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Distribution of spermatogenesis in the testicles of azoospermic men: the presence or absence of spermatids in the testes of men with germinal failure

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The aim of the study was to determine whether a prior diagnostic testicle biopsy can predict success or failure of testicular sperm extraction (TESE) with intracytoplasmic sperm injection (ICSI) in patients with non-obstructive azoospermia caused by testicular failure, and what is the minimum threshold of sperm production in the testis which must be surpassed for spermatozoa to reach the ejaculate. Forty-five patients with non-obstructive azoospermia caused by testicular failure underwent diagnostic testicle biopsy prior to a planned future TESE–ICSI procedure. The diagnostic testicle biopsy was analysed quantitatively, and correlated with the quantitative findings of spermatogenesis in patients with normal spermatogenesis, as well as with the results of subsequent attempts at TESE–ICSI. Men with non-obstructive azoospermia caused by germinal failure had a mean of 0–6 mature spermatids/seminiferous tubule seen on a diagnostic testicle biopsy, compared to 17–35 mature spermatids/tubule in men with normal spermatogenesis and obstructive azoospermia. These findings were the same for all types of testicular failure whether Sertoli cell only, maturation arrest, cryptorchidism, or post-chemotherapy azoospermia. Twenty-two of 26 men with mature spermatids found in the prior testis biopsy had successful retrieval of spermatozoa for ICSI, 12 of their partners became pregnant, and are either ongoing or delivered. The study suggests that 4–6 mature spermatids/tubule must be present in the testis biopsy for any spermatozoa to reach the ejaculate. More than half of azoospermic patients with germinal failure have minute foci of spermatogenesis which are insufficient to produce spermatozoa in the ejaculate. Prior diagnostic testicle biopsy analysed quantitatively (for the presence of mature spermatids) can predict subsequent success or failure with TESE–ICSI. Incomplete testicular failure may involve a sparse multi-focal distribution of spermatogenesis throughout the entire testicle, rather than a regional distribution. Therefore, it is possible that massive testicular sampling from many different regions of the testes may not be necessary for successful TESE–ICSI.

Key words: ICSI/maturation arrest/non-obstructive azoospermia/quantitative spermatogenesis/Sertoli cell only

Introduction

Testicular sperm extraction and intracytoplasmic sperm injection (TESE–ICSI) was first introduced in 1993 for the treatment of obstructive azoospermia (Schoysman *et al.*, 1993; Devroey *et al.*, 1994; Silber *et al.*, 1994, 1995a). It was soon discovered that this technique could also be used for azoospermic men who appeared to have absent spermatogenesis, i.e. 'non-obstructive azoospermia' (Devroey *et al.*, 1995; Silber *et al.*, 1995b). The theoretical basis for attempting to retrieve spermatozoa for ICSI, from the testes of men with apparent absence of spermatogenesis, is based on a study from 1981 with quantitative analysis of spermatogenesis in testicle biopsy specimens (Silber and Rodriguez-Rigau, 1981; Zuckerman *et al.*, 1978). In many cases of non-obstructive azoospermia, an occasional spermatid was noted in the testis biopsy despite being categorized as absent spermatogenesis. There thus appeared to be a certain threshold of a minimum amount of spermatogenesis necessary for spermatozoa to reach the ejaculate (Silber *et al.*, 1995c).

It was not until the availability of intracytoplasmic sperm injection (ICSI) that this finding achieved practical clinical application for these patients (Van Steirteghem *et al.*, 1993; Palermo *et al.*, 1992). The technique (TESE–ICSI) was found to be equally successful in azoospermia caused by either 'incomplete' maturation arrest or by 'incomplete' Sertoli cell only (Devroey *et al.*, 1995; Silber *et al.*, 1996). It was noted that in all cases of maturation arrest, the spermatogenic defect was in meiosis, and there was no need to search for round spermatids when performing TESE–ICSI. Wherever meiosis was completed and round spermatids were formed, mature spermatids and spermatozoa could also be observed.

The qualitative histology of non-obstructive azoospermia cases undergoing TESE–ICSI has been reported to be highly variable (Tournaye *et al.*, 1996a, 1997). The testicular histology was often performed at the same time as the TESE procedure (not as a prior diagnostic biopsy), and was never analysed quantitatively for the presence of mature spermatids using the methods referred to earlier.

