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Diagnostic Value of Differential Quantification of Spermatids in Obstructive Azoospermia

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ABSTRACT: Testicular biopsies from 80 azoospermic young men were revised and the average numbers per cross-sectioned tubule of each germ cell type were calculated and compared with those of control normal testes. In 53 patients, azoospermia had an obstructive cause, and in 22 of those 53 patients more adult spermatids were found by testicular biopsy than young spermatids (over 100% in some testes), in one or both testes. However, in normal testes fewer mature spermatids than young spermatids (23.3%) were found. In the 22 patients, the causes of azoospermia were: vasectomy (7 patients), bilateral agenesis of the vas deferens (3 patients), Young syndrome (3 patients), bilateral cysts in the caput epididymidis (1 patient), bilateral inguinal herniorrhaphy (1 patient), left varicocele (1 patient), and unknown causes (6 patients). Biopsies were bilateral except for 3 cases (a vasectomized patient, a patient with Young syndrome, and a patient with obstruction due to an unknown cause). Hormonal levels were normal in the 22 patients. In addition, testicular biopsies of 3 twisted testes from 3 young adult men showing a number of adult spermatids higher than that of young spermatids were also included in the study. All testicular biopsies—including those of the twisted testes—showed an obstructive histologic pattern, consisting of a mosaic distribution of testicular lesions: mainly tubular ectasis and germ cell sloughing into the adluminal compartment of seminiferous tubules. The increase in the number of adult spermatids was bilateral in 1 of the 6 vasectomized men who underwent bilateral biopsy, and in 7 of the 11 bilaterally biopsied patients with obstructive azoospermia due to other causes. The most probable explanation for the increased number of adult spermatids is stagnation of testicular fluid, caused by sperm excretory duct obstruction. The unilateral increase in the number of adult spermatids in vasectomized men might be related to the occurrence of a spermatic granuloma (a frequent finding in vasectomy) in the proximal end of the sectioned ductus deferens ipsilateral to the testis with nonincreased adult spermatid numbers, and the absence of spermatic granuloma in the ductus deferens ipsilateral to the testis with increased adult spermatid numbers. This granuloma would produce, in addition to spermatozoon destruction, reabsorption of the testicular and epididymal fluids. The higher rate of bilateral increase, in the number of young spermatids observed in the patients with congenital lesions of the ductus deferens or the ductus epididymidis, might be related to the absence of spermatic granulomas in congenital obstructions.

Key words: Male infertility, testicular biopsy, germ cell quantitation.

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The introduction of quantitative methods in the evaluation of the seminiferous epithelium has shown to be an important step in the understanding of the behavior of the seminiferous epithelium against several noxas and has led to revise the diagnostic terms used in the past (Steinberger and Tjioe, 1968; Johnsen, 1970; Skakkebaek et al, 1973; Skakkebaek and Heller, 1973; Makler and Abramovici, 1978; Silber and Rodriguez-Rigau, 1981; Francavilla et al, 1990; Guarch et al, 1992; Nistal and Paniagua, 1997). These methods have enabled us to determine the main cell type lesioned, and to evaluate the degree of the lesion and its repercussion on the germ cells that derive from the lesioned cell.

Comparison of the germ cell values calculated in testicular biopsies with control values permits the evaluation of the spermatogenetic capacity of the testes. The number of adult spermatids (Sc+Sd) in the testis can be compared with spermatozoa in the spermiogram by means of a power curve that correlates both sets of data (Silber and Rodriguez-Rigau, 1981; Nistal et al, 1987). These comparisons have revealed that sperm excretory duct obstruction is a frequent cause of azoospermia and severe oligozoospermia (Galmes-Belmonte and Nistal, 1998). If the absence of spermatozoa in the spermiogram is associated with the presence of adult spermatids in testicular biopsy, such azoospermia has an obstructive cause. If testicular biopsy reveals more adult spermatids than spermatozoa in the ejaculate, the oligozoospermia is considered obstructive.

