or different degrees of germ cell depletion. Varicocele was only associated with increased numbers of diverticula when it was also associated with obstruction. The formation of diverticula in human seminiferous tubules seems to be an obstructive process related to increasing age.

Key words: human seminiferous tubule diverticula, obstruction of male excretory ducts, aging, varicocele, germ cell depletion.

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The occurrence in the seminiferous epithelium of evaginations surrounded by a thin lamina propria has been known for many years (Spangaro, 1902; Stieve, 1930; Johnson, 1934; Hatakeyama et al, 1962; Schulze, 1979). These diverticula have been related to aging (Stieve, 1930); however, their causes and their relationships with pathologic conditions of the testis have not been studied. The aim of this report was to investigate the possible correlation between the occurrence of diverticula in human seminiferous tubules and several physiologic (aging) or pathologic (germ cell depletion, obstruction of the male excretory ducts, varicocele and arteriosclerosis) conditions that are presumably involved in their origin. The results suggest that the formation of diverticula is an obstructive process related to increasing age.

Materials and Methods

The material consisted of 76 testicles with each epididymis and spermatic cord obtained from autopsies (60) or

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post-mortem surgery (16) (1 to 2 hours after death, before autopsy, in infertile patients with the consent of their families), plus 38 testicular biopsies. The average age of the males was 60 years (from 40 to 92 years of age). The testicular specimens were grouped as follows:

1) Twenty-eight testis specimens obtained from autopsies of males who died from causes other than testicular, endocrine or related diseases and presented no histories of systemic arteriosclerosis, varicocele or obstruction of the male excretory ducts. The tissue from these organs showed a normal histologic pattern except for a variable degree of hypospermatogenesis in men older than 60 years of age. The tubuli recti and rete testis were slightly dilated in about half of these elderly men.

2) Twenty-eight testicular specimens from men who suffered from obstruction of the male excretory ducts at the level of: a) cauda epididymidis-initial portion of the vas deferens (nine males: five autopsy, two surgical, and two biopsy specimens); b) rete testis — ductuli efferentes (nine males: five autopsy, two surgical and two biopsy specimens); and c) both extra- and intratesticular level (10 males: five autopsy, two surgical and three biopsy specimens). A marked dilatation of the tubuli recti and rete testis was observed in all autopsy and surgical specimens from these men. In the biopsies, the obstructions were first diagnosed by spermiograms (azoospermia) and confirmed with deferentographs. In the autopsy and surgical specimens, the obstructions were diagnosed by post-mortem deferentographs and by light microscope examination of serial sections of the epididymis and spermatic cords obtained as indicated below.

3) Twenty-one testicular biopsies from oligozoospermic men without obstruction of the excretory ducts; the diagnosis of oligozoospermia was based on the correlation between the spermiogram and the number of mature spermatids in histologic sections, according to the Silber and Rodríguez-Rigau (1981) method. The degree of germ cell depletion was evaluated following Atkinson's index modified by Makler and Abramovici (1978). The occurrence of obstruction was excluded by examination of spermiograms.

4) Seventeen testicular specimens from patients with systemic arteriosclerosis affecting the testis (12 autopsy and five surgical specimens).

5) Twenty testicular specimens from patients with clinical and histologic diagnosis of varicocele; these patients were classified as: a) without histologic signs of obstruction (five males: one autopsy, one surgical, and three biopsy specimens); b) with diffuse dilatation of the rete testis (five males: one autopsy, one surgical, and three biopsy specimens); c) with focal dilatation of the rete testis (five males: one autopsy, one surgical and three biopsy specimens); and d) with extratesticular obstruction (five males: one autopsy, two surgical and two biopsy specimens). The occurrence of obstruction was diagnosed as indicated in Group 2.

Groups 1 and 2 were subdivided according to age (decade of life) (Table 1).

