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INVITED REVIEW

Sperm retrieval for azoospermia and intracytoplasmic sperm injection success rates – A personal overview

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Abstract
It is often questioned whether sperm parameters, including whether retrieved or ejaculated, have any effect on intracytoplasmic sperm injection (ICSI) results. Do severe spermatogenic defects affect embryo quality or pregnancy rate? Further, does it matter in azoospermic patients whether the sperm source is testicular or epididymal? Our studies show there is no significant difference in results with ICSI related to any sperm count parameters either with patient's sperm or even with donor sperm. No matter how poor the sperm count, there was no difference from patients with high sperm counts nor even patients using donor sperm. There is no significant difference between results with epididymal sperm, either fresh or frozen, in comparison to results with ejaculated or donor sperm. However, both pregnancy rate and delivery rate were considerably lower with testicular sperm (testis sperm extraction) than with epididymal sperm (microsurgical epididymal sperm aspiration). This was true for overall results as well as in each category of the female partner's age. It is obvious that in all these cycles the female partner's age was the most important determinant of delivery rate, but testicular sperm always yielded lower results than epididymal sperm. These results show that it is the origin of the sperm rather than the spermatogenic defect that determines success rate with ICSI.

Keywords: *Azoospermia, intracytoplasmic sperm injection (ICSI), microsurgical sperm aspiration (MESA)*

Introduction

Approximately one out of every 200 men in any population (excluding those who have had a vasectomy) is azoospermic (Hull et al., 1985) (Figure 1). Approximately 20% of couples in the United States are infertile (Mosher, 1985, 1987), and approximately 25% of all infertile couples have a low sperm count (Hull et al., 1985). About 2% of infertile couples have azoospermia (Hull et al., 1985). Thus, azoospermia represents approximately 8% of the cases of male infertility, and we classify azoospermia as 'obstructive' and 'non-obstructive.'

Sperm retrieval, whether for obstructive or non-obstructive azoospermia (NOA), should be a quick outpatient procedure under local anaesthesia (Silber, 2000a). The spermatic cord is injected with about 6 ml of 0.5% marcaine (bupivacaine) via a 25-gauge needle just distal to the external inguinal ring. Then an additional 2 ml of 0.5% marcaine is injected over the anterior scrotal skin in the area where a 1–2 cm incision is made and carried down to the tunica vaginalis. The testis and epididymis are then extruded

and fully exposed. For testis sperm extraction (TESE) an incision is made in the tunica albuginea, and all anatomic lobules of the testis are exposed and sampled. This is a thoroughly painless clinical procedure (except for the initial injection of local anaesthetic). The patient is able to get up and walk away immediately afterward with no more pain than if he would have had a vasectomy. For obstructive cases, epididymal sperm (microsurgical epididymal sperm aspiration (MESA)) retrieval is preferred to TESE, and the data supporting this will be explained.

Needle biopsy is another alternative, but it is no less painful than the open biopsy as described above, and the open biopsy can always sample every anatomic lobule of seminiferous tubules so as to be certain that if there are any sperm, they will be found. Needle biopsy cannot accomplish this unless performed multiple times, which is then ironically more invasive and painful than the open biopsy technique, and the results much less certain.

There has been considerable unnecessary confusion about the interpretation and counting of mature spermatids in the testis biopsy. The mature

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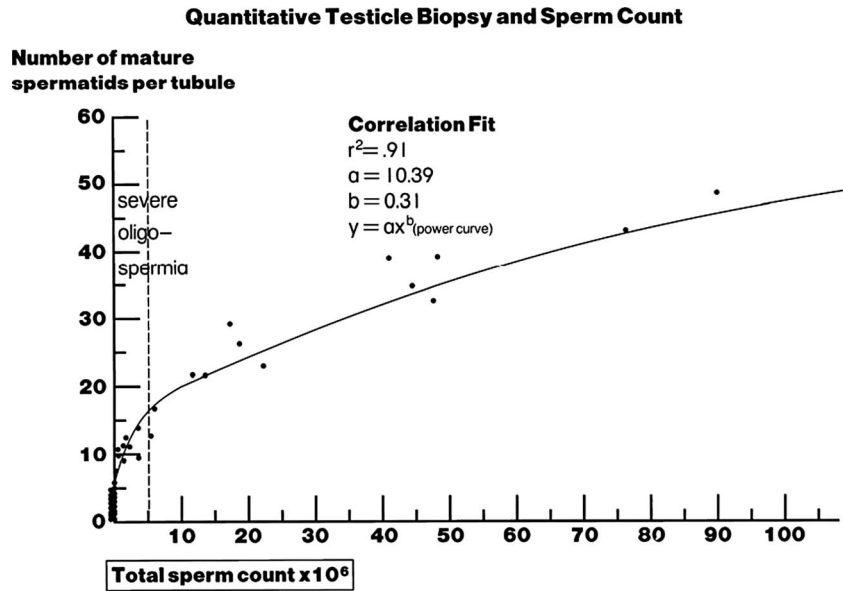
Quantitative Testicle Biopsy and Sperm Count

