AVAILABLE RETRIEVAL PROCEDURES, INDICATIONS AND OUTCOMES:

FOCUS ON MICRO-TESE

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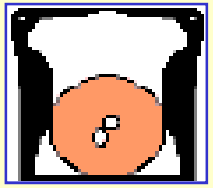

Disclosure: potential conflicts of interest= none

Agenda

- ✓ Definition and Epidemiology of azoospermia
- ✓ Azoospermia: Obstructive (OA) vs. Non-Obstructive (NOA)
- ✓ Sperm retrieval in OA
- ✓ Sperm retrieval in NOA
- ✓ A glimpse on how retrieved sperm does in ICSI

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2.9 Low sperm numbers: cryptozoospermia and suspected azoospermia

- ✓ If no spermatozoa are observed in the replicate wet preparations, azoospermia can be suspected.
- ✓ … the term azoospermia can only be used if no spermatozoa are found in the sediment of a centrifuged sample (Eliasson, 1981).

Epidemiology of AZOOSPERMIA

Azoospermia is found in **5–15%** of infertile men

Jarow et al., 1989
Thonneau et al., 1991
Mazzilli et al., 2000

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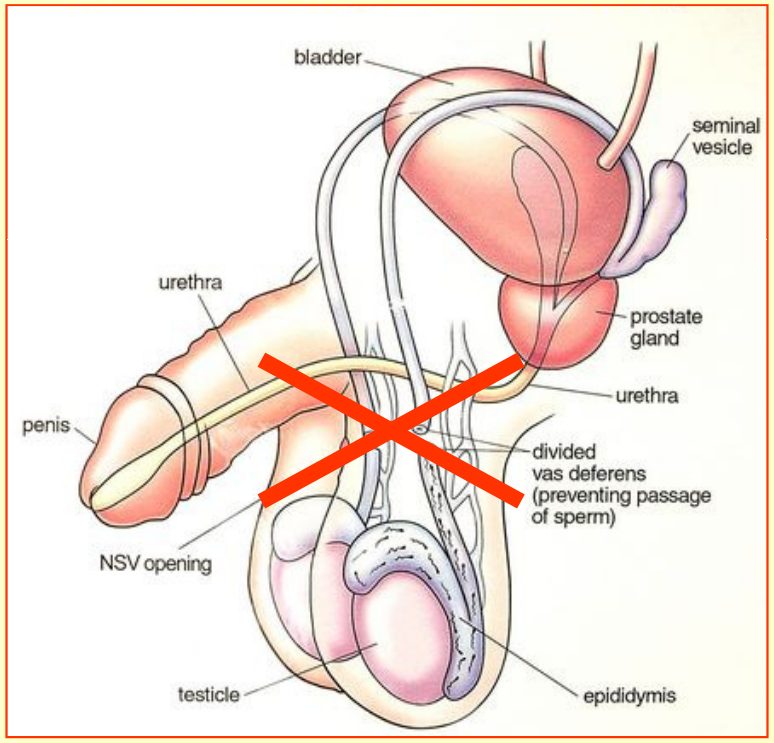
Classification of AZOOSPERMIA

Azoospermia can be differentiated in:

- **OBSTRUCTIVE Azoospermia – "OA"**
  (15-20% of all azoospermias), and:
- **NON-OBSTRUCTIVE Azoospermia – "NOA"**
  (80-85% of all azoospermias)

GR Dohle, et al., 2007

Obstructive Azoospermia – OA (15-20%)

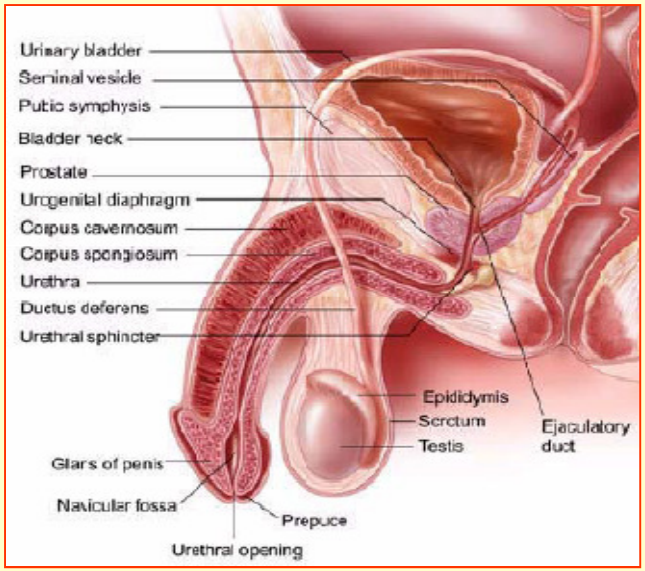


- **Biologically:**
(normo)functioning testis that produces sperm

- **Clinically:**
good volume testis (>15 ml) with dilated epididymis

- **Lab:**
FSH in the normal range

Classification of obstructive azoospermia on the basis of level of obstruction



Intratesticular obstruction (15% of OA)

