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Assisted reproductive technologies have dramatically advanced the opportunities for treating the most challenging cases of male infertility. In no case has this opportunity been clearer than for men with the most severe defect in spermatogenesis: nonobstructive azoospermia (NOA). In men with NOA, sperm production is so defective that no sperm reach the ejaculate. The morphology of these sperm routinely is abnormal, and our experience is that many in vitro fertilization (IVF) laboratories consider sperm obtained from these men to be unusable for assisted reproductive techniques. Sperm retrieval requires invasive techniques to identify limited foci of sperm production within the testis. Clearly, the appearance of sperm from these men is abnormal, but few data have been published on the effect of using these sperm on IVF outcomes, including embryo quality. In this chapter, we describe the outcomes of intracytoplasmic sperm injection (ICSI) using testicular sperm from men with NOA, comparing the results with those obtained with testicular sperm from men with obstructive azoospermia (controlling for testicular source) and from men with sperm in the ejaculate using donor oocytes (con-

trolling for oocytic factors). We also present data on the use of fresh and frozen testicular sperm, as well as sperm retrieved on the day of versus the day before oocyte retrieval. The results show that use of testicular sperm from men with NOA has a substantial effect on fertilization rates (72.6 % vs. 53.3 %; $P < 0.05$, compared with obstructed testicular sperm) but with similar embryo development (66.2 % day 3 “good” embryos [grade 1–2] vs. 53.8 % with obstructed testicular sperm). Clinical pregnancy rates were statistically better (52.8 % vs. 41.4 %) using testicular sperm from men with obstruction versus those with NOA.

Sperm Retrieval

Testicular sperm may be retrieved with a variety of techniques, including fine-needle aspiration, biopsy, or multiple biopsies (testicular sperm extraction [TESE]). We have developed a refined technique of microdissection TESE (microTESE) that allows a detailed search of these dysfunctional testes to identify the very rare sites of sperm production that are present within them. This approach limits the amount of tissue that must be removed from the testes and appears to increase the sperm retrieval rate by 50 % over that obtained with multiple testis biopsies, while limiting the effects on the testicle. Because less tissue is delivered to the embryology laboratory with a higher yield of sperm, the work required for tissue processing and sperm identification may be limited. The microsurgical

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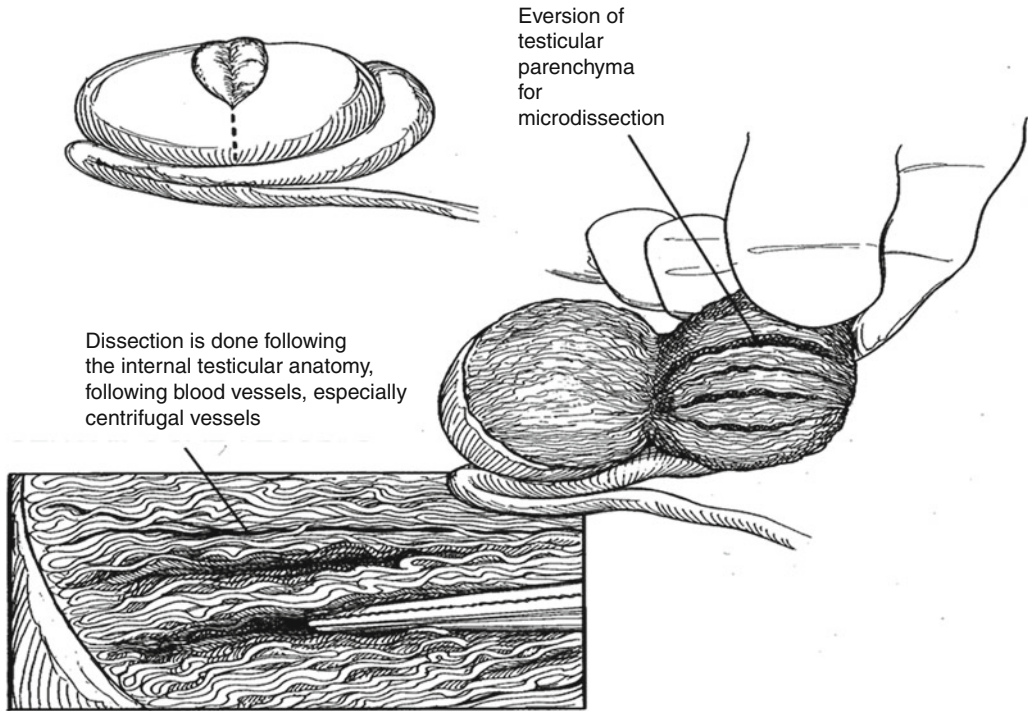
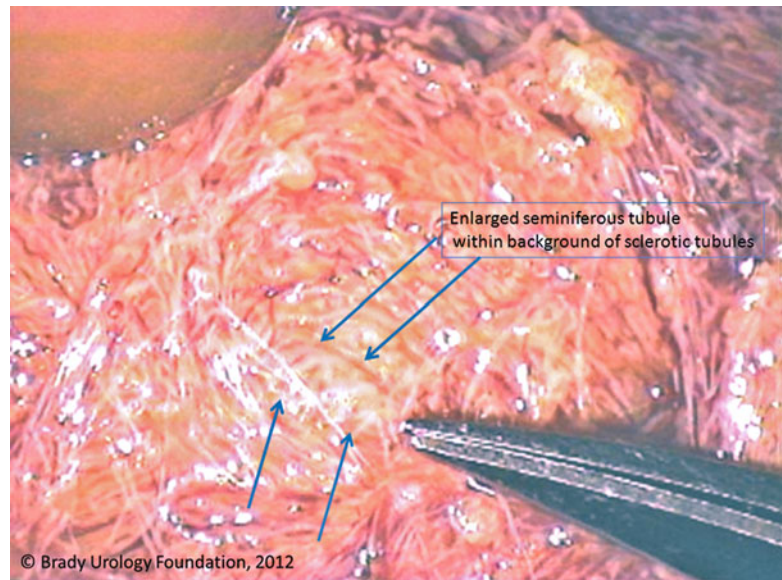