Figure 1. An exponential curve relating sperm count in the ejaculate to the average number of mature spermatids seen in each seminiferous tubule. A threshold of three to six mature spermatids per tubule had to be exceeded in order for sperm to appear in the ejaculate (Silber et al., 1997b).

spermatid always has a tail, but this is rarely seen on histological section, because the sperm head is 4 microns wide and likely to be in the cut of the microtome's thin section, but the sperm tail is less than 1 micron in thickness and unlikely to be in the cut section of the microtome. Therefore, when viewing a histologic section, the spermatids will appear to be without tails even though with TESE, they will appear just like sperm.

Some clinicians have attempted to use the serum follicle-stimulating hormone (FSH) level to monitor the amount of spermatogenesis. A normal FSH level in an azoospermic patient would supposedly indicate obstruction. Unfortunately, this correlation is very poor (De Kretser et al., 1974). Patients with maturation arrest causing azoospermia have a normal FSH level. Furthermore, even the tiniest number of sperm in the testis (not enough to reduce the FSH level) is adequate for successful intracytoplasmic sperm injection (ICSI). Ironically, it is the scattered mosaic arrangement of the various stages of spermatogenesis in the human seminiferous tubule (as opposed to the orderly wave moving across the tubule in most other species) that makes quantifying the human testicular biopsy so simple. In rats, a cut through any particular seminiferous tubule shows only one particular stage (Figure 2a and b). In humans, a cut through any area of the testicle reveals a scattered array of all the various stages of spermatogenesis. Thus, in humans, unlike most other animals, it requires only 25 seminiferous tubules from anywhere in the testis for a good statistical sample of the total range of spermatogenesis in the entire testicle.

Sperm retrieval and ICSI for obstructive azoospermia (MESA and TESE)

Since the first successful use of epididymal sperm aspiration and IVF for CBVAD was reported, ICSI has now made it possible for all of these men to have children (Silber et al., 1987, 1988, 1990a,b, 1994, 1997a,b; Tournaye et al., 1994). In fact, with ICSI, the pregnancy rate with microsurgical epididymal sperm retrieval (MESA) is only related to female factors (Silber et al., 1994, 1995a,b,c, 1997a,b). Frozen epididymal sperm gives results with ICSI no different from fresh, and one MESA procedure should provide enough sperm for virtually an infinite number of ICSI-IVF cycles. Under $10 \times$ to $40 \times$ magnification with an operating microscope, a 0.5-cm incision is made with microscissors into the epididymal tunic to expose the tubules in the most proximal portion of the congenitally blind-ending epididymis. Sperm are aspirated with a micropipette (0.7 mm/22mm; Cook Urological, Spencer, IN) on a tuberculin syringe directly from the opening in the epididymal tubule. The specimens are immediately diluted in HEPES-buffered medium, and a tiny portion is examined for motility and quality of progression. If sperm motility is absent or poor, another aspiration is made 0.5 cm more proximally. Sperm are obtained from successively more proximal regions until progressive motility is found (Figure 3).