- Congenital forms — Dysjunction rete testes – efferent ductules)
- Acquired forms — Post-infective / post-traumatic

Epididymal obstruction (30-67% of OA)

- Congenital forms — Idiopathic epididymal obstruction
- Acquired forms — Post-infective - Post-surgical

Vas deferens obstruction

- Congenital forms — Congenital absence of the vas deferens
- Acquired forms — Post-vasectomy Post-surgical (hernia, scrotal surgery)

Ejaculatory duct obstruction (1-3% of OA)

- Congenital forms — Prostatic cysts (Müllerian cysts)
- Acquired forms — Post-surgical (bladder neck surgery) Post-infective

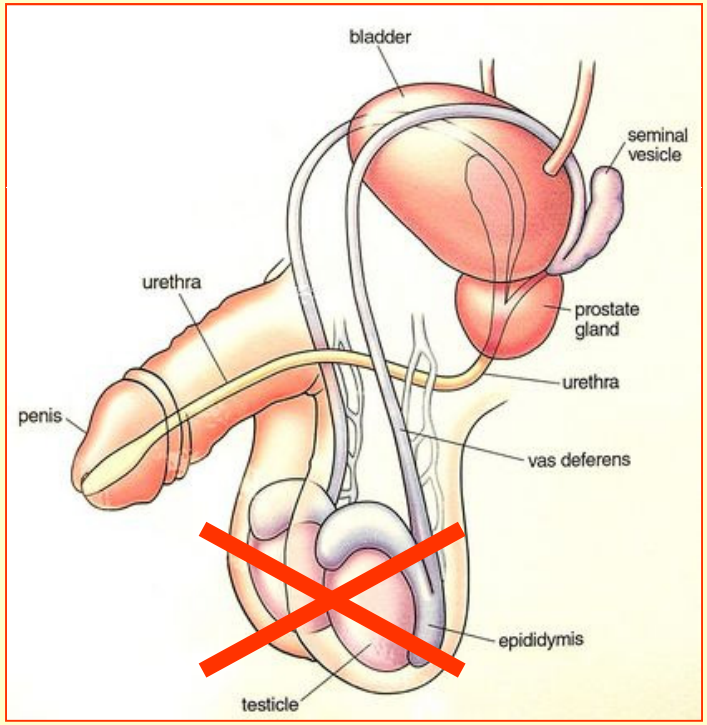
Functional obstruction of the distal seminal ductus

- Acquired forms — Local nuropathy (diabetes, etc.)

GR Dohle et al., 2007

Non-Obstructive Azoospermia – NOA

(15-20%)



- **Biologically:**
dysfunctional testis with severe alteration of sperm production