Previous studies of patients with obstructive azoospermia seen in our hospital have shown that the most frequent lesions occur in the adluminal compartment of the seminiferous epithelium, which showed more adult spermatids than young spermatids. This finding is surprising

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Table 1. *Mean tabular diameter (MTD) and germ cell numbers per cross-sectioned tubules in azoospermic men*

	MTD		Spermato-gonia		Spermato-cytes		Young Spermatids		Adult Spermatids		Difference Adult Sper—Young Sper.		Percentage (Difference ×100/Young Sper.)		Percentage + 23.3	
Patient	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
Vasectomy																
1	253	240	18.2	16.7	27.2	24.4	24.8	15.2	29.5	15.7	4.7	0.5	18.9	3.2	41.9	26.5
2	225	264	19.5	21.8	27.5	30.3	15.6	39.3	36.0	25.5	20.4	−13.8	130.0	−35	153.3	−11.7
3	218	N.B.	17.8	N.B.	17.5	N.B.	12.1	N.B.	17.6	N.B.	5.5	N.B.	45.4	N.B.	68.7	N.B.
4	211	206	14.3	14.5	15.1	14.8	16.9	7.9	10.2	15.5	−6.7	7.6	−42.0	96	−18.7	119
5	200	199	16.1	14.3	29.4	23.0	21.5	22.0	30.4	21.6	8.9	−0.4	41.3	−1.8	64.6	21.5
6	179	195	17.2	17.5	26.4	28.2	29.0	50.3	33.0	18.3	4	−32	13.8	−63.6	37.1	−40.3
7	230	212	19.3	17.6	40.3	40.1	24.3	27.4	39.3	19.4	15	−8	62.5	−29.6	85.8	−6.3
Other causes																
8 (a)	216	218	20.1	21.8	33.5	41.6	45.7	41.8	30.2	43.8	−15.5	2	−33.9	4.8	−10.6	28.1
9 (a)	228	221	17	17.5	25.6	29.8	16.0	30.5	22.1	31.7	4.1	1.2	25.6	3.9	48.49	27.2
10 (a)	143	137	7	4.8	0	11.4	3.5	1.4	2	5.1	−1.5	3.7	−42.8	264.2	−19.5	287.5
11 (b)	169	N.B.	18.4	N.B.	21.3	N.B.	11.6	N.B.	16.8	N.B.	5.2	N.B.	44.8	N.B.	68.1	N.B.
12 (b)	191	211	21.0	16.7	29.7	27.0	24.7	34.3	22.3	37.9	−2.4	3.6	−9.7	10.5	−13.6	33.8
13 (b)	164	N.V.	28.1	N.V	30.7	N.V.	8.2	N.V.	9.6	N.V.	1.4	N.V.	17	N.V.	40.3	N.V.
14 (c)	159	103	17.5	0	16.1	0	7.1	0	12.7	0	5.6	0	78.8	0	102.1	0
15 (d)	165	191	14.7	25.0	25.1	32.8	20.3	26.1	25.7	27.2	5.4	1.1	27	4.2	50.3	27.3
16 (e)	197	151	17.9	11.7	25.2	4.3	18.5	0.2	27.8	0	9.3	−0.2	50.2	−100	73.5	−76.5
17 (f)	156	179	18.9	19.4	28.8	25.0	18.0	28.7	21.8	29.4	3.8	0.7	21.1	2.4	44.4	25.7
18 (f)	202	175	24.5	23.5	35.4	26.0	24.4	23.0	36.0	30.4	11.6	7.4	47.5	32.1	70.8	55.4
19 (f)	223	218	27.6	23.4	42.6	29.5	26.3	15.2	28.9	24.7	2.6	9.5	100	63.3	123.3	86.6
20 (f)	227	199	24.1	19.4	42.1	32.6	32.0	31.6	43.6	32.0	11.6	0.4	36.2	1.2	59.5	24.5
21 (f)	202	190	18.7	14.9	22.3	22.3	17.1	15.9	27.0	23.8	9.9	7.9	57.9	49.7	81.2	73
22 (f)	254	N.B.	18.0	N.B.	31.5	N.B.	27.7	N.B.	32.5	N.B.	4.8	N.B.	17.3	N.B.	40.6	N.B.
Test. torsion																
23	188	N.B.	17.5	N.B.	15.0	N.B.	13.6	N.B.	16.4	N.B.	2.8	N.B.	20.5	N.B.	43.8	N.B.
24	220	N.B.	17.4	N.B.	40.0	N.B.	42.4	N.B.	66.8	N.B.	24.4	N.B.	57.5	N.B.	80.8	N.B.
25	227	N.B.	25.9	N.B.	36.1	N.B.	39.7	N.B.	43.5	N.B.	3.8	N.B.	9.6	N.B.	32.9	N.B.