Motile sperm are usually not obtained until the most proximal portion of the caput epididymis or vasa efferentia is reached. This is the opposite of what you would find in a normal, non-obstructed
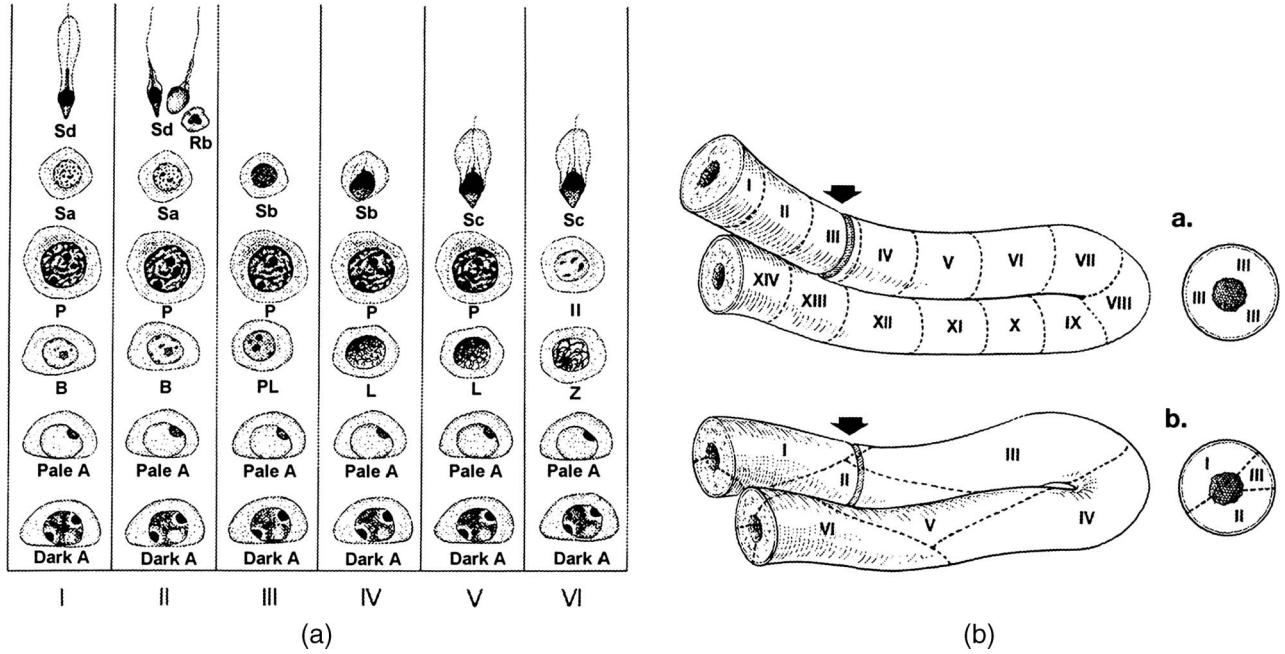
Figure 2. (a) The six stages of spermatogenesis in the human testicle (Silber, 1984). (b) Drawings of the progression of stages of spermatogenesis in the rat seminiferous tubule (a) and in the human seminiferous tubule (b). In most animals there is a wave of spermatogenesis going in an orderly fashion down the seminiferous tubule. In the human, however, there is a mosaic arrangement of the six stages of spermatogenesis (Silber, 1984).
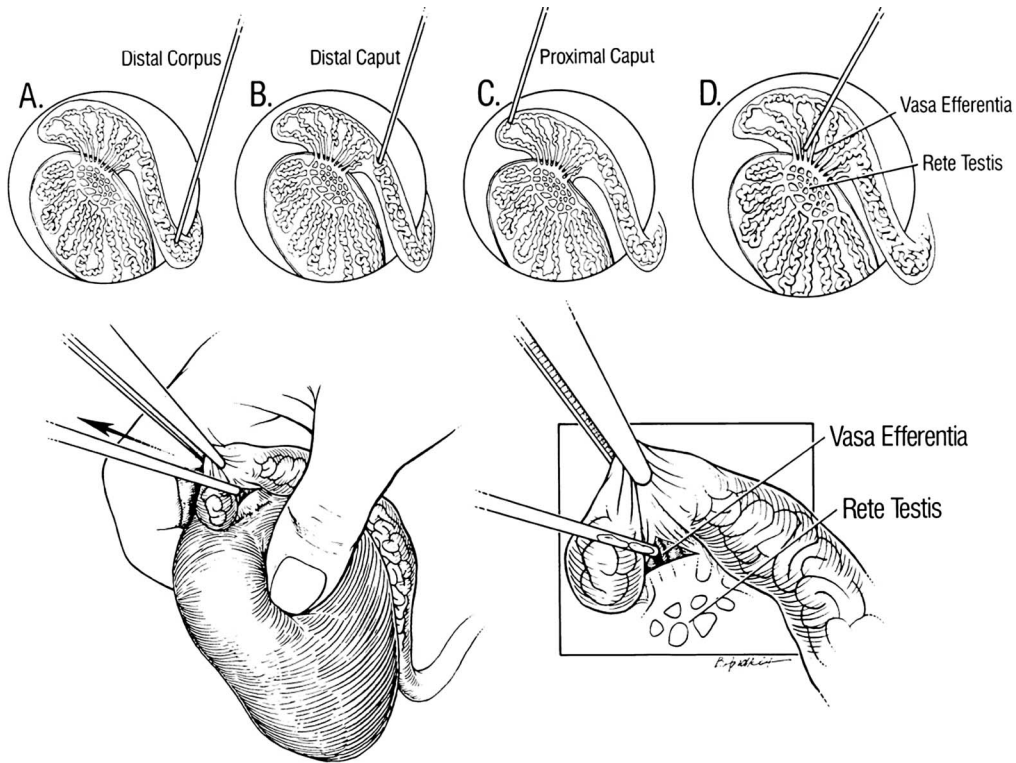

Figure 3. A depiction of microsurgical epididymal sperm aspiration (MESA) beginning at the distal corpus (a) and moving proximally to the distal caput, the proximal caput, and the vasa efferentia (b, c and d). With obstructive azoospermia, there is an inversion of the usual physiological location of greatest and least sperm motility. With obstruction, the most motile sperm are always the most proximal. Distal sperm, because of senescence, are the least motile (Silber et al., 1990a).