Fig. 2.1 Testicular tissue exposure for microTESE (*Courtesy of* © the Brady Urology Foundation, 2012)

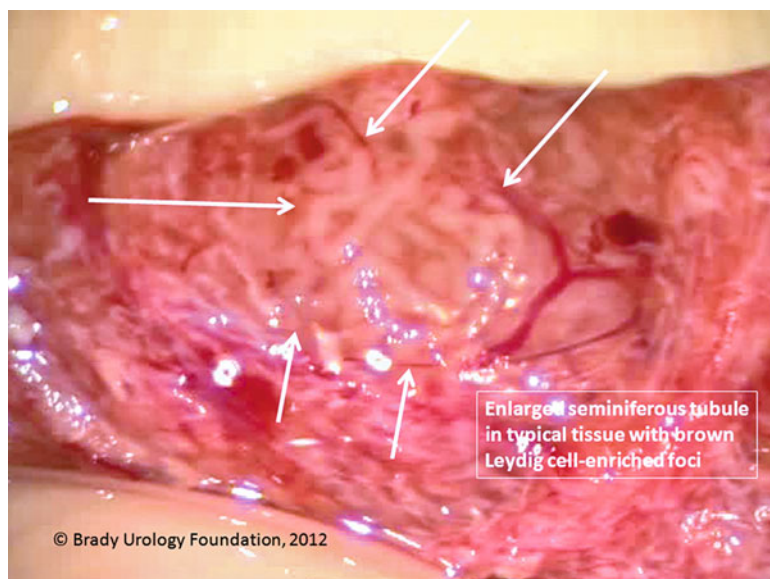
Fig. 2.2 Intraoperative demonstration of enlarged seminiferous tubules in a patient with NOA during a microTESE procedure. In this case, as well as in Fig. 2.3, the entire extent of the enlarged tubule is removed, if possible without vascular compromise (*Courtesy of* © the Brady Urology Foundation, 2012)



search for sites of sperm production involves the wide opening and exposure of testicular tissue, following the natural vascular and tissue planes of the testicle (Fig. 2.1). Individual seminiferous tubules

most likely to contain sperm can be identified with an operating microscope at $\times 10$ to $\times 15$ magnification, allowing selection of the widest and most opaque tubules identifiable (Figs. 2.2 and 2.3).

Fig. 2.3 Intraoperative demonstration of enlarged seminiferous tubules in a patient with NOA during a microTESE procedure. In this case, as well as in Fig. 2.2, the entire extent of the enlarged tubule is removed, if possible without vascular compromise (Courtesy of © the Brady Urology Foundation, 2012)



We have attempted sperm retrieval on the day of, as well as the day before, oocyte retrieval. In both cases, a final semen specimen is examined using an extended sperm preparation technique, allowing us to identify sperm and avoid surgery in 5–10 % of men previously diagnosed as having NOA. Sperm retrieval on the day before oocyte retrieval allows greater flexibility to process tissue and identify sperm during the nearly 24 h before oocyte retrieval is done. The effect of such an approach on IVF outcomes is described.

With microTESE, we have been able to retrieve sperm from 57 % of men with NOA at Weill Cornell Medical Center, despite most patients having had prior failed attempts at treatment elsewhere (and despite the exclusion of 5–10 % of men thought to have NOA but actually having cryptozoospermia).

Obstructive Versus Nonobstructive Azoospermia

Testicular sperm are obtained primarily from male patients with either obstructive azoospermia (OBST; congenital or acquired obstructions) or NOA azoospermia. The comparison of ICSI efficiency between OBST and NOA has limitations, as sperm from various surgical sources

(epididymal vs. testicular) are compared. However, when we compared these two groups at Cornell (Table 2.1), we observed an overall lower fertilization rate in the NOA versus the OBST group. This is expected, as epididymal and TESE sperm samples from men with obstruction generally have more sperm and greater motility, which may reflect greater fertilizing ability. Other centers also have shown this trend, with fertilization rates of 70 % for OBST and 50 % for NOA. Interestingly, if motile epididymal or testicular sperm are injected, no differences in fertilization or pregnancy rates are observed in patients with obstructive azoospermia. Furthermore, embryos created from TESE sperm have apparently higher developmental potential than epididymal sperm in obstructed cases. At Cornell, higher fertilization rates in OBST also are reflected in higher pregnancy and implantation rates and higher percentages of frozen embryos (blastocyst stage), with no differences in miscarriage rates (Table 2.1). Similar results were obtained elsewhere in a meta-analysis that revealed no difference in implantation and miscarriage rates. Regarding embryo quality, Vernaev et al. (2003) observed no differences in the average percentage of good-quality embryos between OBST and NOA (58 % vs. 52 %) or among types of NOA. Weill Cornell data for 2010 also show no differences in

Table 2.1 Obstructive versus nonobstructive azoospermia (1995–2010)