Each testis, epididymis and spermatic cord obtained from autopsy or post-mortem surgery was cross-sectioned into 5-mm-wide slices. These slices plus the testicular biopsies were fixed in 10% buffered formaldehyde and embedded in paraffin. Six-μm-thick sections were stained with hematoxylin-eosin, Masson trichrome, periodic acid-Schiff, van Gieson stain, orcein, and methenamine-silver.

For quantitative studies, 10 histologic sections from each testis were selected at random. On each of these sections the number of diverticula was counted in 10 randomly selected microscopic fields (346,000 μm^2). Only clearly recognizable diverticula were counted. This requisite included the following conditions: 1) continuity between the diverticulum and the seminiferous tubule from which the diverticulum arose, 2) the thickness of the tunica propria in the diverticulum was less than 3.5 μm (the tunica propia thickness in the seminiferous tubules was at least 7.5 μm), and 3) the diverticulum could be distinguished from small protrusions of the tubular wall (only diverticula measuring at least 50 μm in diameter were counted). The mean values and standard deviations for each subgroup and group were calculated from the average values for each testis. The differences between means were evaluated by either the student's *t* test (for

TABLE 1. Variations in the Number of Diverticula in Human Seminiferous Tubules in the Non-obstructed (Group I) and Obstructed (Group II) Testis in Relation to Age*

Testis Type	Decade of Life	Average Age (years)	n	Number of Diverticula per 10 mm² Testis
Nonobstructed	5th	44.0 ± 4	7	13.3 ± 7
Obstructed	5th	43.8 ± 4	7	44.0 ± 25†
Nonobstructed	6th	54.8 ± 3	7	76.6 ± 37
Obstructed	6th	55.7 ± 4	7	128.2 ± 85
Nonobstructed	7th	63.3 ± 3	8	57.5 ± 21
Obstructed	7th	64.0 ± 3	8	204.3 ± 86†
Nonobstructed	8th	75.3 ± 5	6	155.1 ± 80
Obstructed	8th	76.8 ± 7	6	357.8 ± 132†
Total nonobstructed		60.1 ± 11	28	88.3 ± 21
Total obstructed		60.5 ± 13	28	184.7 ± 45†

*Values are expressed as means ± standard deviation.

†Indicates that differences between the obstructed and nonobstructed testis are significant at this age ($P < 0.05$).

paired values) or analysis of variance (for three or more values). The correlation between the number of diverticula and age was determined by a linear regression test.

Results

The diverticula appeared as evaginations of the seminiferous epithelium towards the testicular interstitium (Fig. 1A–D). The diverticula were connected to the seminiferous tubules by a narrow neck (Fig. 1A) or by a wide base (Fig. 1B). Diverticulum size varied widely from small buds (Fig. 1C) to diverticula looking like small seminiferous tubules (Fig. 1A). When the plane of the section did not cut the base of the diverticula, these could be misinterpreted as small seminiferous tubules (Fig. 1C and D). However, when serial sections were performed, the connection between these apparently small tubules and true seminiferous tubules could be observed. Even in the absence of serial sections, these diverticula could be identified because their seminiferous epithelium was surrounded by a thin tunica propria with scanty peritubular myoid cells (Fig. 1B). Germ cell development in the diverticula was similar to that found in the seminiferous tubules from which they arose (Fig. 1A–D); however, some diverticula, principally the small ones, only contained the cells located in the basal compartment of the seminiferous epithelium. Diverticula were found in all the male groups studied.

Comparison of the number of diverticula per 10 mm^2 testis in Group 1 men (without testicular pathology) and Group 2 men (with obstruction of the excretory ducts) in relation to age revealed that: 1) the number of diverticula was higher in the testis with obstruction than in that without obstruction for any age considered (Table 1), and 2) the number of diverticula increased linearly with age in both the obstructed and nonobstructed testis (Table 1 and Fig. 2).

No relationship between the level of damage to the seminiferous epithelium and the number of diverticula was found in testicular biopsies from oligozoospermic men (Group 3) (Table 2). Additionally, no significant differences between men with systemic arteriosclerosis (Group 4) and Group I men were found (Table 3). Varicocele was only associated with increased numbers of diverticula when it was also associated with obstruction (Table 4). No significant differences were found among the various types of obstruction (Table 4).