- **Clinically:**
hypotrophic (>15 ml) and dystrophic testis

- **Lab:**
elevated FSH

ORIGINAL ARTICLE

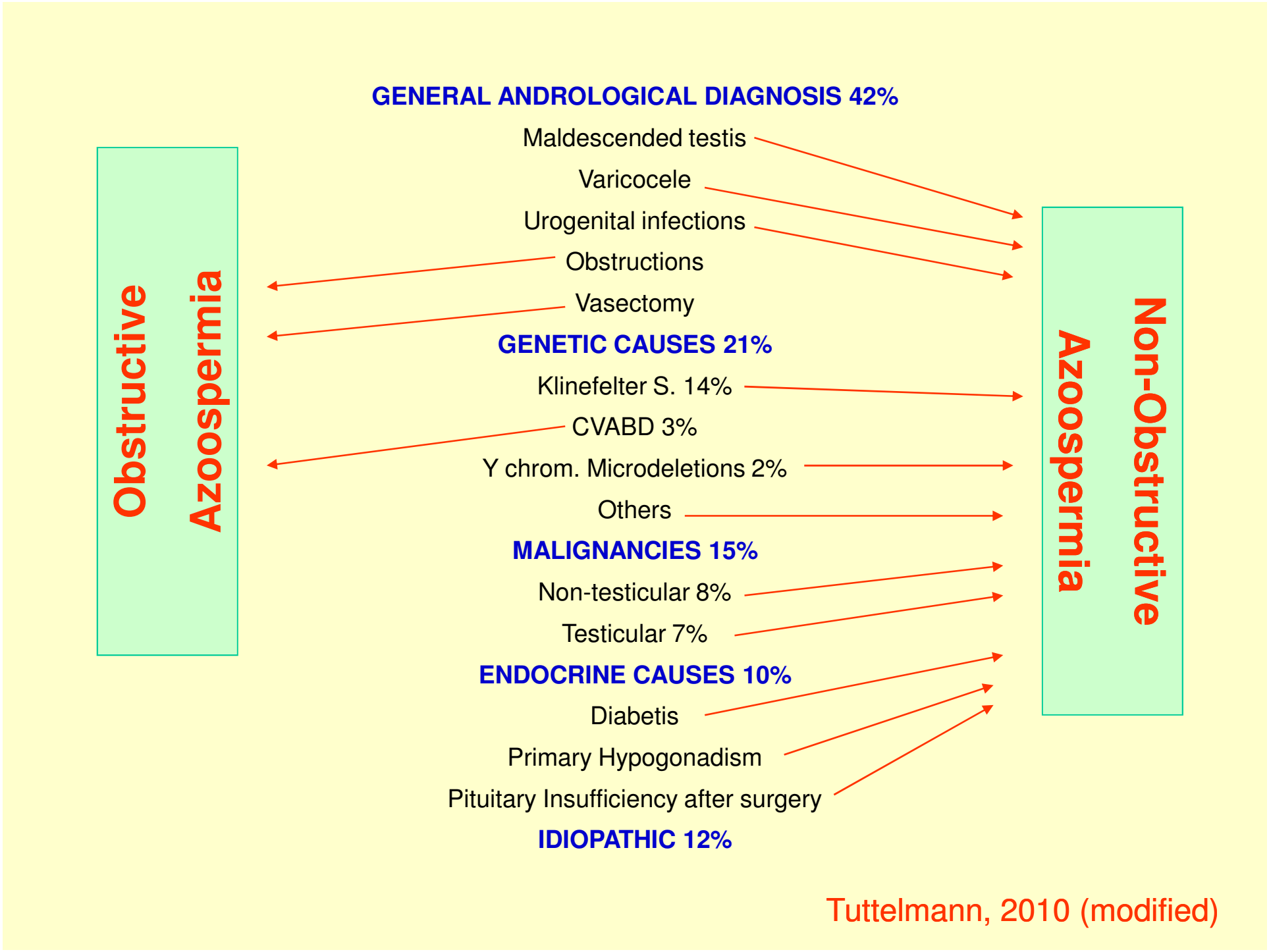
Clinical experience with azoospermia: aetiology and chances for spermatozoa detection upon biopsy

F. Tüttelmann,*† F. Werny,† T. G. Cooper,† S. Kliesch,† M. Simoni†‡ and E. Nieschlag†

International Journal of Andrology ***2010***

Retrospective analysis of **1583** consecutive patients with azoospermia betw. 1976 – 2009

the largest reported cohort of azoospermic pts



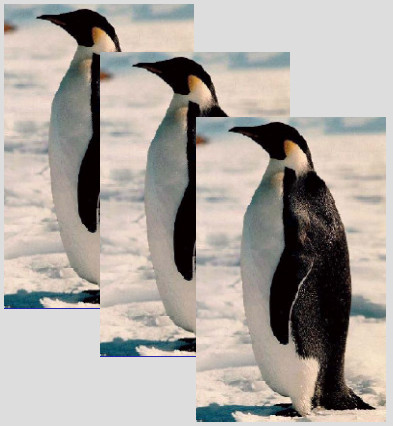
Tuttelmann, 2010 (modified)

Late ’90s: shift in paradigm in azoospermia

From primarily DIAGNOSTIC to primarly RETRIEVAL act

Never do a biopsy
for diagnostic purpose alone

FREEZE!