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epididymis and is the cause of much confusion in the literature on this subject. In the obstructed epididymis, the most recently produced sperm are the most proximal, and therefore the most viable and motile. The distal epididymal sperm are the most senescent and clearly non-viable. Therefore, distal epididymal sperm have much greater sperm DNA fragmentation so obvious as to be readily observable under electron microscopy (Asch et al., 1992). Once the area of motile sperm is found, an aliquot of epididymal fluid is used for ICSI, and the remainder is frozen.

For obstructive azoospermia (OA) we prefer to use epididymal sperm, although testicular sperm often works just as well. One advantage of epididymal sperm as a first choice is that it freezes so easily and represents such a simple, clean, easy and indefinite supply of sperm for the laboratory to use for that particular patient, without any need for future invasive procedures. More importantly, our data now show that a higher ICSI success rate is achieved with motile epididymal sperm as opposed to less mature testis sperm.

There have been many trivial debates over how to best collect epididymal or testicular sperm from azoospermic patients for ICSI. For OA, there is usually some epididymis present no matter how severe the congenital defect. In these instances, we prefer microsurgical epididymal sperm aspiration (MESA). We do all sperm retrieval under local anaesthesia without sedation. Although the approach is microsurgical and careful, it is an outpatient procedure performed with minimal postoperative discomfort. Once the tunica vaginalis is entered, the epididymis and testicle are exposed and brought into the field of an operating microscope. The patient, indeed, can watch the whole procedure on a video monitor and should be wide-awake and comfortable. The advantage of epididymal sperm retrieval performed in this fashion is the huge number of the most motile sperm that can readily be obtained from the most proximal duct and frozen for an unlimited number of future ICSI cycles.

There is often only one specific area of the proximal epididymis where motile sperm can be retrieved, and this can be found more easily through microsurgery than via a blind needle stick (which, in truth, is a more painful than this microsurgical MESA procedure).

An important warning is that for NOA, epididymal sperm can never be retrieved, because the walls are collapsed and there is no obstruction to allow epididymal sperm collection to take place. For NOA, an open testicular biopsy, performed under the microscope, must be performed under the same type of local anaesthetic, with the patient wide-awake, and with minimum postoperative discomfort.

Testicular sperm extraction (TESE) for non-obstructive azoospermia

Shortly after introducing sperm retrieval for OA, we made the observation that even in men with the most severe spermatogenic defects, causing complete azoospermia, there were often a very minute number of sperm sparsely present in an extensive testicular biopsy, and these occasional testicular sperm could be used for ICSI (Devroey et al., 1995a,b; Silber et al., 1995a,b,c, 1996, 1997a,b; Silber, 1998). We coined this procedure testicular sperm extraction (TESE). This approach was based on quantitative studies of spermatogenesis dating back to the late 1970s (Steinberger & Tjioe, 1968; Zukerman et al., 1978; Silber & Rodriguez-Rigau, 1981; Silber et al., 1990a,b). The examination of the testicular histology of azoospermic, oligospermic and normospermic men shows that the number of sperm in the ejaculate is directly correlated to the number of mature spermatids found quantitatively in the testis. The average mature spermatid count per tubule in a large number of tubules is predictive of the sperm count in the ejaculate. Intriguingly, about 60% of patients with complete azoospermia have a few mature spermatids in their testis histology (Figure 4).

An extremely diminished quantity of sperm production in the testis will result in absolute absence of sperm in the ejaculate even though there is some sperm being produced in the testicle. There is a minimal quantitative threshold of sperm production that is necessary before any sperm can actually 'spill over' into the ejaculate. Thus, severe oligospermia, which is readily treated with ICSI, is just a quantitative variant of azoospermia where more than three mature spermatids per tubule are found in the testis. There is some minute presence of
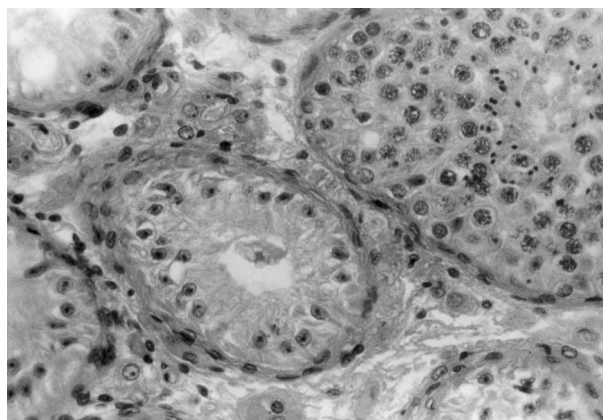

Figure 4. This is a histologic section of testicle biopsy in a patient with Sertoli cell only, elevated FSH, and occasional tubules with normal spermatogenesis. Upper right-hand tubule exhibits normal spermatogenesis, but all of the other tubules are Sertoli cell only (Silber et al., 2000).

spermatogenesis in 60% of azoospermic men. However, the amount of spermatogenesis is below the threshold (three mature spermatids per tubule) necessary for these few sperm to 'spill over' into the ejaculate (Silber et al., 1997a,b).