Because 60% of azoospermic cases with germ cell failure have been found to have spermatozoa in the testis, the diagnosis of 'Sertoli cell only' has had to be reconsidered and re-defined in many cases (Silber *et al.*, 1995c). The apparent absence of spermatogenesis appears in a majority of cases to be only a partial or incomplete defect. A better understanding of spermatogenesis has thus led to the successful treatment of many otherwise sterile azoospermic men with testicular failure of a variety of causes (Clermont, 1972; Steinberger and Tjioe, 1968; Heller and Clermont, 1964; Silber, 1995; Tournaye *et al.*, 1996b; Staessen *et al.*, 1996).

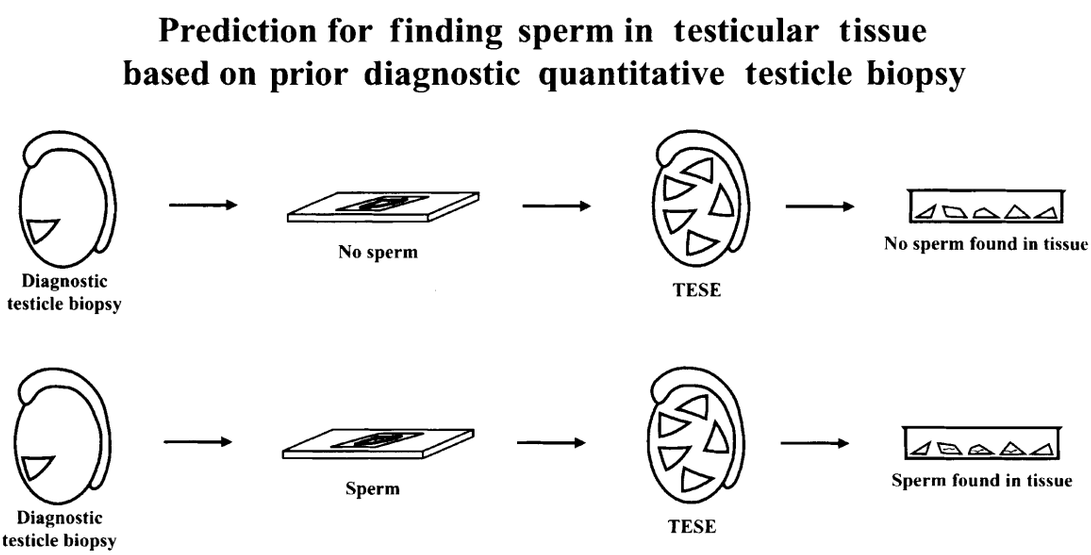

Figure 1. All patients in this study underwent a small diagnostic testicle biopsy before the relatively massive testicular sperm extraction (TESE)–intracytoplasmic sperm injection (ICSI) procedure.

The purpose of the present study is to answer several questions regarding spermatogenesis, and the pathology and treatment of non-obstructive azoospermia. The first question is: what is the maximum threshold number of mature spermatids/tubule found in the testis biopsy, below which no spermatozoa will appear in the ejaculate? The second question is: can quantitative analysis of a prior routine diagnostic testicle biopsy give any prognosis for the potential success of a future TESE–ICSI?' Finally, what can we conclude about the distribution of spermatids in such cases, and would this information help in the surgical strategy for sperm retrieval and TESE in non-obstructive azoospermia?

Materials and methods

Patient population

The patient population consisted of 45 consecutive male patients with a finding of azoospermia who were subjected to diagnostic testicle biopsy prior to their planned TESE–ICSI procedure (Figure 1). In all patients, the azoospermic semen was subjected, on three separate occasions, to centrifugation at 1800 *g*, with careful examination to determine the presence of even a single spermatozoon. If any spermatozoa at all were detected after centrifugation, these patients were excluded from the current study and underwent a standard ICSI procedure under the category of 'pseudo-azoospermia'. Patients were not considered to be azoospermic if even a single spermatozoon could be found after centrifugation at 1800 *g*. The absence of ductal obstruction was verified in all patients at the time of the diagnostic biopsy. All patients in this study then underwent, along with their wives, a TESE–ICSI procedure as has been previously described (Devroey *et al.*, 1995; Silber *et al.*, 1995a; Palermo *et al.*, 1992). In summary, the selection of patients for this study were those couples suffering from non-obstructive azoospermia who underwent a TESE–ICSI attempt after having undergone a prior diagnostic testicle biopsy.