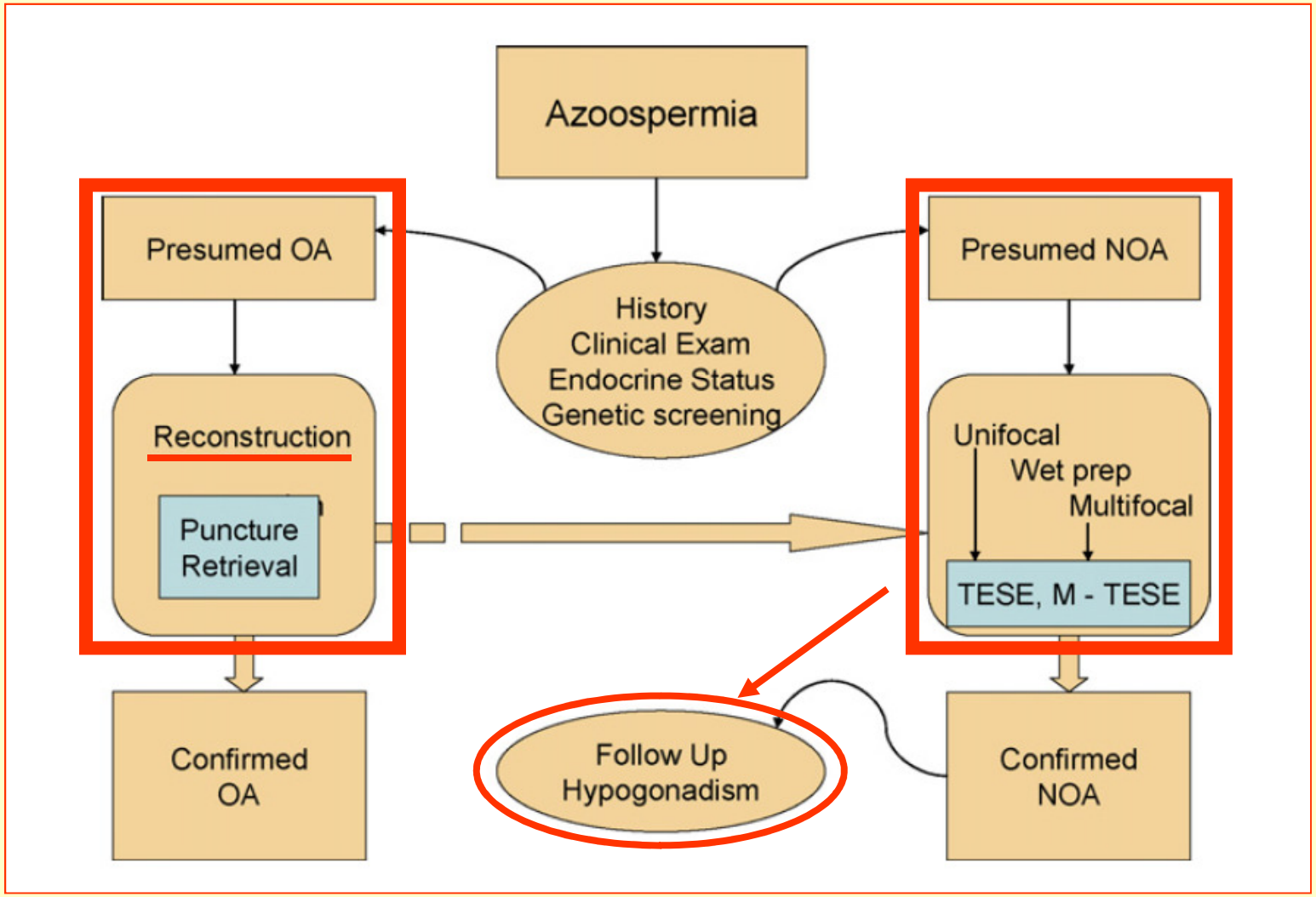


Human R

Tro
E, Vita

Int., 2000

Diagnostic-Therapeutic Algorythm in Azoospermia



P Pantke, **2008**

"**Identifying the underlying aetiology of azoospermia** and predicting the chances of finding spermatozoa by testicular sperm extraction (TESE) are essential for counselling patients desiring paternity.

However, predictions are often unreliable and **a final answer can frequently only be achieved by testicular biopsy**"

Schlegel, 2004

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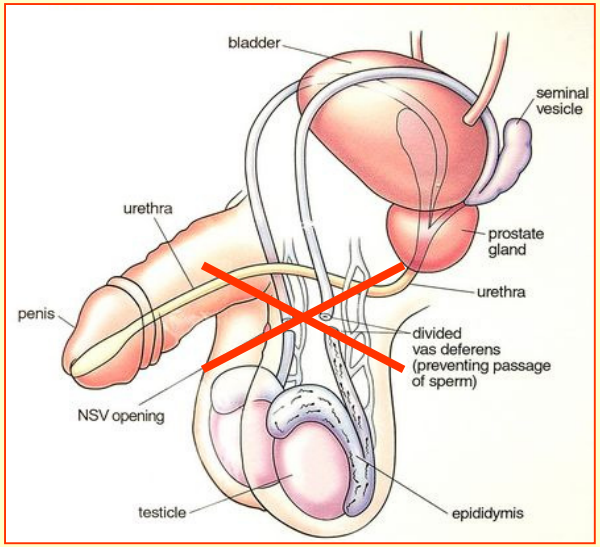


Sperm retrieval for obstructive azoospermia

The Practice Committee of the American Society for Reproductive Medicine

Fertility and Sterility® Vol. 90, Suppl 3, November 2008

*Almost all men with obstructive azoospermia have **abundant sperm** in the testes that can be retrieved successfully using **a variety of different techniques***

Sperm retrieval for obstructive azoospermia

The Practice Committee of the American Society for Reproductive Medicine

Fertility and Sterility® Vol. 90, Suppl 3, November 2008

Microsurgical reconstruction *should be offered to men having a reparable reproductive tract obstruction.*