Parameter	OBST (TESE, frozen TESE, EPI, frozen EPI)	NOA (TESE, frozen TESE)	<i>P</i> value (Fisher or χ^2)
Cases, <i>n</i>	638	801	–
Avg. age, years	34.6	32.6	–
Oocytes, <i>n</i> (avg.)	7,743 (12.1)	9,984 (12.5)	–
Mature/injected, <i>n</i> (%)	5,995 (77)	7,824 (78)	–
Fertilizations, <i>n</i> (%)	4,353 (72.6)	4,174 (53.3)	<0.05
Day 3 embryos transferred, <i>n</i> (avg. grade)	559 (2.4)	686 (2.0)	–
Day 5 embryos transferred, <i>n</i>	39	27	–
Embryos transferred, <i>n</i> (avg.)	1,796 (3)	1,805 (2.5)	–
Frozen embryos, <i>n</i>	804	404	–
Freezing rate, %	18.5	9.7	<0.05
Clinical pregnancies, <i>n</i> (%)	316 (52.8)	295 (41.4)	<0.05
Babies, <i>n</i> (% implantations)	451 (25)	390 (21.6)	<0.05
Losses and biochemical pregnancies, <i>n</i> (%)	98 (16.4)	113 (15.8)	NS
Good embryos (grade 1–2) on day 3, <i>n</i> (%)	13 (53.8)	68 (66.2)	–
Cycles, <i>n</i>	6	33	–
Embryos grade $\geq$ 2BB on day 5 (2010 only), <i>n</i> (%)	73 (78.1)	53 (71.7)	–
Cycles, <i>n</i>	39	27	–

Avg. average, *EPI* epididymal sperm, *NS* not significant

the percentage of good-quality embryos transferred on day 3 versus day 5 (Table 2.1).

Fresh Versus Frozen TESE

In cases in which spermatozoa are found with TESE, surplus sperm and testicular tissue may be cryopreserved for future use and possibly represent the only option for preserving fertility in severe cases of NOA. In comparing cases using fresh TESE (OBST and NOA) with those using frozen TESE, we found no difference in fertilization rates (Table 2.2); however, pregnancy and implantation rates, as well as the percentage of frozen embryos, were higher in the fresh versus frozen sperm patients. These data reflect sperm obtained from both OBST and NOA cases, as well as all female age groups. It is not immediately clear why frozen sperm have poorer outcomes, as even the percentage of good-quality embryos did not differ between groups. Nevertheless, it is possible that sperm DNA damage due to freezing and thawing might influence postimplantation embryo development. Of note, many, but not all, patients had a first attempt at

ICSI using freshly retrieved testicular spermatozoa. In contrast, our observations show that miscarriage rates did not increase in cases using frozen TESE samples (Table 2.2). These results are consistent with other reports comparing first-attempt IVF–ICSI cases. The same occurrences were observed in comparing fresh versus frozen TESE OBST cases, with significantly higher fertilization and pregnancy rates using motile versus nonmotile spermatozoa.

Day-of Versus Day-Before TESE

The timing of the microTESE sperm procedure is important if freshly retrieved spermatozoa are to be used, to synchronize with oocyte retrieval and optimal patient (female and male) treatment. If microTESE is done the same day as oocyte retrieval (day of), pressure is generated on the patient, surgeon, and laboratory staff to find enough sperm for ICSI. Time constraints in completing the male surgery (which may take between 2 and 4 h or longer) may influence the outcome, as the best chance of sperm retrieval is within the first 2–4 h of operative time. In addition, at Weill Cornell, in analyzing

Table 2.2 Fresh versus frozen testicular sperm extraction (1995–2010)

Parameter	Fresh TESE	Frozen TESE	<i>P</i> value (Fisher or χ^2)
Cases, <i>n</i> (avg.)	643 (12.9)	202 (10.6)	–
Avg. age, years	32.0	35.3	–
Oocytes, <i>n</i>	8,315	2,149	–
Mature/injected, <i>n</i> (%)	6,526 (78)	1,679 (78)	–
Fertilizations, <i>n</i> (%)	3,557 (54.5)	876 (52.1)	NS (0.08)
Day 3 embryos transferred, <i>n</i> (avg. grade)	557 (2.1)	165 (1.9)	–
Day 5 embryos transferred, <i>n</i>	23	7	–
Embryos transferred, <i>n</i> (avg.)	1,459 (2.6)	455 (2.7)	–
Frozen embryos, <i>n</i>	379	63	–
Freezing rate, %	10.7	7.2	<0.05
Clinical pregnancies, <i>n</i> (%)	256 (44.1)	57 (33.1)	<0.05
Babies, <i>n</i> (% implantations)	337 (23)	77 (17)	<0.05
Losses and biochemical pregnancies, <i>n</i> (%)	91 (16)	27 (15.7)	NS
Good embryos (grade 1–2) at day 3 transfer, <i>n</i> (%)	49 (62.2)	22 (68.2)	–
Cases, <i>n</i>	6	11	–
Embryos grade $\geq$ 2BB on day 5 (2010 only), <i>n</i> (%)	45 (71.1)	17 (88.2)	–
Cases, <i>n</i>	23	9	–

sperm retrieval success and the time spent finding the sperm postoperatively (extensive laboratory search), only 7 % of the time was sperm found after extensive searches in the laboratory when spermatozoa were not identified in the operating room (OR). Experienced embryologists are essential to identify the rare sperm identified after tissue digestion. Another crucial factor in TESE–ICSI outcome is the optimal window of sperm injection. Oocytes must be injected within 4–6 h after retrieval for optimal outcome; difficult cases of sperm retrieval with microTESE and subsequent tissue processing on the day of oocyte retrieval might cause that window to be missed.

Sperm retrieval on the day before oocyte retrieval appears to be the more optimal approach (and is our preferred strategy for microTESE cases). This approach gives us the flexibility to examine TESE samples adequately. We then have time to cancel oocyte retrieval in cases of no sperm, to use donor sperm as a backup, or to obtain the patient’s consent for oocyte cryopreservation. To evaluate the efficiency of performing TESE on the day of or the day before oocyte retrieval, we compared the combined data for the last 7 years in OBST and NOA patients (Table 2.3).

The fertilization rate is decreased slightly in the day-before TESE group (51.7 % vs. 55.2 %); on the contrary, pregnancy and implantation rates were no different, and no major differences in embryo quality were found (Table 2.3).