Microsurgical TESE

When extensive multiple biopsies from every area of the testis are performed in an effort to find sufficient sperm for TESE, a great deal of testicular damage can result, and may limit 'successful' patients to only one attempt (Tournaye et al., 1996, 1997). An attempt to limit damage by using multiple needle sticks rather than open biopsy to obtain sperm for ICSI is just as invasive and quite risky as well (Craft et al., 1997). Furthermore, control studies have shown that for difficult cases of NOA, where spermatogenesis is very meager, needle biopsy is much less likely to find the rare foci of spermatogenesis than open biopsy (Friedler et al., 1997; Rosenlund et al., 1998).

We studied the distribution of spermatogenesis in azoospermic men, and have outlined a *microsurgical* approach to TESE that minimises tissue loss and pain, and makes TESE very easily repeatable for an indefinite number of cycles. Knowledge of the distribution of spermatogenesis and use of microsurgical technique helps to prevent testicular damage and post-operative pain, making multiple repeat TESE procedures (if needed) safe and reliable (Figure 5) (Silber et al., 1997a,b; Silber 2000b).

The solution to cases where there are no sperm to be seen on TESE is not to look for 'round spermatids' (Silber & Johnson, 1998; Silber et al., 2000). We never see round spermatids in the absence of mature spermatids, which at TESE are what just appear to be sperm (Figure 6a–c) (Silber & Johnson, 1998; Silber et al., 2000; Holstein &

Roosen-Runge, 1981). The solution is to search for the few sperm with tails that are very sparsely and diffusely present.

Technique of microsurgical tese procedure

There has been a great deal of confusion created by the term 'microsurgical TESE'. One extremely damaging approach is the so-called 'micro-dissection'. In this approach, the entire testis is exposed, not just the periphery, and every millimetre is dissected looking for 'dilated' tubules. The idea is to try to limit somniferous tubule loss, but in truth, the opposite results, and many of these cases result in virtual castration (Schlegel & Chang, 1991; Schlegel & Su, 1997; Schlegel et al., 1997; Schlegal 1999). The safe and equally effective alternative microsurgical approach is as follows (Silber 2000a,b). Under the operating microscope, a longitudinal incision is made in the tunica albuginea exposing all anatomic lobules of the testis. Tissues from only the peripheral loops of the seminiferous tubules are sampled. This avoids damage to the testis and yet samples every corner from which sperm might be present. The tunica albuginea is closed with 9-0 nylon interrupted sutures, after meticulous haemostasis with micro-bipolar forceps (Figures 7 and 8). This prevents any increase in intratesticular pressure, resulting in minimal pain and no subsequent atrophy.

Of the total cases subjected to microsurgical TESE for NOA, about 60% yield sperm sufficient for ICSI. In *maturation arrest*, often a larger amount of testicular tissue has to be removed because all tubules are normal size, whereas with Sertoli cell only, the tubules are very thin. Nonetheless, in either case, minimal damage is incurred with micro TESE because blood supply is not interrupted, microscopic bleeders are meticulously coagulated, and tunica albuginea is not encroached upon because of the
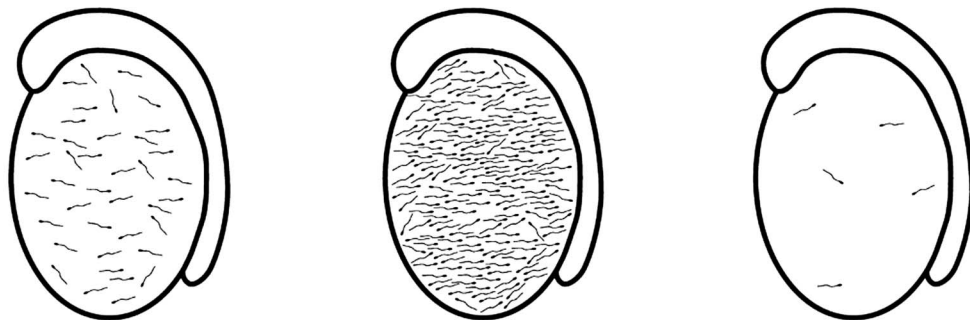
| Non-Obstructive Azoospermia (One in 20 tubules have sperm) | Normal Spermatogenesis (All tubules have sperm) | Non-Obstructive Azoospermia (One in 100 tubules have sperm) |

Figure 5. Various 'degrees' of azoospermia. Normal spermatogenesis (centre drawing) is associated with obstructive azoospermia. With non-obstructive azoospermia, TESE may be easy as in the drawing depicted on the left, or very difficult as depicted in the drawing on the right.