In all cases, standard deviations were lower than 10%. (a) Bilateral agenesis of the ductus deferens; (b) Young syndrome; (c) bilateral epididymal cysts; (d) bilateral inguinal herniorrhaphy; (e) left varicocele (1 patient); (f) unknown causes. N.B., not biopsied; N.V., not valuable. For normal testes, the germ cell number ranges were: spermatogonia, 17 to 25; primary spermatocytes, 25 to 37; young spermatids, 30 to 44; and adult spermatids, 23 to 29.

because, in normal testes, the number of adult spermatids is about 23.3% lower than that of young spermatids (Nistal and Paniagua, 1997). The aim of this study was to evaluate the frequency of this finding and provide an explanation for it.

Materials and Methods

Testicular biopsies and andrological histories from 80 azoospermic young men (from 27 to 48 years of age) evaluated in La Paz Hospital (Madrid) during the last 5 years were reviewed. In 53 of these patients, azoospermia had an obstructive cause because their testicular biopsies showed complete spermatogenesis. The testicular biopsies of the remaining 27 azoospermic patients showed Sertoli cell–only tubules, tubular hyalinization, or tubular sclerosis. In 22 of the 53 patients with obstructive azoospermia (27.5% of all azoospermic men studied and 41.5% of men with obstructive azoospermia), aged 30 to 48 years, the number of adult spermatids in testicular biopsy was higher than that of young spermatids.

In the 22 men selected for the study, the causes of azoospermia were: vasectomy performed between 3 and 17 years before biopsy (7 patients); bilateral agenesis of the vas deferens (3 patients); bilateral epididymal enlargement associated with respiratory diseases (Young syndrome, 3 patients), bilateral cysts in the caput epididymidis (1 patient), bilateral inguinal herniorrhaphy (1 patient), left varicocele (1 patient), and unknown causes (normal physical exploration, 6 patients). Before and after surgery, the 22 patients presented normal basal serum levels of follicular stimulating hormone (FSH), luteinizing hormone (LH), testosterone, prolactin, and inhibin B. Testicular and semen volumes and levels of both fructose and citric acid were also normal.

Testicular biopsy was bilateral in 19 patients and unilateral in 3 patients: case 3, a vasectomized man with history of left orchidoepididymoectomy owing to testicular trauma; case 11, a man who lost his left testis because of neonatal torsion; and case 22, a bilaterally biopsied man whose left testis biopsy was poorly fixed and was excluded from the study (Table 1). Therefore, a total of 41 biopsies were available.

The study also included unilateral testicular biopsies from

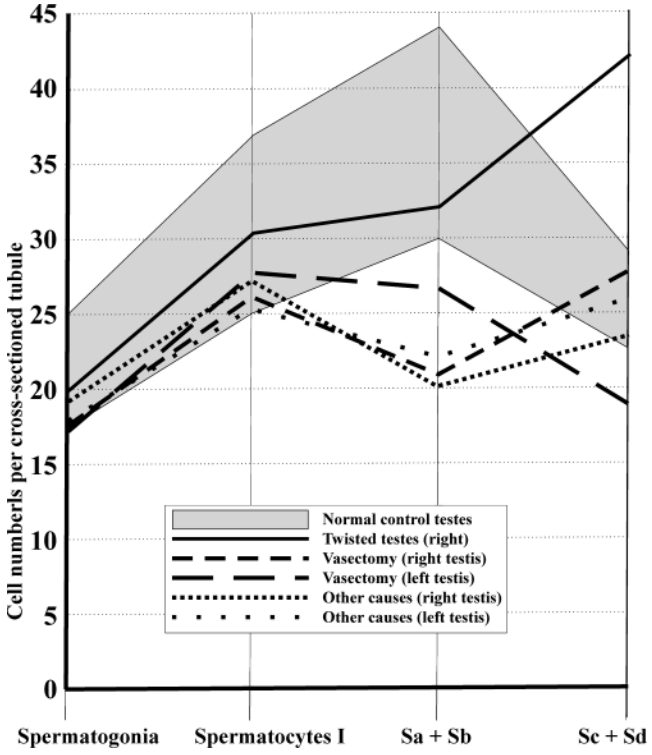
Table 2. *Average values of mean tubular diameter (MTD) and germ cell numbers per cross-sectioned tubules in three groups of azoospermic men (vasectomized, other causes, and testicular torsion)*