There are ongoing questions whether sperm retrieved from NOA patients with microTESE have a lower fertilization capacity than ejaculated sperm and what role of the male gamete is in embryo development and implantation. To shed light on these questions, we compared data from shared oocyte donors in cases in which one recipient used ejaculated sperm and the other used TESE sperm using the same oocyte cohort, removing the female factor (Table 2.4). Although the sample size was small, these preliminary results show fertilization rates slightly lower using TESE sperm (63 % vs. 79 %). Pregnancy and implantation rates did not differ, resulting in overall good embryo quality on days 3 and 5.

To our knowledge, there are no studies adequately addressing embryo quality using testicular sperm from men with NOA. These studies would be very difficult to perform because of the differences among patient populations and male and female factors. In general, viable sperm from

Table 2.3 Day-of versus day-before testicular sperm extraction (2003–2010)

Parameter	Day-of TESE	Day-before TESE	<i>P</i> value (Fisher or χ^2)
Cases, <i>n</i>	149	265	–
Avg. age, years	32.2	30.9	–
Oocytes, <i>n</i> (avg.)	1,888 (12.7)	3,497 (13.2)	–
Mature/injected, <i>n</i> (%)	1,446 (77)	2,791 (79.8)	–
Fertilizations, <i>n</i> (%)	799 (55.2)	1,444 (51.7)	<0.05
Day 3 embryos transferred, <i>n</i> (avg. grade)	126 (1.9)	219 (2.3)	–
Day 5 embryos transferred, <i>n</i>	4	18	–
Embryos transferred, <i>n</i> (avg.)	305 (2.3)	515 (2.2)	–
Frozen embryos, <i>n</i>	53	186	–
Freezing rate, %	6.7	12.9	<0.05
Clinical pregnancies, <i>n</i> (%)	56 (43)	106 (45)	NS
Babies, <i>n</i> (% implantations)	72 (23.6)	138 (26.7)	NS
Losses and biochemical pregnancies, <i>n</i> (%)	16 (12.3)	38 (16)	NS (0.07)
Good embryos (grade 1–2) on day 3, <i>n</i> (%)	17 (76.5)	29 (58.6)	–
Cases, <i>n</i>	8	14	–
Embryos grade $\geq$ 2BB on day 5 (2010 only), <i>n</i> (%)	8 (50)	36 (15)	–
Cases, <i>n</i>	4	18	–

Table 2.4 Ejaculated versus testicular-extracted sperm in split donor oocyte cycles^a

Parameter	Ejaculated sperm	TESE/frozen TESE	<i>P</i> value (Fisher or χ^2)
Cases, <i>n</i>	6	6	–
Avg. age, years			
Recipient	41.0	44.3	–
Donor	27.8	27.8	–
Oocytes, <i>n</i>	64	62	–
Mature/injected, <i>n</i> (%)	53 (83)	54 (87)	–
Fertilizations, <i>n</i> (%)	42 (79.2)	34 (63)	NS (0.08)
Day 3 embryos transferred, <i>n</i>	3	4	–
Day 5 embryos transferred, <i>n</i>	3	2	–
Embryos transferred, <i>n</i> (avg.)	14 (2.3)	13 (2.2)	–
Frozen embryos, <i>n</i>	11	11	
Freezing rate, %	26.2	32.4	NS
Clinical pregnancies, <i>n</i> (%)	4 (67)	4 (67)	NS
Babies, <i>n</i> (% implantations)	7 (50)	5 (38.5)	NS
Losses and biochemical pregnancies, <i>n</i>	2	1	–
Good embryos (grade 1–2) on day 3, <i>n</i> (%)	3 (100)	4 (100)	–
Embryos grade $\geq$ 2BB on day 5, <i>n</i> (%)	3 (100)	2 (100)	–

^aSix donors split among 12 recipients

TESE (OBST and NOA) have a similar, if not the same, fertilization and embryo developmental capacity as ejaculated sperm. More large case studies are needed with selection based on male history, infertility, and sperm characteristics, with careful analysis of embryo development.

Preparation of Testicular Tissue (TESE)

At Weill Cornell, microTESE generally is limited to NOA cases in which low sperm numbers are anticipated. The main goal is to retrieve an adequate amount of sperm, preferably motile,

that may be used for ICSI. In the OR, the urologist performing the TESE procedure rinses blood from the tissue piece before mincing and mechanically processing the testicular sample suspension sequentially through a 24-gauge angiocatheter to confirm adequate disruption of the testicular tissue. The tissue then is passed to the IVF andrologist for evaluation. A detailed description of the TESE technique performed by Peter N. Schlegel at Weill Cornell may be found in the article by Ostad et al. (1998). After the microtubules are minced and broken up, the sample is placed into either a 1.5-mL microcentrifuge tube or a 5-mL round-bottom culture tube, depending on the volume of each tissue sample. The IVF andrologist in the OR places a small aliquot (5–7 μ L) of the liquid portion of the sample on a disposable counting chamber slide and examines it under phase-contrast microscopy at $\times 200$ magnification. The surgeon is given information on whether sperm are present, the morphologic characteristics of the sperm identified, and the presence or absence of germ cells observed. Additional samples are taken using microdissection as necessary until adequate sperm are observed, or until all areas of tissue that can be safely accessed are examined without positive results.

Sperm Observed in the OR

After the number of matured oocytes for injection has been determined, a decision may be made regarding how much of the sample should be processed to retrieve adequate sperm for ICSI. Depending on the sperm concentration and the total volume of the sample, samples may be either combined or processed separately. The tissue suspension from the sample tube(s) is mixed gently, and the liquid portion from the sample tube(s) is removed carefully. The tissue particles may be combined with the remaining tissue for cryopreservation. The liquid portion is washed with sperm wash medium and centrifuged for 5 min at $1,800 \times g$. The supernatants are removed and pellets resuspended in a small amount of medium. The processed sample is ready for ICSI. When very rare sperm are seen in perhaps one tube, the

use of enzymatic digestion can maximize the number of sperm recovered.