(a)

(b)
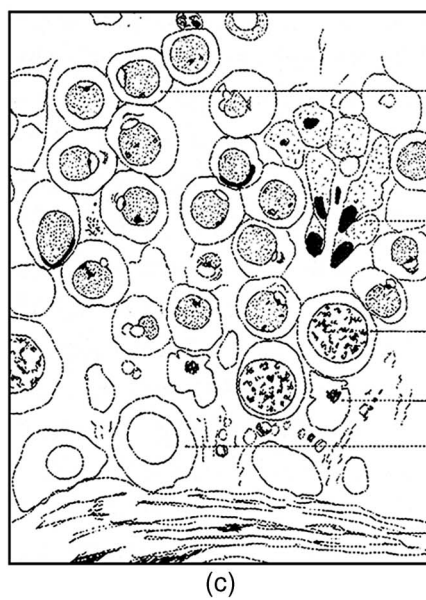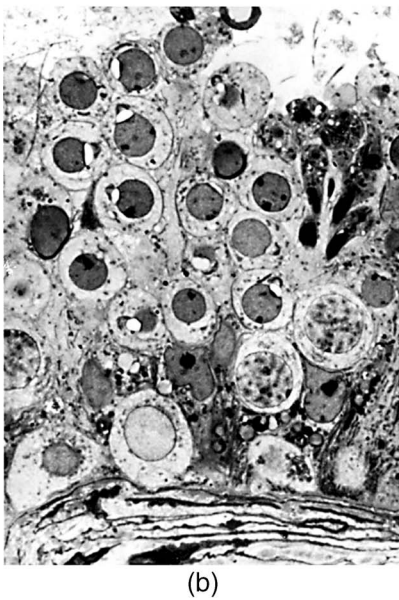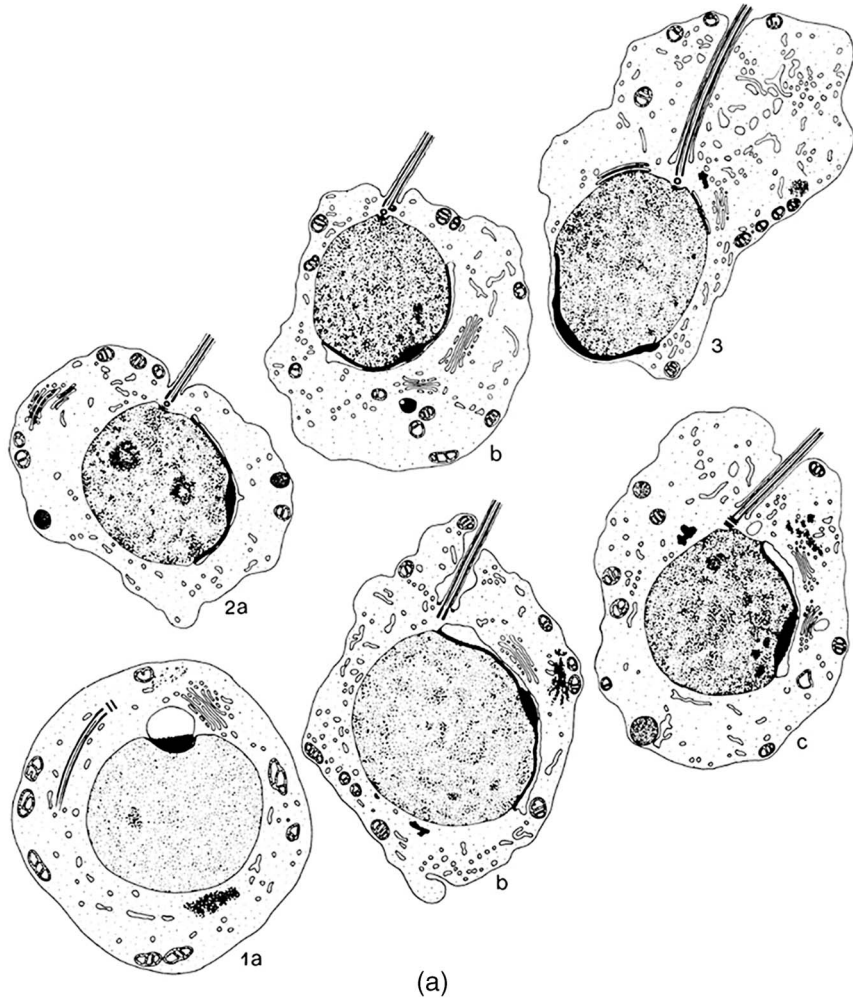
early spermatids (step 1)
with round shaped nuclei

mature spermatids
(step 7)

primary spermatocyte (zygotene)

Sertoli cell nucleus

A pale tale spermatogonium

lamina propria

(c)