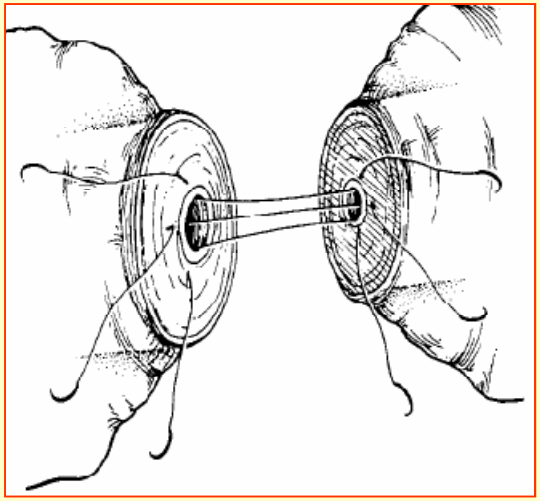
In general, ***the optimal treatment would allow the couple to conceive naturally****.*

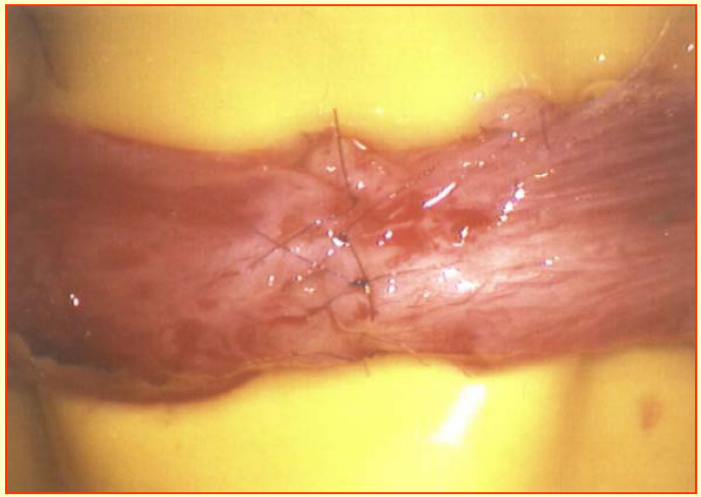
Surgical sperm retrieval: what not to do

Khaldoun Sharif, F.R.C.O.G., and Samer Ghunaim, M.Sc.

Fertility and Sterility Vol. 89, No. 1, January 2008

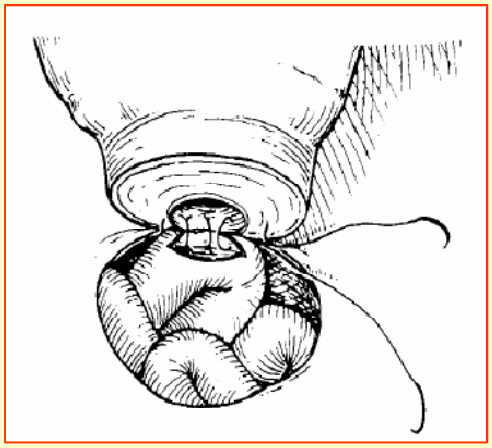
Do not do SSR in post-testicular (obstructive) azoospermia without first considering ***surgical correction***





Epididymovasostomy as the first-line treatment of obstructive azoospermia in young couples with normal spermatogenesis

Smrkoli T et al, Repr Biom Online **2010**



- Testicular biopsy and microsurg. side-to-side epididymovasostomy were performed in 34 azoospermic men with OA mostly due to inflammation. Ductal system patency was recovered in 21 **(63.6%)** men and natural pregnancy achieved in 13 **(38.2%)** of couples.

- The pregnancy rates obtained after surgery were not statistically different from those obtained by TESE-ICSI, but when also considering multiple pregnancies, miscarriages and side effects, the results obtained with surgery are **better** than those obtained with TESE-ICSI.

Sperm Retrieval Techniques in OA

1. **Intraoperative** testicular sperm retrieval during vaso-vasostomy/vasoepididymostomy
2. **MESA** (Microsurgical Epidydimal Sperm Aspiration)
3. **PESA** (Percutaneous Epididymal Sperm Aspiration)
4. **TESE** (TEsticular Sperm Extraction)
5. **TESA** (TEsticular Sperm Aspiration) / **TEFNA** (TEsticular Fine Needle Aspiration)

1. Intraoperative testicular sperm retrieval during vasovasostomy/vasoepididymostomy

✓ Since only 20% to 40% of couples conceive after attempted vasoepididymostomy despite patency rates of 60% to 80%, it is reasonable to consider sperm retrieval at the time of surgical reconstruction.

✓ If motile sperm are found at the site of reconstruction, they may be aspirated and **cryopreserved**.