All categories of non-obstructive azoospermia, based on the classical histological evaluation and clinical history, were included in this study. These categories were complete or incomplete Sertoli cell only, maturation arrest, cryptorchid testicular atrophy, post-chemotherapy azoospermia, and mixed types of deficient spermatogenesis. The diagnosis of testicular failure was based on the finding of azoospermia, the absence of obstruction, and classic pathological interpretation of histology. Then the biopsies were subjected to a time-consuming quantitative analysis.

Quantitative analysis of testicular biopsy

The method of quantitative analysis has already been described (Zuckerman *et al.*, 1978; Silber and Rodriguez-Rigau, 1981; Silber *et al.*, 1990). Histologic sections were prepared by excising the seminiferous tubules with wet, sharp, Iris scissors using a no-touch method, and allowing the specimen to fall into Zenker's solution. At no stage was the sample handled. The specimens were stained with haematoxylin and eosin and cut into thin sections.

At least 20 seminiferous tubules (usually more) were evaluated on each side. All of the steps of spermatogenesis were counted, but only the most common, pre-meiotic and post-meiotic cells, for the purpose of this report, were tabulated, i.e., pachytene spermatocyte (pre-meiotic), and mature, or maturing, spermatids (slightly to completely elongated). A biopsy showing a single, isolated tubule with a few spermatids in a field of tubules that were otherwise strictly Sertoli cell only was, for simplicity, defined as 'Sertoli cell only'. 'Maturation arrest' was defined as an absence of mature spermatids despite normal early stages of spermatogenesis. Again, the presence of a rare spermatid in an entire slide of otherwise total maturation arrest was defined as maturation arrest. The totals of pre-meiotic spermatocytes (pachytene) and post-meiotic cells (spermatids) were tabulated and expressed as the 'mean' number/seminiferous tubule. The contents of all seminiferous tubules in the histologic section were included in the tabulation.

If the biopsy showed normal spermatogenesis as defined by classical methods, the patient was deemed to have obstructive azoospermia, was thereby excluded from this study, and underwent microsurgery to correct the obstruction combined with microsurgical epididymal sperm aspiration (MESA). If obstruction was discovered, the patient was not included in this study, which is limited to pathology and treatment of non-obstructive azoospermia.

Technique of diagnostic testicle biopsy

The technique for diagnostic testicle biopsy was very simple compared to that used for TESE–ICSI. For diagnostic testicle biopsy, the

spermatic cord was injected with 5 ml of 0.5% local anaesthetic via a 27 gauge needle just distal to the external inguinal ring. Then an additional 2 ml of 0.5% local anaesthetic was injected over the anterior scrotal skin in the area where a 0.5 cm incision was made all the way into the tunica albuginea. This is a fairly painless clinic procedure from which the patients are recovered within 15–20 min. Although the procedure is no more painful than a needle biopsy, it always yields a sufficient number of seminiferous tubules to perform an adequate quantitative analysis.

The technique used for TESE–ICSI, however, involved a larger incision, usually a general anaesthetic, and an extensive exposure of the surface of the tunica albuginea in order to obtain samples from all regions of the testis. Thus, a prior testicle biopsy was a very minimal procedure compared to the extensive procedure employed for TESE–ICSI.

TESE–ICSI procedure

The technique for TESE–ICSI in these cases of non-obstructive azoospermia was more extensive than in cases of obstruction, and has previously been described in great detail (Silber *et al.*, 1996). A 2 cm horizontal incision was made in the scrotal skin and carried through the peritoneal tunica vaginalis, and then small incisions were made through the tunica albuginea in different regions of the testis. A small piece of extruding testicular tissue was excised and placed in a Petri dish with 3 ml HEPES-buffered media. After each biopsy, the tunica albuginea was closed with 3–0 vicryl stitches and the tissue was prepared and examined in the IVF laboratory before any subsequent biopsies were made in other areas of the testicle or testicles. If no spermatozoa were found after an initial search, then another biopsy was performed. The extensive search for spermatozoa in the ICSI lab would take too long for the patient to remain in the operating room waiting for a decision as to whether another TESE biopsy should be obtained from a different region of the testis. Therefore, if no spermatozoa were seen initially (before concentration in multiple 5 µl droplets), further biopsies were taken. This is the standard approach we have used from the beginning for such cases. Often 4–8 biopsies were obtained if deemed necessary.