No Sperm Observed in the OR: Enzymatic Digestion

Enzymatic Digestion of Testicular Tissue (Collagenase/DNase Treatment)

Pretreating with an erythrocyte-lysing buffer for 5 min at 37°C may be beneficial before proceeding with digestion. Prepared collagenase/DNase is stored frozen in small cryovials, each containing 1 mL of prepared enzyme solution. The enzyme solution is thawed at 30°C and added at a 1:1 (v:v) ratio to the TESE tissue suspension. The tissue is incubated at 37°C for 1 h (agitating the tubes every 10–15 min to facilitate digestion). After incubation is complete, centrifugation is performed at $50 \times g$ for 5 min to pellet the undigested tissue and separate the supernatant. Enzymes are removed by adding an equal amount of sperm wash medium. To remove the supernatant, centrifugation is carried out at $1,800 \times g$ for 5 min ($\times 2$). Resuspended pellets are mixed thoroughly, and a small aliquot of the sample is examined for the presence of sperm. Microdroplets are made for sperm searching and ICSI by mixing the final digested sample. The total volume of the final suspension is critical to the success of the search throughout the microdroplets. If the suspension is too concentrated, the samples will be too dense and difficult to search. We prefer to see a monolayer of cells/debris with a little space between each of the components. For frozen TESE samples, the same tissue digestion may be used successfully with cryoprotectants completely removed before enzymatic digestion.

Pentoxifylline Treatment

Pentoxifylline is a phosphodiesterase inhibitor shown to increase motility and enhance the acrosome reaction (Terriou et al. 2000). Prolonged exposure of sperm to pentoxifylline may be damaging and must be avoided. One milliliter of the

sample is diluted in sperm wash medium, 20 μL of 180-mM pentoxifylline solution (final concentration = 3.53 mM) is added, and the sample is incubated for 20 min at 37 °C. The sample is washed twice by centrifugation/resuspension ($1,800\times g$ for 5 min). Alternatively, pentoxifylline may be added directly to the microdroplet in cases of extremely low sperm concentrations (TESE). Two microliters of the originally prepared pentoxifylline solution (180 mM) is added to 1 mL of sperm wash medium. One microliter of this diluted pentoxifylline solution is then added to a 5- μL microdroplet containing the tissue/sperm suspension. Motility should be enhanced after approximately 20 min of treatment. Sperm selected from the microdroplet are

washed in 7 % polyvinylpyrrolidone before injection (ICSI).

TESE Performed the Day Before Oocyte Harvest

When TESE is performed the day before oocyte retrieval and no sperm are found, one has the option of cancelling oocyte retrieval, freezing oocytes, or proceeding immediately with donor backup once oocytes are collected. Once it is determined that at least a few sperm are present, the digested sample may be resuspended in sperm wash medium, sealed tightly, and placed in the dark at room temperature (20–25 °C) until the next day.

Fig. 2.4 Testicular biopsy sample after tissue digestion. Various spermatozoa forms are seen, featuring mature and immature (cytoplasmic neck droplet) sperm cells (*circled areas*). Some spermatozoa have a neck defect and others have a small acrosome ($\times 400$)

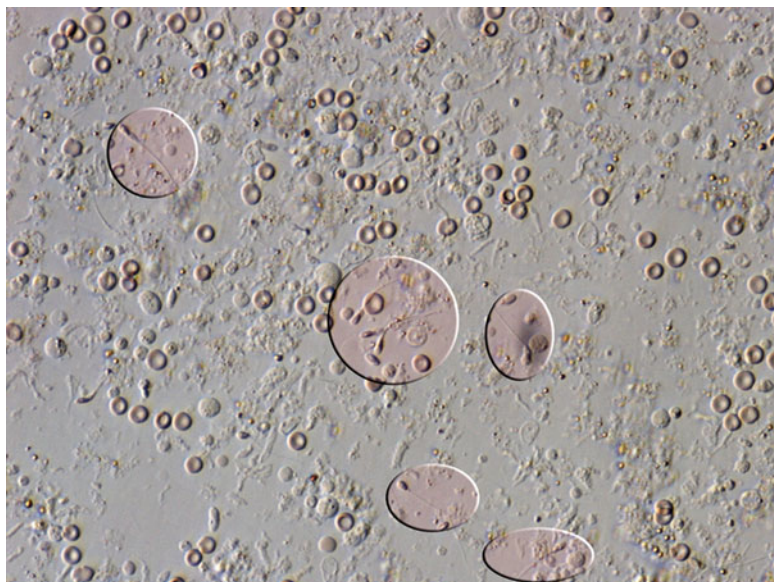


Fig. 2.5 Another TESE sample. Most of the spermatozoa have a neck defect and cytoplasmic droplet ($\times 400$)

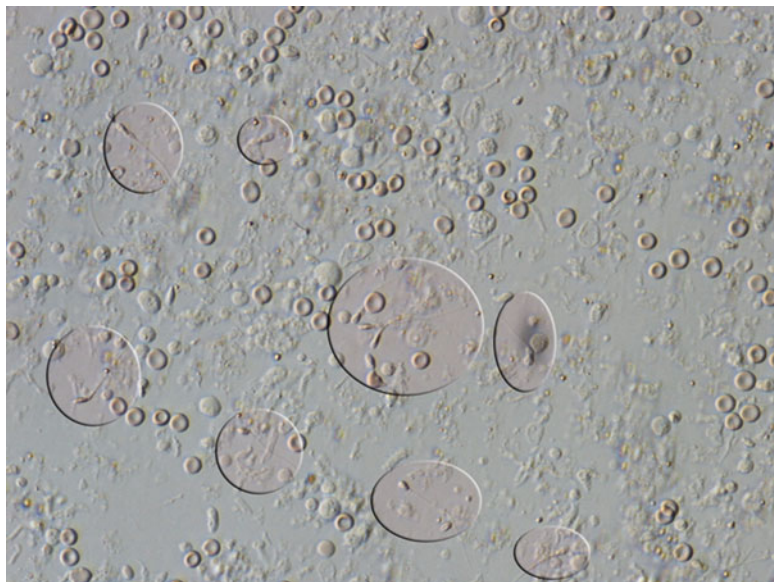


Fig. 2.6 TESE sample after digestion. More mature forms of spermatozoa are seen. With the addition of pentoxifylline, some of them show motility ($\times 400$)