Discussion

Tubular diverticula should be distinguished from branching tubules. A first approach is that diverticula are connected to the seminiferous tubules by a narrow neck, whereas branching tubules do not show a markedly reduced tubular diameter at the point of branching. These features are not constant and there are many exceptions. According to Holstein and Roosen-Runge (1981), the most reliable criterion is that the tunica propria is much thinner in the tubular diverticula than in seminiferous tubules, branching or not. The identification of tubular diverticula in this study was done following this criterion.

Both obstruction of the male excretory ducts and advancing age are associated with an increase in the number of diverticula in human seminiferous tubules. Of these two factors, obstruction seems to be the most important since, at any age considered, the number of diverticula is higher in the obstructed than in the nonobstructed testis. Obstruction probably contributes to the formation of diverticula through an increase in intraluminal pressure. In addition, the development of diverticula in the human seminiferous tubule is another morphologic alteration to be added to the physiologic (Royer et al, 1984; Vermeulen and Deslypere (1985) and morphologic (Johnson et al, 1984a; Paniagua et al, 1985; 1986; Rui et al, 1986) changes reported in the aging human testis. Age itself seems to be associated with some degree of obstruction, since a dilatation of the rete testis and tubuli recti, similar though less pronounced to that found in men with testicular obstruction at the level of the intra- or extratesticular excretory ducts, was observed in many men older than 60 years of age without apparent obstruction. These aging men probably had an intrinsic testicular obstruction, perhaps due to a progressive thickening of testicular fluid with age, as has been reported in the rat (Laporte and Gillet, 1984) and hamster (Cisneros and Franklin, 1985). Similarly, varicocele can increase the development of diverticula through the obstructive changes that accompany this disease (Nistal et al, 1984).

The presence of diverticula in young adult men with normal testicular histology suggests that factors other than obstruction can be involved in the formation of diverticula. These factors are probably related to the tunica propria since diverticulum formation should be favoured by the occurrence of zones of weak resistence in the tubular wall. These zones might correspond to focal areas of altered peritubular myoid cells, which possess the usual cytologic components of contractile cells (Bustos-Obregón and Holstein, 1973; Virtanen et al, 1986). Their function