✓ Alternatively, sperm may be retrieved via testicular biopsy. *Sperm retrieved directly from the testis are generally limited in numbers and often exhibit no motility or nonprogressive motility but nevertheless typically still are viable and almost always functional for use in ART.*

*Practice Committee of the Am Soc Repr Med, **2008***

2. MESA

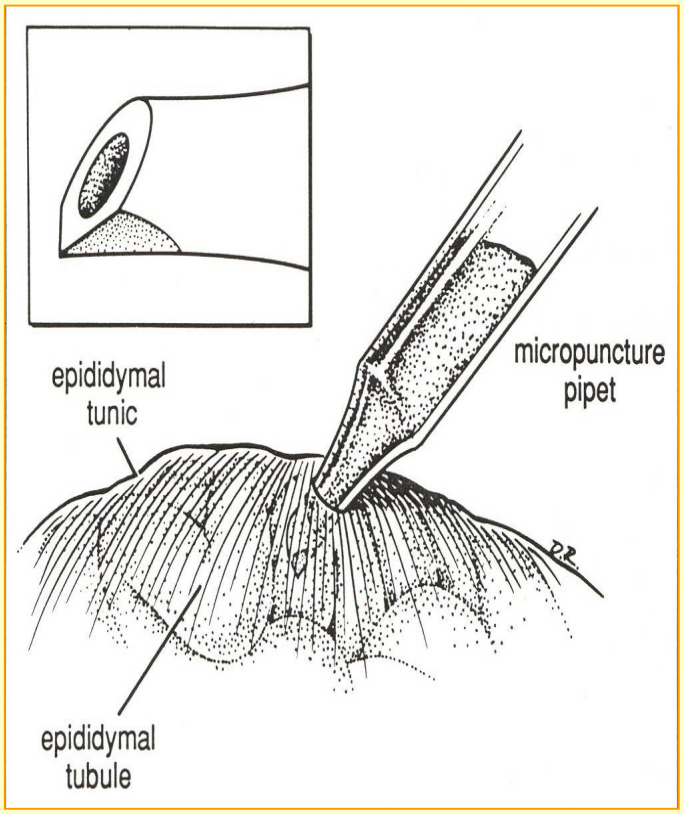
(Microsurgical Epidydimal Sperm Aspiration)

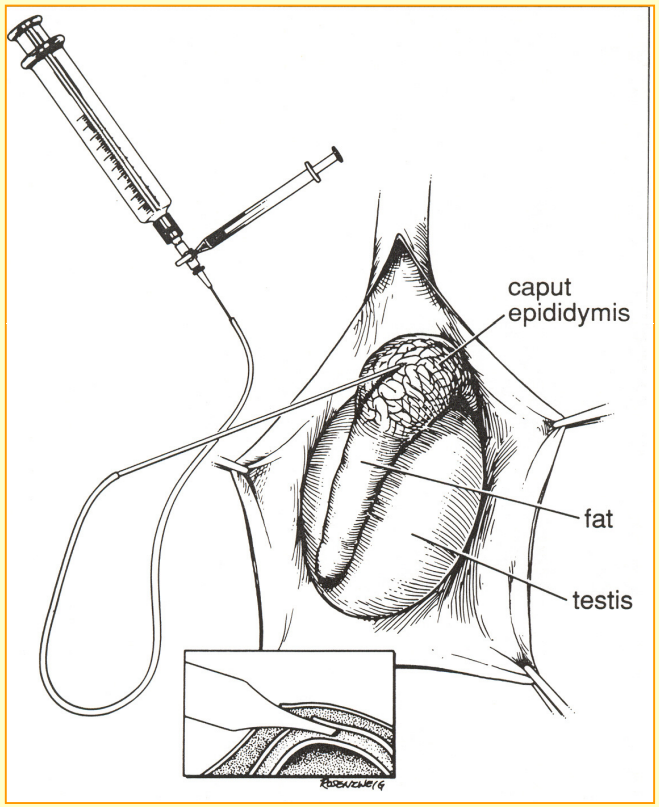
- ✓ The first sperm-retrieval technique for ART, introduced by Silber in 1988
- ✓ MESA allows for the best **quantity/quality** sperm retrieval in NOA: **>95%.** The best sperm is usually obtained from the most proximal epididymis. Sperm is highly concentrated in the epididymal fluid (approx. 1 million sperm/µL); the microsurgical approach allows for selection of the largest tubuli with minimal blood contamination.