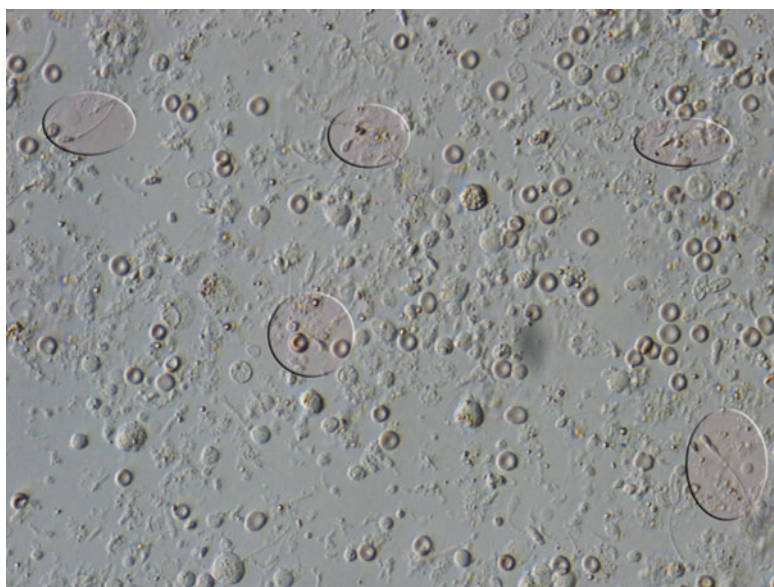


Fig. 2.7 TESE sample with abundant cells and tissue. A few spermatozoa may be seen, most with amorphous heads or having only a head (*bottom right*; $\times 400$)

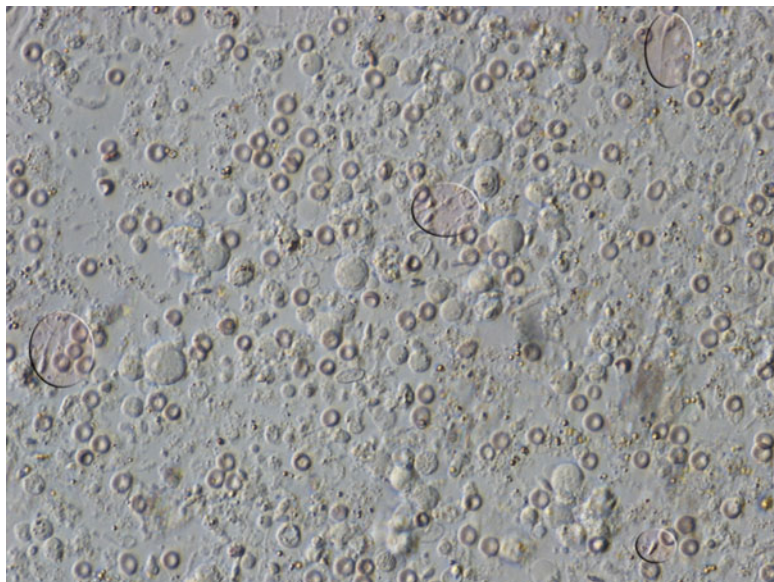


Fig. 2.8 TESE tissue after digestion. Large Sertoli cells are seen with some connective tissue. No germ cells or spermatozoa could be recorded ($\times 400$)

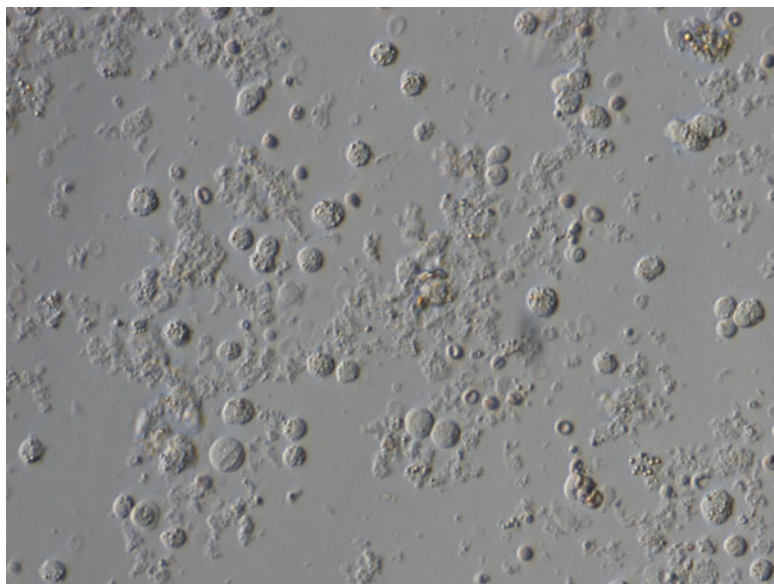


Fig. 2.9 Testicular tissue after digestion. The tissue is very depleted with no germ cells or spermatozoa and few red blood cells (*RBCs*; $\times 400$)