The testicular tissue was minced finally in HEPES-buffered media and, after initial inspection in the IVF lab, placed in a 5 ml falcon tube and centrifuged for 5 min at 300 *g*. The supernatant was removed and the pellet resuspended in 50 µl of medium. For very difficult cases, the testicular suspension was incubated in red blood cell lysing buffer, and then washed again with HEPES-buffered medium (Ogura and Yanigamachi, 1993). All of the testicular suspension was examined completely by dividing it into multiple microdroplets in the ICSI injection dish with a volume of 5 µl/droplet. In this manner, the entire effluent obtained by mincing testicular tissue was examined *in toto* over the course of many hours, in an attempt to retrieve spermatozoa for ICSI. The ICSI procedure was then performed in the manner which has already been described in great detail (Silber *et al.*, 1996).

Results

Threshold of spermatogenesis for spermatozoa to reach the ejaculate

Tables I, II, and III summarize the results of the quantitative tabulation of pachytene spermatocytes and spermatids found in the seminiferous tubules on histologic examination of the patients with Sertoli cell only, with maturation arrest, and with mixed causes of non-obstructive azoospermia. In previous studies it has been demonstrated that with obstruction and normal spermatogenesis, the mean number of mature spermatids/tubule ranges from 17.4–31.4 (Silber and Rodriguez-Rigau, 1981; Silber *et al.*, 1990). Wherever spermatids are present, a similar number of spermatocytes are also found. As can be seen from Table I, in patients with the classic histologic diagnosis of Sertoli cell only, nine out of 23 (39%) were found to have a very small number of spermatids present (0.5–6.4 spermatids/seminiferous tubule) and thus were, in a sense, incomplete Sertoli cell only. This tiny focus of spermatogenesis in 39% of men who otherwise had classic Sertoli cell only is dramatically less than the number of spermatids seen in men with obstructive azoospermia who are presumed to have normal spermatogenesis (Figure 2).

A similar finding is noted in Table II in the number of spermatids seen in the histology of men with azoospermia caused by maturation arrest. In this case, there is a mean of 0.4 spermatids/tubule to 5.6 spermatids/tubule seen in 10 of the 12 cases, and no spermatids whatsoever seen in two of the 12 cases. Again, this represents a dramatic decrease in the presence of spermatids compared to that seen in men with obstructive azoospermia and presumably normal spermatogenesis. Table III demonstrates the same phenomenon in cases of miscellaneous non-obstructive azoospermia including cryptorchidism and post-chemotherapy azoospermia, with anywhere from 0 spermatids/tubule to a maximum of 6.6 spermatids/tubule.

In the vast majority of these 45 cases of non-obstructive azoospermia, <4 mature spermatids/tubule were noted in the quantitative histological analysis, and in none of these 45 patients with non-obstructive azoospermia was there >6.5 mature spermatids/tubule noted. This represents a 6–30-fold reduction in the number of mature spermatids/tubule seen in the two-dimensional histologic section from men with non-obstructive azoospermia compared to men with normal spermatogenesis.

As was explained in 1981, the quantitation of spermatids/seminiferous tubule on histologic section represents a two-dimensional evaluation, whereas the amount of spermatozoa in the ejaculate relates to the three-dimensional testicular volume. Therefore, the correlation between spermatogenesis seen on the two-dimensional histological slide and the number of spermatozoa to reach the ejaculate is exponential.

Therefore the testicular histology does not have to be absolute zero in order for no spermatozoa to reach the ejaculate. Rather, a threshold of approximately 4–6 mature spermatids/tubule must be exceeded for any spermatozoa to reach the ejaculate.