Figure 6. (a) Drawings of the stages of spermiogenesis after the second meiotic division has occurred. Prior to the formation of the tail, the round spermatid can always be recognised by the prominent acrosomal vesicle (1a). As the acrosomal vesicle recedes, the tail begins to form. (Holstein & Runge, 1981). (b) Electron micrograph of a section of human spermatogenesis demonstrating pale 'Type A' spermatogonia, Sertoli cell nuclei, pachytene spermatocytes, early round spermatids with acrosomal vesicle, and mature spermatids with an oval, dark staining head. (Holstein & Roosen-Runge, 1981). (c) Diagrammatic depiction of Figure 13b with labelling of the specific cells involved in spermatogenesis. (Holstein & Roosen-Runge, 1981).
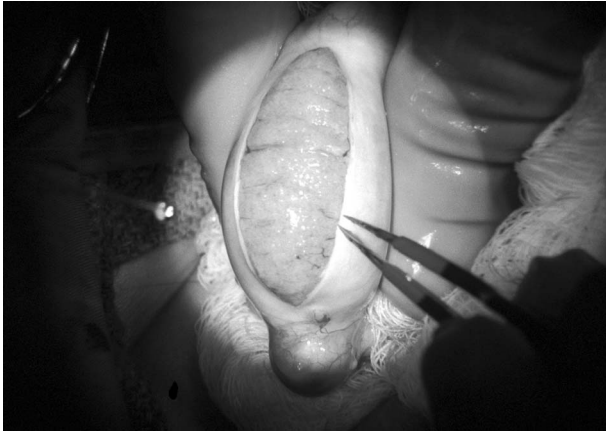
Figure 7. A single large testicular incision for a TESE procedure under the operating microscope results in minimal to no testicular damage, minimal to no post-operative pain, and an ability to analyse each specific seminiferous tubule for the presence of spermatogenesis. (Silber, 2000b).

Figure 8. Microsurgical closure of the tunica albigunea of the testes after a microsurgical TESE procedure results in no increase in intratesticular pressure and subsequently no loss of testicular function. (Silber, 2000b).

closure with 9–0 nylon interrupted stitches. Consequently there is no increase in intra-testicular pressure (Figures 7 and 8). This results in no testicular damage, and minimal pain.

Our direct mapping gives evidence for a diffuse rather than regional distribution of spermatogenesis in NOA (Silber & Rodriguez-Rigau, 1981; Silber et al., 1997a,b; Silber 2000a,b). Furthermore, the variation in sparseness of spermatogenesis verified by observation of contiguous strips of testicular tissue, explains why a single random biopsy may or may not yield sperm, and why with OA removal of very small amounts of tissue blindly with a needle might have an success rate, but has a very low success rate with NOA.

The formidable testicular deterioration that has been observed with overly aggressive TESE procedures is caused by either direct interference with microvascular supply of the seminiferous tubules or even more commonly, increased intratesticular pressure caused by minor amounts of bleeding within the enclosed tunica albuginea. The tunica albuginea is a very non-flexible enclosure. A small degree of intratesticular bleeding causes a noticeable increase in intratesticular pressure. This can be readily observed by anybody doing conventional, multiple testicle biopsy samplings for TESE. Furthermore, the closure of open biopsies with the usual non-microsurgical suture, particularly in a running fashion with conventional TESE, further compromises the intratesticular volume and thereby adds to the increased pressure.

ICSI–IVF results with testis sperm vs. epididymal sperm vs. ejaculated sperm

In our IVF programme, all oocytes undergo ICSI without any selection bias based on semen parameters or other sperm testing. This afforded an excellent opportunity to evaluate the degree to which ICSI–IVF results are or are not affected by he degree of spermatogenic defect. One thousand eight hundred forty-nine consecutive unselected ICSI cycles with ejaculated sperm were analysed retrospectively for success rates based on sperm count ranging from below 2 million/ml to greater than 20 million/ml to donor sperm. These results were compared to ICSI success rates for 719 azoospermic cycles using testicular sperm versus using epididymal sperm. For NOA, only testicular sperm could be obtained (TESE). For OA, epididymal sperm (MESA) was used in most cases, but in many cases testicular sperm (TESE) was used for obstructive (OA). MESA and TESE cases were divided and analysed according to fresh and frozen, as well as OA versus NOA categories. Results were analysed with chi square, and were reported and reviewed at the British Andrology Society Meeting, Belfast, Northern Ireland, November 2009.

Tables I and II summarise the pregnancy rate and delivery rate for ICSI cycles using ejaculated sperm according to differences in sperm count, and even for ICSI with donor sperm, further broken down also by the age of the wife. There is no significant difference in results with ICSI related to any sperm count parameters either with patient's sperm or even with donor sperm. No matter how poor the sperm count, there was no difference from patients with high sperm counts nor even patients using donor sperm.

Tables III and IV summarise the pregnancy and delivery rates for ICSI cycles in azoospermic men using sperm retrieved either from the epididymis (MESA) or testis (TESE). There is no significant difference between results with epididymal sperm, either fresh or frozen, in comparison to results with

Table I. Ejaculated sperm pregnancy rates for Ejaculated sperm in 1849 consecutive cases.