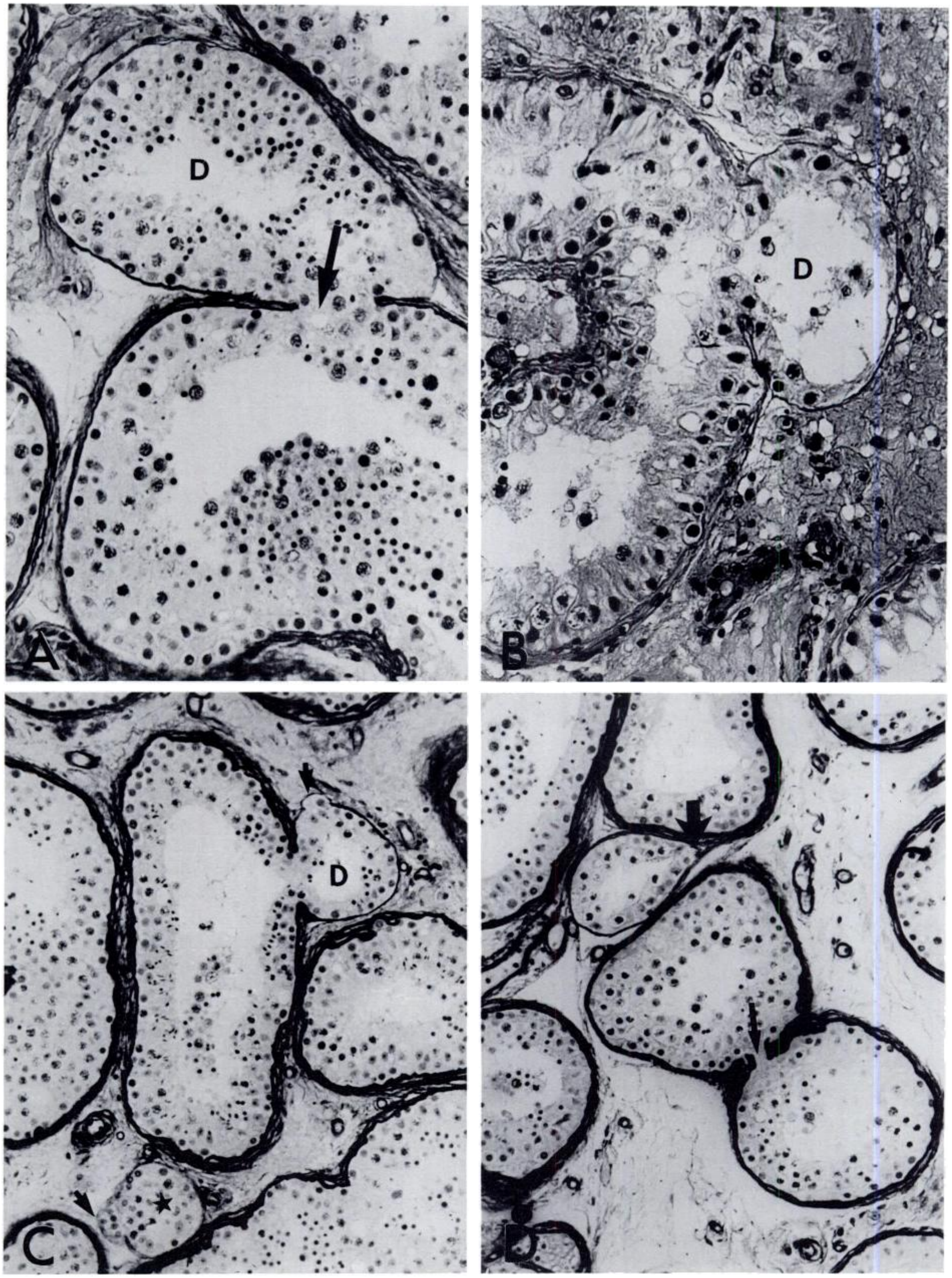
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Fig. 1A-D. **A:** Seminiferous tubule from a 53-year-old man with varicocele and diffuse dilatation of the rete testis showing a diverticulum (D) that is joined to the seminiferous tubule by a narrow neck (arrow). The diverticulum is surrounded by a tunica propria thinner than that of the tubule from which the diverticulum arises. Methenamine-silver. (X 320). **B:** Diverticulum (D) in a seminiferous tubule from a 63-year-old man with obstruction at the level of the cauda epididymidis. The seminiferous epithelium shows development of germ cells up to the spermatocyte level in the seminiferous tubule and its diverticulum. Masson trichrome. (X 320). **C:** Seminiferous tubule with complete spermatogenesis showing a diverticulum (D) that is joined to the seminiferous tubule by a wide base. Another cross-sectioned tubule (star) seems also to be a diverticulum sectioned throughout a plane that does not contain the connection between the seminiferous tubule and the diverticulum, since the tunica propria of this diverticulum is thinner than that of the adjacent tubules. A small protrusion (arrows) suggesting an initial stage in diverticulum formation arises from both diverticula. From a 55-year-old man without testicular pathology. Methenamine-silver. (X 160). **D:** Two cross-sectioned seminiferous tubules appear joined by a bridge (small arrow). Both sections do not seem to be true diverticula since their tunica propria is as thick as that of the adjacent tubules. Compare these tubules with a true diverticulum displaying a thin tunica propria (large arrow). The diverticulum is sectioned at the level of connection with the adjacent tubule. From a 59-year-old oligozoospermic man. Methenamine-silver. (X 160).