Variations: - 2a Micropuncture MESA (1994)

-2b Mini-MESA (1996 & 1998)

2a. Micropuncure MESA Schlegel, 1994





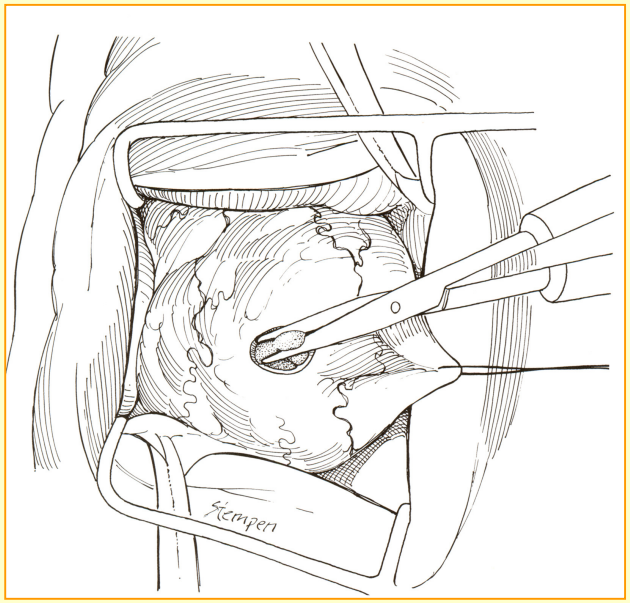
A glass micropuncture pipette connected with a suction system replaces the use of microscissors

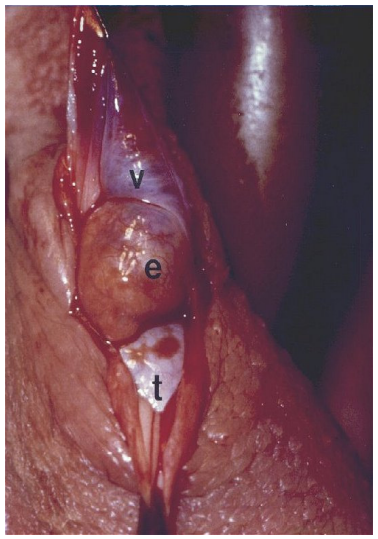
2b. Mini-MESA

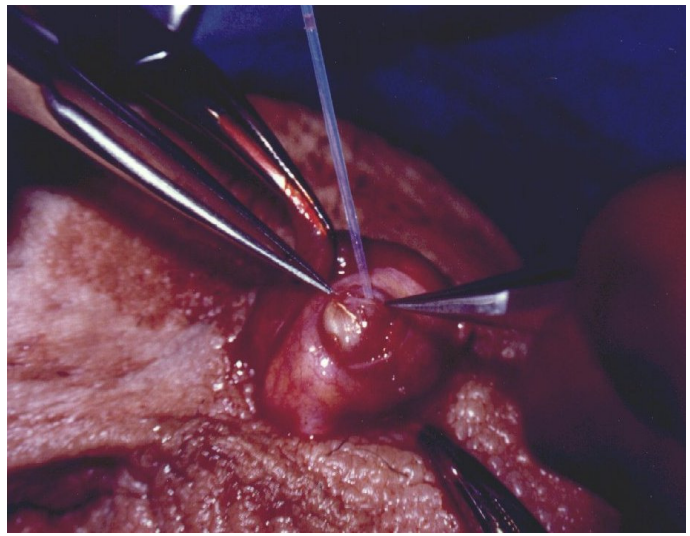
Franco, 1996; Nudell, 1998

Window-incision, avoiding testis extrusion:

- less post-op pain,
- lower chanches of post-surgical adhesions







3. PESA
(Percutaneous Epididymal Sperm Aspiration)

First alternative to MESA, introduced by Craft in 1995

PESA vs MESA:

Plus= quicker, less expensive

Minus= -lower SRR (60-70% vs 90-95%)

-higher chances of iatrogenic obstruction and of scrotal hematoma

4. TESE
(TEsticular Sperm Extraction)

Introduced by Silber in 1995

Plus ***TESE vs Epididymal sperm retrievals***: no risk of iatrogenic obstruction

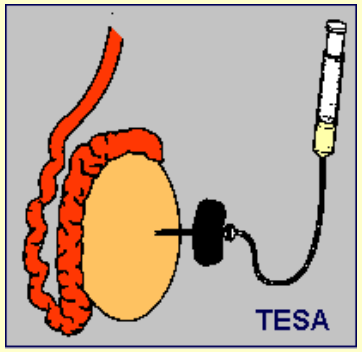
Minus ***TESE vs MESA***: retrieval of less/lower quality sperm (nevertheless viable and funcional for use in ART); loss of testicular parenchima (follow-up for low T)

AVOID MULTIPLE TESTICULAR INCISIONS:

testicular arteries are end-arteries: injury at biopsy: possible partial testicular infarction!