Comparison of quantitative evaluation of histology in a diagnostic testicle biopsy and the successful retrieval of spermatozoa for TESE–ICSI

Of the 45 patients included in this study, 26 (58%) had detectable spermatids present in the diagnostic biopsy performed prior to the attempt at TESE–ICSI. Of those 26 patients with spermatids present in the prior biopsy, 22 (85%) had a successful TESE–ICSI procedure performed later. Of those 22 patients in whom spermatozoa could be retrieved and used to

Table I. Azoospermia caused by Sertoli cell only

Patient no.	Mean no. pachytene spermatocytes/ seminiferous tubule[a]	Mean no. spermatids/ seminiferous tubule[a]	Spermatozoa found for TESE–ICSI?	
			yes	no
1	3.3 ± 8.6	1.70 ± 6.0	X[b]	
2	3.1 ± 8.0	4.10 ± 13.6	X	
3	0	0		X
4	0	0		X
5	6.3 ± 10.2	.30 ± 7.1	X[b]	
6	2.1 ± 3.2	1.73 ± 2.5	X[b]	
7	0	0		X
8	0	0		X
9	3.3 ± 8.8	2.70 ± 7.6	X	
10	0	0		X
11	0	0	X	
12	1.6 ± 4.8	1.50 ± 5.1	X	
13	0	0		X
14	0	0		X
15	0	0		X
16	0	0		X
17	3.9 ± 12.2	2.70 ± 8.9	X[b]	
18	8.1 ± 13.5	6.40 ± 11.2	X[b]	
19	0	0		X
20	0	0		tX
21	0	0		X
22	0	0		X
23	1.5 ± 4.9	0.50 ± 1.8	X	

[a]±SD.
[b]Pregnant, ongoing >20 weeks.
TESE = testicular sperm extraction.
ICSI = intracytoplasmic sperm injection.

Table II. Azoospermia caused by maturation arrest

Patient no.	Mean no. pachytene spermatocytes/ seminiferous tubule[a]	Mean no. spermatids/ seminiferous tubule[a]	Spermatozoa found for TESE–ICSI?	
			yes	no
1	25.4 ± 5.8	3.1 ± 4.2	X[b]	
2	19.4 ± 9.2	0	X	
3	17.6 ± 5.4	0	X	
4	22.1 ± 5.6	5.6 ± 2.6	X	
5	17.1 ± 7.0	1.8 ± 4.2		X
6	28.7 ± 9.7	2.1 ± 4.1	X	
7	33.5 ± 7.6	1.4 ± 1.9	X[b]	
8	25.5 ± 6.9	1.2 ± 1.8	X[b]	
9	15.9 ± 5.1	1.6 ± 3.7		X
10	22.8 ± 5.7	3.2 ± 3.4	X	
11	24.9 ± 7.0	0.4 ± 1.0	X	
12	17.4 ± 5.1	1.3 ± 1.9	X[b]	

[a]±SD.
[b]Pregnant, ongoing >20 weeks.
TESE = testicular sperm extraction.
ICSI = intracytoplasmic sperm injection.

fertilize their wife's oocytes, 12 (55%) had a successful pregnancy. Of the 19 patients without any spermatids seen on an exhaustive quantitative evaluation of the prior biopsy, only one had a successful TESE–ICSI procedure (defined by retrieving spermatozoa which were used successfully to fertilize their wife's oocytes), but none became pregnant. Thus, a prior positive testicle biopsy yielded an 85% chance for successful TESE–ICSI, and a negative prior testicle biopsy reduced the possibility for successful TESE–ICSI to only 5%.

Comparison of Sertoli cell only, maturation arrest, and other miscellaneous causes of non-obstructive azoospermia

With azoospermia caused by Sertoli cell only, as noted in Table I, the mean number of pachytene spermatocytes is roughly equivalent to the mean number of spermatids/seminiferous tubule. This one-to-one relationship is the same as is seen in normal spermatogenesis (Silber *et al.*, 1990). However, in Table II, it is observed that with azoospermia caused by maturation arrest, there is a normal number of pachytene

Table III. Miscellaneous non-obstructive azoospermia: cryptorchidism, chemotherapy, combined maturation arrest and Sertoli cell only

Patient no.	No. pachytene spermatocytes/ seminiferous tubule[a]	No. spermatids/ seminiferous tubule[a]	Spermatozoa found .for TESE–ICSI?	
			yes	no
1	7.6 ± 12.1	0		X
2	8.9 ± 9.2	3.3 ± 7.1	X	
3	0	0		X
4	6.4 ± 7.1	0		X
5	6.2 ± 8.5	6.5 ± 9.9	X[b]	
6	10.8 ± 9.4	2.5 ± 5.4	X	
7	1.7 ± 5.5	1.4 ± 4.6	X[b]	
8	18.1 ± 6.8	3.1 ± 3.9	X	
9	10.7 ± 9.5	3.8 ± 6.2	X[b]	
10	11.5 ± 10.1	3.8 ± 5.6	X	

[a]±SD.
[b]Pregnant, ongoing >20 weeks.
TESE = testicular sperm extraction.
ICSI = intracytoplasmic sperm injection.