	MTD		Spermato-gonia		Spermato-cytes		Young Spermatids		Adult Spermatids		Difference (Ad. Sper.– Young Sper.)		Percentage (Difference ×100/Young Sper.)		Percentage + 23.3	
Patient	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
Vasectomy	217	219	17.4	17.0	26.0	26.7	20.5	26.9	27.9	19.2	9.7	4.0	52.0	49.6	75.2	55.6
Other causes	183	183	19.5	17.9	27.2	25.5	20.0	22.6	23.9	26.0	6.3	3.7	43.6	43.6	66.9	66.9
Test. torsion	212		19.9		30.3		31.9		42.3		10.3		29.2		52.5	

For normal testes, the germ cell number ranges were: spermatogonia, 17 to 25; primary spermatocytes, 25 to 37; young spermatids, 30 to 44; and adult spermatids, 23 to 29.

three patients (aged 18, 21, and 23 years) who presented because of testicular torsions of less than 8 hours of evolution, and because the quantitative study of biopsies from the removed twisted testes revealed that the number of adult spermatids was higher than young spermatids (Table 1).

All testicular biopsies were fixed in Bouin solution for 24 hours and embedded in paraffin. Eight 6-μm thick, nonconsecutive sections were obtained from each testis, mounted on the same slide, and stained with hematoxylin-eosin. In each of these sections, the number of spermatogonia, primary spermatocytes, young spermatids (Sa + Sb), and adult spermatids (Sc + Sd) were counted in 20 cross-sectioned seminiferous tubules per section, and the average values were calculated. Spermatid classification was done according to Clermont's criteria (Clermont, 1963). Testicular biopsies obtained 6 to 8 hours after death from



Average germ cell numbers per cross-sectioned tubule in testes from azoospermic men.

10 males (20 to 49 years of age), who died because of myocardial infarct or in traffic accidents, had not suffered from testicular or related diseases, and had proven fertility were used as controls (Nistal et al, 1987). These control values were similar to those reported by other authors (Johnsen, 1970; Skakkebaek and Heller, 1973; Skakkebaek et al, 1973; Makler and Abramovici, 1978; Silber and Rodriguez-Rigau, 1981; Nistal and Paniagua, 1997). According to these controls, the lowest limits of normal testicular parameters were the following: 17 spermatogonia, 25 primary spermatocytes, 30 young spermatids, and 23 adult spermatids. This means that the number of adult spermatids was 23.3% lower than the number of young spermatids: (30–23)100/30. In addition, the average tubular diameter was evaluated in the same cross-sectioned tubules (the lowest normal value was 185 μm).

In order to determine whether the etiology of the obstruction was related to the evaluated parameters, testicular biopsies were classified into 3 groups: vasectomized patients (7 men, 13 testes), other causes of obstructive azoospermia (15 men, 27 testes), and testicular torsion (3 men, 3 testes) (Table 1).

Results

All testicular biopsies showed an obstructive pattern, according to previously reported criteria (Nistal and Paniagua, 1999). This pattern consisted of a mosaic distribution of testicular lesions consisting mainly of tubular ectasis and germ cell sloughing into the adluminal compartment of seminiferous tubules.