Age	< 2 million sperm	2–5 million sperm	6–20 million sperm	> 20 million sperrn	Donor sperm	Overall
< 30	61/121–50%	22/39–56%	29/59–49%	94/162–58%	19/35–54%	225/416–54%
30–35	102/247–41%	53/94–56%	61/127–48%	161/322–50%	27/68–40%	404/858–47%
36–40	51/118–43%	18/46–39%	28/73–38%	70/185–38%	14/34–41%	181/456–40%
> 40	6/42–14%	3/13–23%	4/19–21%	8/39–21%	0/6–0%	21/119–18%
Overall*	220/528–42%	96/192–50%	122/278–44%	333/708–47%	60/143–42%	831/1849–45%

*No statistical difference.

Table II. Ejaculated sperm delivery rates for ejaculated sperm in 1849 consecutive ICSI cycles.

Age	< 2 million sperm	2–5 million sperm	6–20 million sperm	> 20 million sperm	Donor sperm	Overall
< 30	45/121–37%	20/39–51%	20/59–34%	67/162–41%	17/35–49%	169/416–41%
30–35	74/247–30%	35/94–37%	39/127–31%	114/322–35%	20/68–29%	282/858–33%
36–40	25/118–21%	11/46–24%	17/73–23%	46/185–25%	9/34–26%	108/456–24%
> 40	2/42–5%	0/13–0%	1/19–5%	4/39–10%	0/6–0%	7/119–6%
Overall*	146/528–28%	66/192–34%	77/278–28%	231/708–33%	46/143–32%	566/1849–31%

*No statistical difference.

Table III. ICSI pregnancy rates for azoospermic patients by sperm source (testis vs. epididymis).

Age	MESA OA fresh	MESA OA frozen	TESE OA fresh	TESE NOA Fresh and frozen	Overall
< 30	26/40–65%	12/28–43%	9/24–38%	24/49–49%	71/141–50%
30–35	24/47–51%	52/79–66%	18/42–43%	18/66–27%	112/234–48%
36–40	8/20–40%	23/48–48%	8/24–33%	15/36–42%	54/128–42%
> 40	0/4–0%	8/14–57%	1/4–25%	0/3–0%	9/25–36%
Overall*	58/111–52%	95/169–56%	36/94–38%	57/154–37%	246/528–47%

*For MESA compared to TESE $p < .001$.

Table IV. ICSI delivery rates for azoospermic patients by sperm source (testis vs. epididymis).

Age	MESA OA fresh	MESA OA frozen	TESE OA fresh	TESE NOA fresh and frozen	Overall
< 30	23/40–58%	11/28–39%	6/24–25%	13/49–27%	53/141–38%
30–35	16/47–34%	40/79–51%	14/42–33%	11/66–17%	81/234–35%
36–40	3/20–15%	15/48–31%	3/24–13%	10/36–28%	31/128–24%
> 40	0/4–0%	2/14–14%	0/4–0%	0/3–0%	3/25–8%
Overall*	42/111–38%	68/169–40%	23/94–24%	34/154–22%	167/528–32%

*For MESA compared to TESE $p < .001$.

ejaculated or donor sperm. However, both pregnancy rate and delivery rate were considerably lower with testicular sperm (TESE) than with epididymal sperm (MESA). This was true for overall results as well as in each category of the female partner's age. It is obvious that in all these cycles the female partner's age was the most important determinant of delivery rate, but testicular sperm always yielded lower results than epididymal sperm.

Of course, the question arises as to why there have been instances of poorer results using epididymal sperm (MESA) in other centres (Tournaye et al., 1994). The reason for this is the paradox that most centres retrieve distal epididymal sperm under the mistaken notion (derived from the physiology of the non-obstructed state) that the distal sperm will be more mature and, therefore, of better quality. In fact, most of the epididymal sperm in the obstructed state will be non-motile because of degenerative senescence. Blind needle stick PESA usually does not locate the most proximal and most motile non-degenerative sperm. That is the reason for a microsurgical approach (MESA) to epididymal sperm retrieval.

Interestingly, one might have speculated whether NOA (with deficient spermatogenesis) would yield worse results than OA (with normal spermatogenesis). However, these results show that it is the origin of the sperm rather than the spermatogenic defect

that determines success rate with ICSI. Even with normal spermatogenesis and obstructive azoospermia, motile epididymal sperm yield dramatically better results with ICSI than motile testicular sperm. There is no significant difference in results between OA and NOA using testis sperm. However, epididymal and ejaculated sperm give identical ICSI results, which are clearly superior to what is obtained with testicular sperm. Thus, some maturing process must be happening to sperm as they leave the testis and enter the epididymis which results in better pregnancy and delivery rates even with ICSI, and the degree of spermatogenic defect is not relevant. Only the origin of the sperm matters. Of course, even with NOA and testis sperm, favourable pregnancy rates can be achieved, but if it is possible to obtain epididymal sperm, like with obstructive azoospermia, MESA is still preferable to TESE.

Declaration of interest: The author reports no conflicts of interest. The author alone is responsible for the content and writing of the paper.

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