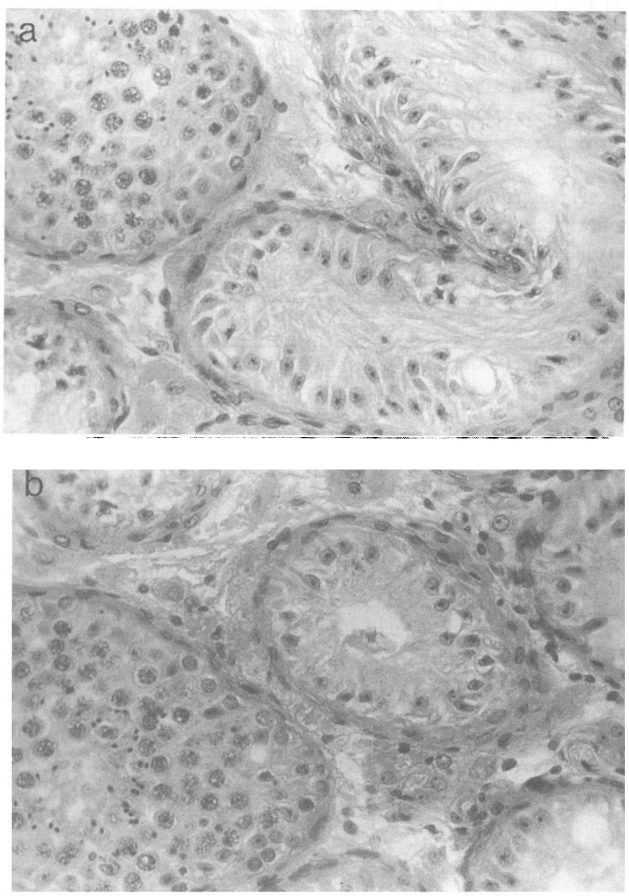

Figure 2. **(a)** Sertoli cell only, **(b)** normal spermatogenesis.

spermatocytes counted per seminiferous tubule despite either zero or a drastically reduced number of spermatids/seminiferous tubule (Figure 3). In the miscellaneous categories of non-obstructive azoospermia, there were a variable number of pachytene spermatocytes despite a consistently reduced number of spermatids.

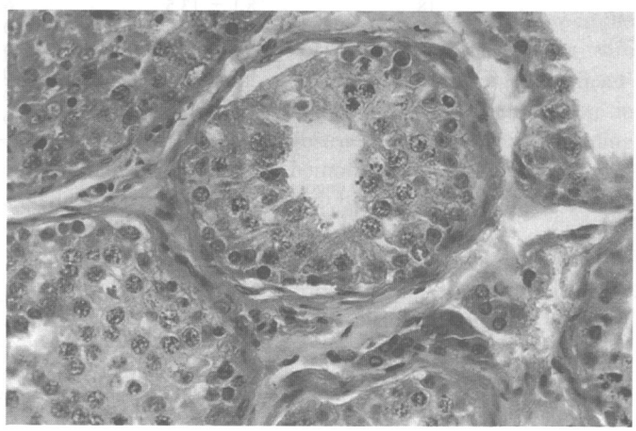

Figure 3. 100% maturation arrest.

Peculiarly, with Sertoli cell only, the presence of any spermatids in the prior biopsy uniformly predicted that TESE–ICSI would be successful. However, with maturation arrest, the finding of spermatids in a prior biopsy was no guarantee of successful retrieval of spermatozoa for TESE–ICSI. Nine out of nine Sertoli cell only cases with spermatids seen at prior histology had successful ICSI sperm retrieval, but only six out of 10 cases of maturation arrest with spermatids seen at prior histology had a successful retrieval of spermatozoa at TESE–ICSI. Furthermore, the histology of patients with maturation arrest usually revealed only 20–40 cross-sections of tubules for counting, whereas in Sertoli cell only cases, there were generally more than 100 tubule cross-sections. This is readily explained by the diminished diameter of seminiferous tubules with Sertoli cell only compared to the completely normal diameter of seminiferous tubules in patients with maturation arrest. As has been noted previously, this phenomenon explains the normal size of testes with maturation arrest, and the generally normal follicle-stimulating hormone (FSH) concentration in those cases (Silber *et al.*, 1996).