Of the 44 biopsies, germ cell development was observed in 42 testes. In one patient (case 13), the left testis showed only completely hyalinized seminiferous tubules, and another patient (case 14) showed Sertoli cell–only tubules in his left testis. The results of germ cell quantitations are shown in Tables 1 and 2, and the Figure.

The 7 vasectomized men showed more adult spermatids than young spermatids in one (6 men) or both (1 man) testes. This increase varied widely among testes, from 0.5 cells (3.2%) to 20.4 cells (130%) per cross-sectioned tubule, with an average value of 8.3 cells (51.4%). Since in normal seminiferous tubules the number of adult sper-

matids is 23.3% that of young spermatids, the actual increase that should be assumed for azoospermic men would vary from 21.5% (3.2 + 23.3) to 153.3% (130 + 23.3), with an average value of 68.7% (Tables 1 and 2, and the Figure).

Of the 27 biopsies from the 15 men with obstructive azoospermia due to causes other than vasectomy, increased numbers of adult spermatids in relation to young spermatids were found in 22 testes. Obstructive azoospermia was bilateral in 7 of the 12 patients whose bilateral biopsies were available for the study. The differences between the number of adult spermatids and the number of young spermatids varied from 0.4 cells (1.2%) to 11.6 cells (264.2%) per cross-sectioned tubule, with an average value of 5.1 cells (43.6%) per cross-sectioned tubule. The corrected percentage (by adding 23.3%) varied from 24.5% to 287.5% (average 66.9%) (Tables 1 and 2, and the Figure).

The biopsies of 3 twisted testes showed characteristic lesions of degree II hemorrhagic infarct, according to Mikuz's classification (Mikuz, 1985). The increases in the numbers of spermatids during maturation were 2.8 cells (20.5 %), 24.4 cells (57.5%), and 3.8 cells (9.6%), respectively, with an average value of 29.2% (52.5% after correction) (Tables 1 and 2, and the Figure).

Discussion

Differential counting of germ cell types in the seminiferous epithelium for evaluation of infertility has provided interesting results. This method, as present results confirm, has revealed that most sperm excretory duct obstructions cause lesions in the adluminal compartment of seminiferous tubules. Germ cell counting also permits us to identify the target cells that suffer the effects of increased intratubular pressure caused by obstruction. In addition, present results reveal that many patients with obstructive azoospermia have more adult spermatids than one would expect, given the number of young spermatids counted in their seminiferous tubules.

This finding poses 3 questions: 1) What is the cause of such increase? 2) What are the differences among patients or between both testes within the same patient? and 3) What is the reliability of the power curves that correlate germ cell numbers in testicular biopsy and sperm numbers in spermiogram, in order to evaluate obstructive azoospermias?

A higher ratio of adult-to-young spermatids has been observed in testosterone-treated rats; this has been interpreted as failure of the spermiation process, caused by the transient drop in testosterone levels (Saito et al, 2000). This was not the hormonal status of the patients studied here, who presented normal levels of gonadotropins and testosterone. In any case, a hormonal change would impair both testes in a similar way. The most probable explanation is a stagnation of testicular fluid, caused by sperm excretory-duct obstruction. Obstruction of the proximal segments of the excretory duct (epididymis and proximal portion of the ductus deferens) in both laboratory animals (Smith, 1962; Lopez et al, 1988) and humans (Nistal and Paniagua, 1999) has been shown to produce the histological testicular lesion known as ''obstructive pattern.'' These lesions start when the capacity of the ductuli efferentes to reabsorb testicular fluid is exceeded; and the closer the obstruction is to the testis, the more severe the lesion. Our 22 azoospermic patients presented this characteristic obstructive histological pattern and, although the anatomical level of obstruction could not be determined in the group of vasectomized patients, the similarity of the histological lesions among the different groups of azoospermic patients studied, together with the absence of data supporting a distal obstruction, suggests that the lesion mechanism is the same in the 22 patients. This conclusion is also supported by the observation of the same findings in the 3 twisted testes. Most adult testicular torsions are intravaginal as torsion involves all structures of the spermatic cord, which becomes surrounded by a high insertion of the tunica vaginalis testis, causing strangulation of the veins and sperm excretory ducts. The accumulation of testicular fluid and spermatozoa in the intratesticular ducts (seminiferous tubule lumen and rete testis channels) and the extratesticular ducts near the testis, caused by obstruction makes spermiation difficult. As a result, adult spermatids accumulate in the seminiferous epithelium.