is very important in the structure of the human seminiferous tubule, which is characterized by the complexity and abundance of peritubular cells. These cells contribute to the regulation of the size of the seminiferous tubule as well as to the progression of the spermatozoa and testicular fluid towards the excretory ducts (de Kretser et al, 1975; Farr and Ellis, 1980; Ellis et al, 1981). Peritubular myoid cells may be affected by hormone alterations that take place with increasing age (Royer et al, 1984; Vermeulen and Deslypere, 1985). It has been reported that the peritubular cells are controlled by testosterone, prostaglandins, and cyclic-GMP (Farr and Ellis, 1980). The effect of age on these hormones might lead to a decline in the ability of peritubular cells to maintain the structure of the seminiferous tubules, or to alterations in their intercellular junctions (Bustos-Obregón and Holstein, 1973; de Kretser et al, 1975), as well as to a deficient synthesis of the collagen and elastic fibers surrounding the seminiferous epithe-

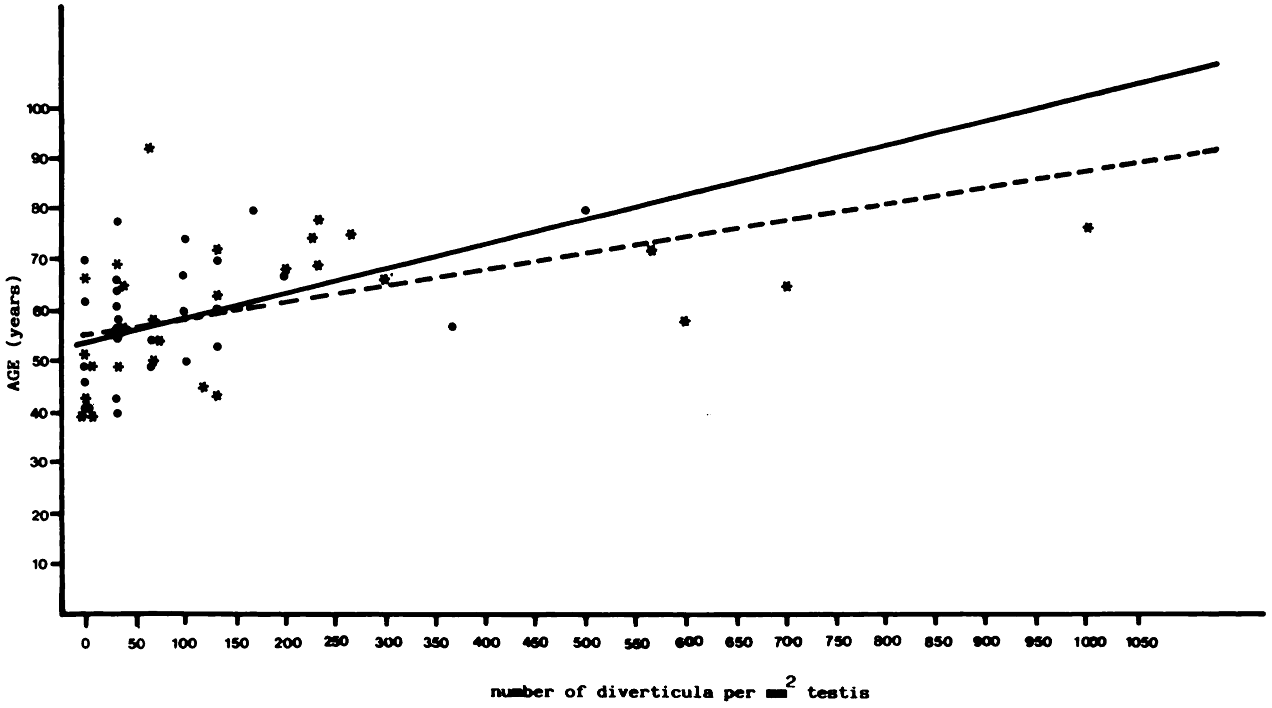

Fig. 2. Correlation between number of diverticula per 10 mm² testis and age in both the non-obstructed (•) and obstructed (*) testis. For nonobstructed testes (——) the regression equation is y = 4.57 X + 54.9 and the correlation coeficient is r = 0.44. For the obstructed testis (---) the regression equation is y = 2.19 X + 56.7 and the correlation coeficient is r = 0.40. For both lines the confidence limits are 95%.