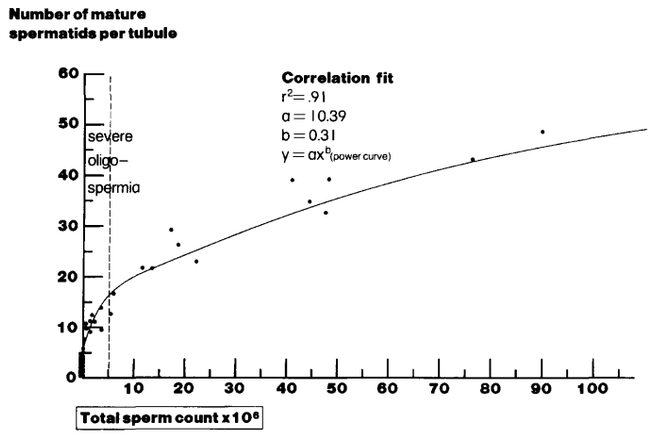

Figure 4. Graph depicting quantitative testicle biopsy and sperm count.

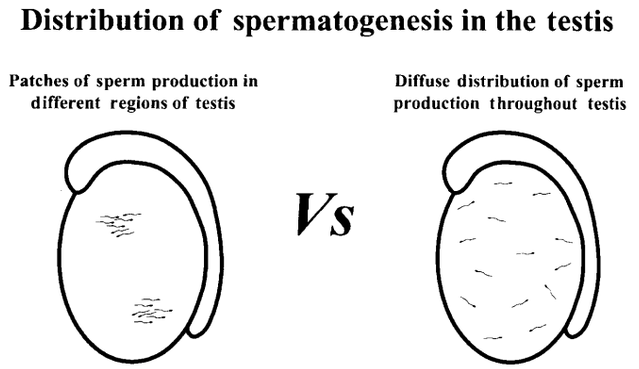

Figure 5. The reliability of prior diagnostic biopsy suggests a homogeneous distribution of spermatogenesis in non-obstructive azoospermia, and not a patchy distribution.

Discussion

The rationale for treating azoospermia caused by germinal failure with TESE and ICSI is based on the consistent observation that in a little over 50% of such patients, a miniscule number of spermatozoa can be extracted from testicular tissue and that this tiny number of often non-motile testicular spermatozoa can be successfully used with the ICSI procedure to achieve fertilization and pregnancy. The present study appears to indicate that there is indeed a minimum quantitative threshold of spermatogenesis which must be exceeded for any spermatozoa to reach the ejaculate. In all cases of non-obstructive azoospermia subjected to a detailed quantitative study there appears to be a mean of <6 mature spermatids/tubule (usually <4). Most likely, when the mean number of mature spermatids/seminiferous tubule (i.e. all of the spermatids found on the slide divided by all of the number of tubules) is <4–6, there is a quantity of spermatozoa insufficient to reach the ejaculate after transit from the testicle. The normal range of spermatogenesis in fertile men, or in obstructive azoospermia, appears to be from 17 spermatids/seminiferous tubule to about 35 spermatids/seminiferous tubule (Silber *et al.*, 1990; Silber and Rodriguez-Rigau, 1981).

Because of the exponential effect of the sperm count in the ejaculate being a function of seminiferous tubule volume, and the quantitative analysis histology being a two-dimensional measurement, the curve expressing the relationship between mean number of spermatids/seminiferous tubule, and sperm count in the ejaculate, is an exponential one which 'breaks' at around 15 spermatids/tubule (Figure 4). Therefore a 6–30-fold decrease in the quantitative production of spermatozoa seen on a two-dimensional seminiferous tubule cross-section can result in as much as a thousand-fold or greater difference in actual sperm production. Thus, the presence of only a few mature spermatids/tubule in germinal failure and non-obstructive azoospermia cases represents an extremely deficient degree of sperm production. It is apparent that an extremely low quantity of sperm production is compatible with azoospermia and that a threshold of probably 4–6 mature spermatids/tubule must be exceeded for any spermatozoa to reach the ejaculate.