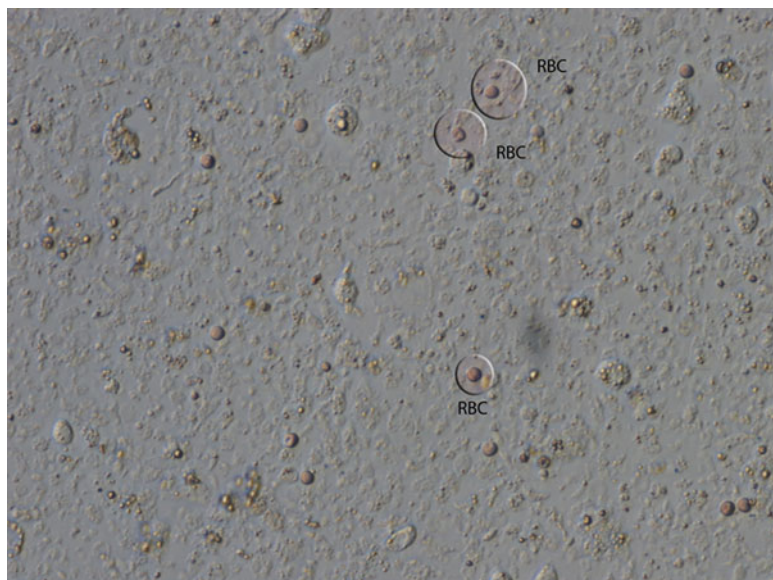


Fig. 2.10 Postdigestion TESE tissue in which only early elongated (immature) spermatids (*red circle*) were found. These cells may be injected with the patient's consent ($\times 400$)



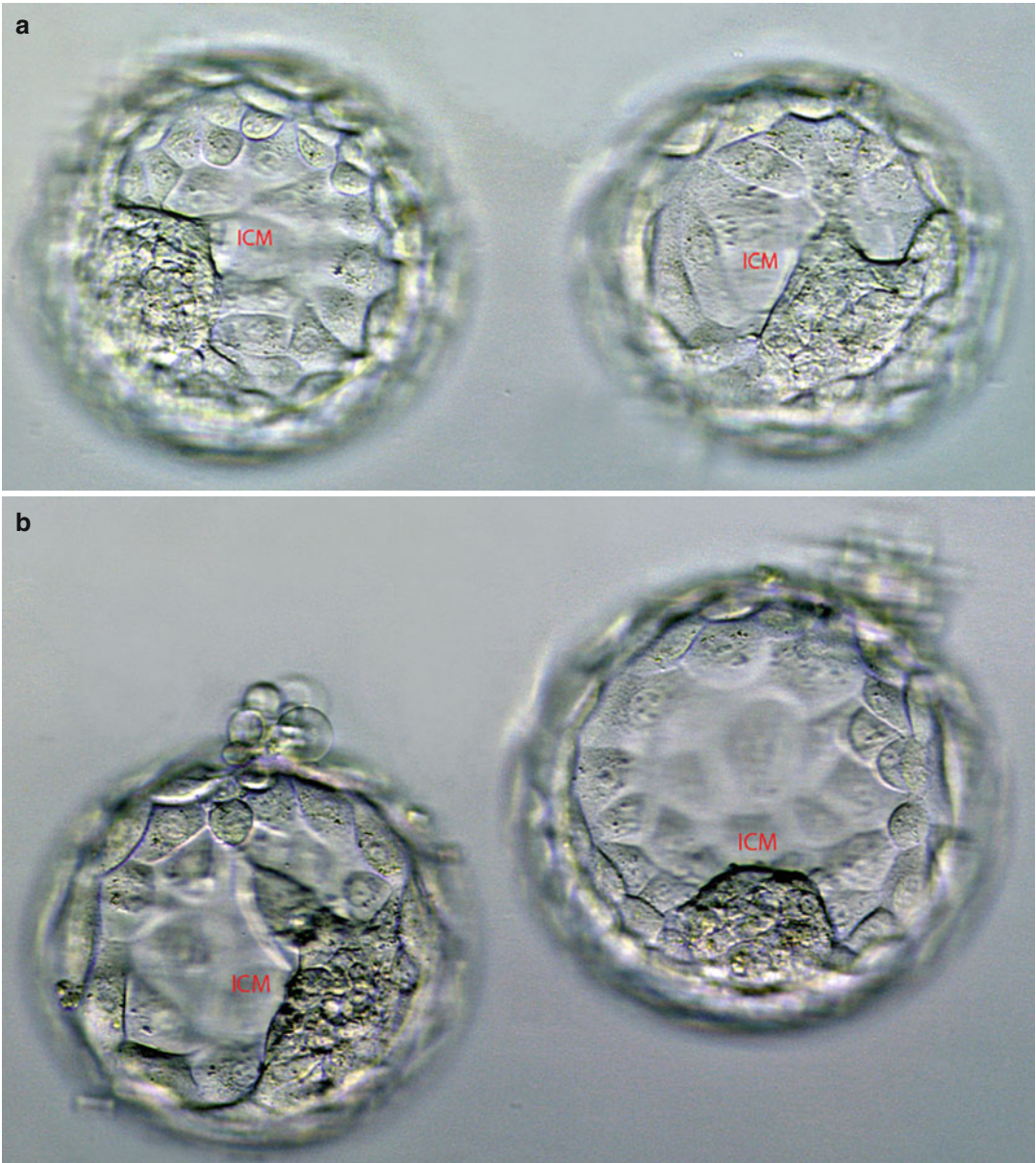


Fig. 2.11 Embryos at the time of transfer (day 5) in donor oocyte recipients (sharing the same egg donor). One patient received ejaculated spermatozoa (**a**), the other TESE spermatozoa (**b**). Both images show a high grade

of blastocysts. The patient in *panel A* (ejaculated spermatozoa) had a miscarriage, whereas the patient in *panel B* (TESE) had two babies. Inner cell mass (*ICM*) is indicated in both panels ($\times 200$)