TABLE 2. Number of Diverticula in Human Seminiferous Tubules in Males with Nonobstructive Oligozoospermia and Different Levels of Germ Cell Development*

Level of Germ Cell Development	Average Age (years)	n	Number of Diverticula per 10 mm² Testis
Spermatids and spermatozoa	54 ± 6	10	73.2 ± 36
Spermatogonia and spermatocytes	54 ± 13	6	55.5 ± 35
Sertoli-cell-only tubules	54 ± 11	5	146.5 ± 103

*Values are expressed as the means ± standard deviation. Differences among groups were not significant ($P < 0.05$).

lium, all of which would contribute to a weakening of the tubular wall.

Besides the peritubular cells, Sertoli cells might also be involved in the formation of diverticula. The Sertoli cells secrete testicular fluid (Laporte and Gillet, 1984; Muffly et al, 1985) and cooperate with the peritubular cells in the production and deposition of intercellular matrix components in the tunica propria (Mather and Phillips, 1984; Skinner et al, 1985). Since human Sertoli cells undergo morphologic alterations with aging (Paniagua et al, 1985) leading to a progressive decline (Johnson et al, 1984b), it is probable that these alterations may compromise Sertoli cell functions, leading to a thickening of the testicular fluid (Laporte and Gillet, 1984; Cisneros and Franklin, 1985) and to an impairment of their interaction with the peritubular cells in the maintenance of the tubular wall.

In conclusion, it may be suggested that the formation of diverticula in human seminiferous tubules seems to be related to: 1) an increase in intratubular pressure caused by extrinsic (at the level of the intra- or extratesticular excretory ducts) or intrinsic (thickening of testicular fluid) testicular obstruction; and 2) a focal weakening of the tubular wall caused by alterations in the peritubular myoid cells and/or Sertoli cells. These lesions increase with advancing age.

TABLE 3. Number of Diverticula in Human Seminiferous Tubules in Men with or without Systemic Arteriosclerosis Affecting the Testis*

Testis	Average Age (years)	n	Number of Diverticula per 10 mm² Testis
Without arteriosclerosis	60.2 ± 11	28	88.3 ± 21
With arteriosclerosis	65.5 ± 11	17	90.1 ± 28

*Values are expressed as means ± standard deviation. Differences among groups were not significant ($P < 0.05$).

TABLE 4. Number of Diverticula in Seminiferous Tubules in Men with or without Varicocele and with or without Testicular Obstruction

Testis Type	n	Number of Diverticula per 10 mm² Testis
Without varicocele and without obstruction (group 1)	28	88 ± 21[a]
With varicocele and without obstruction (group 5a)	5	80 ± 18[a]
With varicocele and obstruction (groups, 5b,c,d)	15	199 ± 58[b]
With varicocele and diffuse dilation of the rete testis (group 5b)	9	216 ± 148[b]
With varicocele and focal dilation of the rete testis (group 5c)	5	191 ± 92[b]
With varicocele and extratesticular obstruction (group 5d)	5	189 ± 75[b]
Without varicocele and with obstruction (group 2)	28	184 ± 45[b]
Without varicocele and with extratesticular obstruction (group 2a)	9	225 ± 126[b]
Without varicocele and with intratesticular obstruction (group 2b)	9	185 ± 60[b]
Without varicocele and with both extra and intratesticular obstruction (group 2c)	10	200 ± 14[b]

*Values are expressed as the mean ± standard deviation. Values with different superscript letters are significantly different ($P < 0.05$). All patients were in the 7th decade of life.

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