The second question is: Why is the lesion not always bilateral and not present in all patients with obstructive azoospermia? Only 1 of the 7 vasectomized patients who underwent bilateral testicular biopsy showed an increased number of adult spermatids bilaterally. There are 2 explanations for the unilateral increase in adult spermatids: 1) differences in the surgical procedure, and 2) the obstruction being followed by reactions that reduce the increased intratubular pressure by variable degrees. The first explanation is not likely in the vasectomized men studied here because the technique used was always the same (ligation of both ends of the ductus deferens), and, in any case, a surgical procedure would only justify differences among patients, but not between testes from the same patient. Since germ cell counts are similar to those observed in vasectomies using the open proximal end technique (Moss, 1992; Whyte et al, 1998), the explanation seems to be related to the occurrence of a spermatic granuloma in the proximal end of the sectioned ductus deferens. The incidence of spermatic granuloma in vasectomized patients varies from 20% to 35% (Belker et al, 1983) to 60% (McDonald, 1966). When the incidence is

compared in different vasectomy techniques, the figures vary from 20.8% to 58.4% (McCallum et al, 2000). Therefore, whatever the technique employed, at least 20.8% spermatic granulomas are to be expected. Studies in humans (Lopez et al, 1988), as well as laboratory animals (Silber, 1977; Sheynkin et al, 2000), lead us to conclude that spermatic granuloma causes a decrease in excretory duct pressure. This granuloma would produce, in addition to spermatozoon destruction, reabsorption of the testicular and epididymal fluids. Spermatic granuloma is a frequent finding in vasectomy (Belker et al, 1983; Lopez et al, 1988). The cause of these granulomas is unknown, but it has been proposed that they occur when the ligature is too tight or when cauterization is excessive (Belker et al, 1983).

In the group of nonvasectomized azoospermic patients, bilateral testicular biopsy was performed in 12 patients; the increased number of adult spermatids was bilateral in 7 of them. This higher bilaterality rate might be related either to the occurrence of congenital lesions of the ductus deferens or of the ductus epididymidis observed in many of these patients (congenital absence of ductus deferens, Young syndrome, epididymal cysts, etc.). Although it is well known that the effects of congenital obstruction on the testes are milder than those of acquired obstructions (Hirsch and Choi, 1990), the absence of spermatic granulomas in congenital obstructions would probably account for less sperm destruction, as well as less reabsorption of testicular fluid.

The third question was whether the increased number of adult spermatids casts doubt on the predictive value of germ cell counts with regard to the number of spermatozoa expected in the spermiogram. It should be taken into account that the estimate of spermatozoa on the basis of the number of adult spermatids in testicular biopsy is biased because the increased number of adult spermatids in these azoospermic men does not measure the actual number of spermatozoa produced by these testes. At first sight, the number of young spermatids seems to be more pertinent to such evaluation. However, the predictive value of this number is also doubtful. The reason is that, although most obstructions produce lesions in the adluminal compartment, including the loss of primary spermatocytes, these lesions are reversible as soon as the patency of sperm excretory ducts is restored. This means that the first consequence of obstruction is an increase in the number of young spermatids and, thereafter, of adult spermatids. Only a long-term study of patients with obstructive azoospermia, whose cause has been detected and treated adequately would provide worthwhile information about this question

In summary, differential counting between young spermatids and adult spermatids is a rapid and simple method of diagnosing the presence of an obstruction. This is so even in the absence of clinical data, when the number of adult spermatids is higher than young spermatids. This finding has been observed in 41.5% of patients with obstructive azoospermia and might be bilateral, mainly in patients with congenital obstruction. A finding of fewer adult spermatids than young spermatids is also consistent with an obstructive process if the testes bear lesions in the adluminal compartment of the seminiferous tubules, and these lesions have a mosaic distribution (obstructive pattern).

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