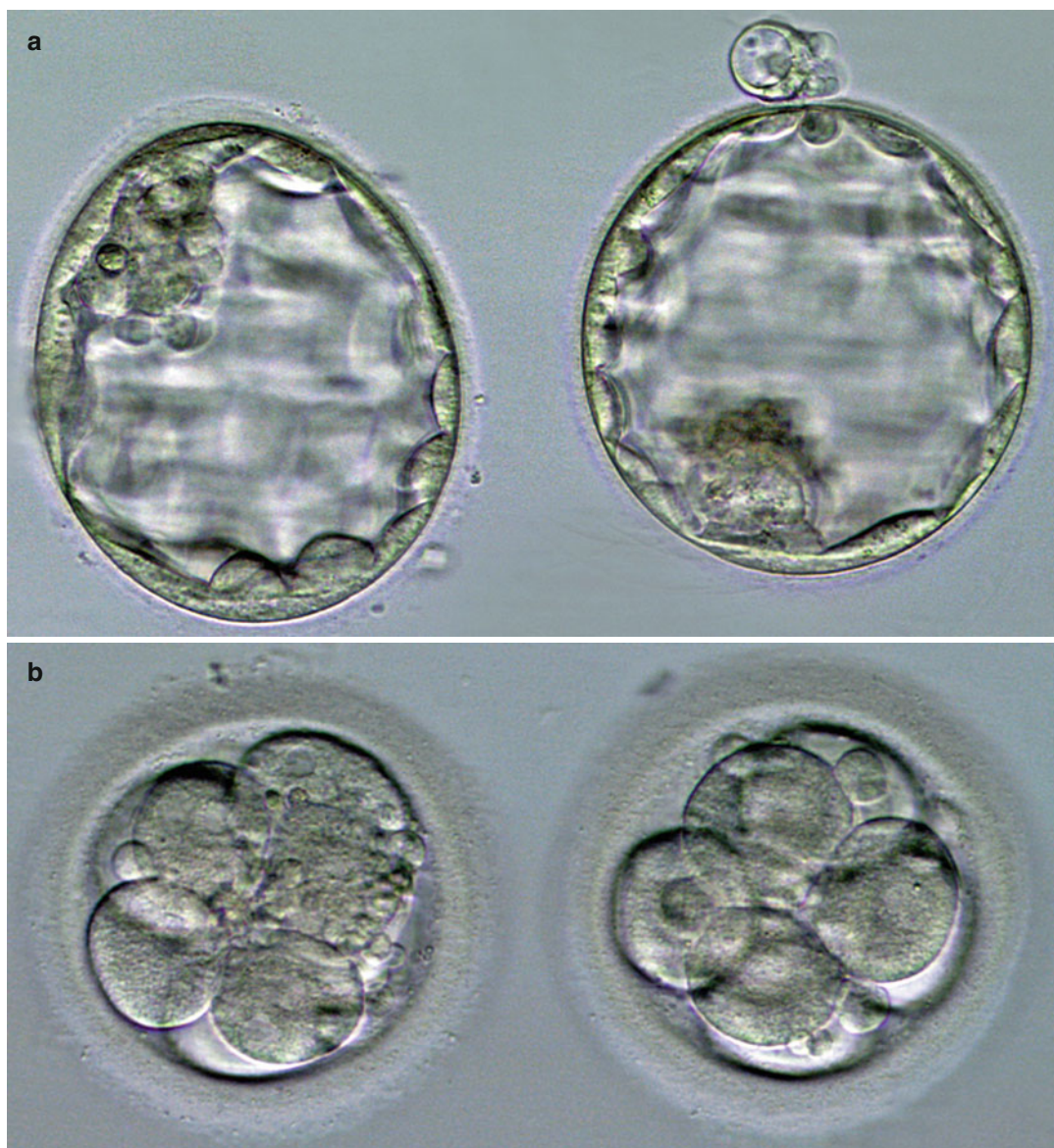


Fig. 2.12 Embryos at the time of transfer in shared donor oocyte recipients. **(a)** This patient used ejaculated sperm, transfer on day 5. High-quality blastocysts resulted in

delivery of one baby. **(b)** This patient used TESE sperm, transfer on day 3. Although the embryos were of good quality in this case no pregnancy resulted ($\times 200$)

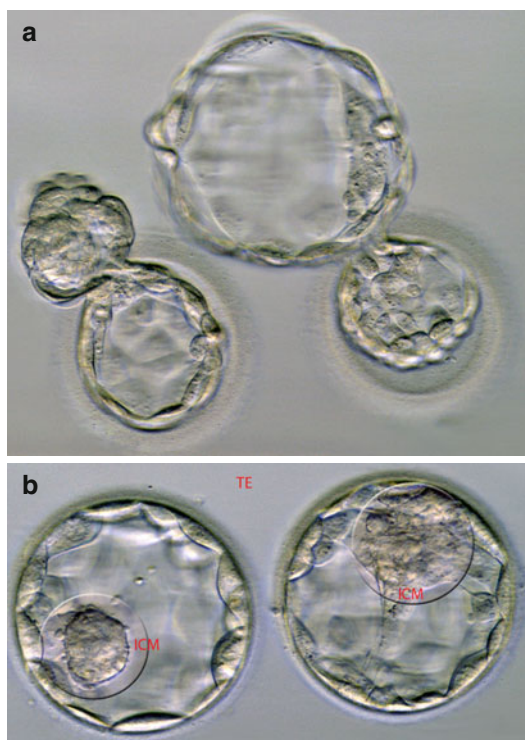


Fig. 2.13 Embryos at the time of transfer on day 5 in shared donor oocyte recipients. (a) In this recipient using ejaculated spermatozoa, preimplantation genetic diagnostic testing resulted in the birth of two babies. (b) This image from recipient using TESE sperm recipient shows high-grade blastocysts with prominent ICMs (circle), which resulted in the birth of one baby ($\times 200$). TE trophectoderm

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Atlas on the Human Testis
Normal Morphology and Pathology
Jezek, D. (Ed.)
2013, XII, 287 p., Hardcover
ISBN: 978-1-4471-2762-8