The findings were similar for cases of Sertoli cell only, maturation arrest, cryptorchidism, post-chemotherapy, and even combinations of maturation arrest and Sertoli cell only. Thus, the type of germinal failure, or the cause of the non-obstructive azoospermia, had no effect on this threshold concept. In fact, the same threshold principle seems to apply equally for all types of germinal failure and non-obstructive azoospermia whatever the cause.

How can a small diagnostic testicle biopsy specimen representing perhaps 1/1000 of the total testicular volume possibly predict the presence of a minute patch of spermatogenesis to be found somewhere in the testicle at a future date during a TESE–ICSI procedure? The fear is that either the diagnostic biopsy could miss the one area where spermatogenesis was occurring, or alternatively, if the diagnostic biopsy was lucky enough to hit the one area of spermatogenesis, then perhaps it could be removed with that diagnostic biopsy. The findings of our present study suggest that these are not serious problems because the distribution of a minute quantity of spermatogenesis in these germinal failure patients must be diffusely multi-focal, distributed evenly throughout the testicle rather than in a few patchy areas (Figure 5). The fact that a single diagnostic testicle biopsy is relatively predictive of successful retrieval of spermatozoa at TESE, and in fact of multiple TESE–ICSI procedures in the same patient, argues for a distribution of spermatogenesis in patients with non-obstructive azoospermia that is multi-focal and relatively homogeneous throughout the testicle.

This homogeneous distribution within the testis has been well established in normal spermatogenesis since the early work of Steinberger, who found that the quantitative determination of the various elements of spermatogenesis determined by a single diagnostic testicle biopsy is representative of what would be found if the entire testicle were removed, homogenized, and counted *in toto* (Steinberger and Tjioe, 1968). If the presence of a minute amount of spermatogenesis in these deficient testicles was of a regional nature, dispersed unevenly in perhaps one or two different areas, rather than sprinkled like pepper throughout the testicle, then a diagnostic testicle biopsy would not be predictive of the potential success or failure of TESE–ICSI. The fact that a diagnostic biopsy is predictive of

success with TESE supports Steinberger's original concept from 1968.

Comparison of the findings in maturation arrest and Sertoli cell only is also of interest. In maturation arrest cases, we found a completely normal distribution of 17.1–33.5 pachytene spermatocytes/seminiferous tubule. This is no different from the number of pre-meiotic cells that would be seen in patients with obstructive azoospermia and normal spermatogenesis. Nonetheless, in maturation arrest, there were either no spermatids, or a very tiny number of spermatids (up to a maximum of 6/tubule), just as in Sertoli cell only. Thus, the maturation arrest cases we have seen do not reflect a failure of the maturing of spermatids. Rather, maturation arrest in our series represented a failure of the spermatocytes to undergo meiosis. Furthermore, like 'Sertoli cell only,' it is not necessarily a 100% defect. In the majority of cases of maturation arrest, there are nonetheless a tiny number of pachytene spermatocytes that complete meiosis into spermatids which are capable of developing into normal children after TESE–ICSI. Finally, it needs to be emphasized that in none of the cases of maturation arrest did we find round spermatids in the absence of elongated spermatids, or mature spermatozoa.

In summary, there is a threshold of spermatogenesis which must be exceeded for any spermatozoa at all to reach the ejaculate. Quantitative analysis of testicle biopsy is able to predict the likelihood of success or failure of TESE–ICSI. Extremely tiny numbers of spermatozoa extracted from a testicle biopsy in azoospermic men appear to yield pregnancy rates using ICSI no lower than those achieved in men with normal spermatogenesis. Finally, prior diagnostic testicle biopsy is recommended for all patients with azoospermia from any cause in order to plan appropriate treatment. Rather than representing an unnecessary extra procedure, this knowledge may prevent the needless removal of excessive tissue from all regions of the testis when attempting TESE–ICSI. If the prior testicle biopsy, evaluated quantitatively, reveals no spermatids, then the couple should be counselled about the possibility of donor sperm back-up.

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