Schlegel, 1997

5. TESA (TEsticular Sperm Aspiration)
TEFNA (TEsticular Fine Needle Aspiration)



- Introduced in 1995 by Bourne
- In OA it usually allowes a sperm retrieval sufficient for ICSI, but not for cryopreservation.
- Intratesticular hematoma has been reported in 7% of cases within 30 min (Lewin, 1999)

The management of infertility due to obstructive azoospermia

Practice Committee of the American Society for Reproductive Medicine in collaboration with the Society for Male Reproduction and Urology

Fertil Steril **2008**;90:S121–4

- The technique of sperm retrieval and the source of sperm *(testis, epididymis, vas or seminal vesicle)* have no significant effect on pregnancy rates achieved with IVF/ICSI. *All methods generally provide sufficient numbers of viable sperm for ICSI* and often also for cryopreservation.
- The choice of method depends primarily on the experience and preferences of the surgeon and the embryologist

Outcomes achieved with ICSI using **frozen-thawed** or **fresh** spermatozoa retrieved from men with OBSTRUCTIVE azoospermia are comparable

Janzen N, 2000

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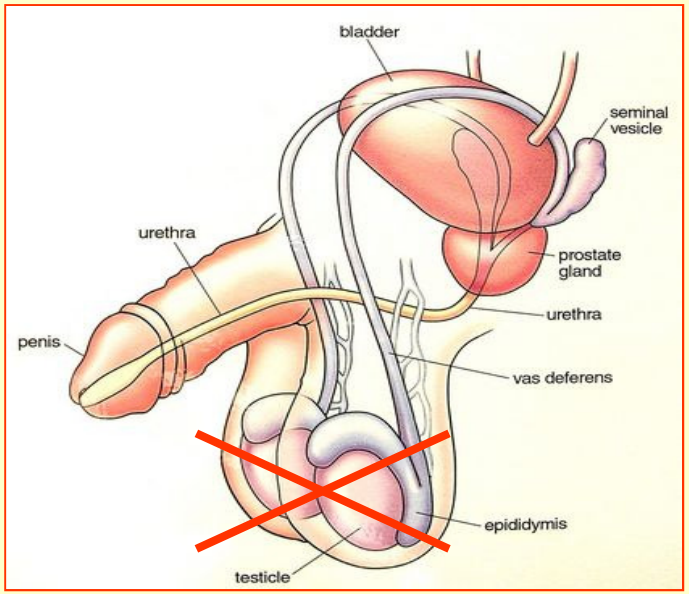


Key reports on residual spermatogenesis in azooospermic men - 1

At histology:

In the case of germ cell aplasia and maturation arrest, focal spermatogenesis may still be present histologically

Levin, 1979

Key reports on residual spermatogenesis in azooospermic men - 2

Histology on TESE samples from NOA:

- More than half of azoospermic patients with germinal failure have minute foci of spermatogenesis which are insufficient to produce spermatozoa in the ejaculate
- Incomplete testicular failure may involve a sparse multi-focal distribution of spermatogenesis throughout the entire testicle.
- 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.

Silber, 1997

“According to current standards a man is considered sterile and cannot father his own genetic offspring if no sperm cells are detected in different locations in the testis”

Hauser et al 2006

Sperm Retrieval Techniques in NOA

- ✓ **TESA** (TEsticular Sperm Aspiration) / **TEFNA** (TEsticular Fine Needle Aspiration)
- ✓ **TESE** (TEsticular Sperm Extraction)
- ✓ **Micro-TESE** (Microdissection TEsticular Sperm Extraction)
  - ➢ Modifications of classic Micro-TESE
  - ➢ Snapshot: retrieval in Klinefelter cases

TESA / TEFNA in NOA

TeFNA is not indicated in NOA because of its **low sperm retrieval rate: 21.1%**

in practice, it works only in cases of hypospermatogenesis

Dohle GR et al., 2007

Fine needle aspiration vs. mTESE in non-obstructive azoospermia

S. El-Haggar,* T. Mostafa,* T. Abdel Nasser,* R. Hany† and A. Abdel Hadi*

2007 Int J Androl 30, 1–7

- The overall **SRR** was 54% by mTESE and 10% by FNA
- The total **complication rate** following mTESE was 10% in the early phase and none in the long-term follow-up compared to 24% of FNA side.

*It is concluded that **mTESE is superior to FNA** as regards sperm retrieval rate and lower incidence of complications in NOA patients.*

Varicocele Repair in Patients With Nonobstructive Azoospermia: A Meta-Analysis

John W. Weedin,*,† Mohit Khera‡ and Larry I. Lipshultz§

J Urol, June **2010**

- ✓ After varicocele repair 91 **(39.1%)** patients had motile sperm in the ejaculate, and **14** spontaneous pregnancies were reported.
- ✓ **Success rates** in patients with ***maturation arrest (42.1%)*** or ***hypospermatogenesis (54.5%)*** were significantly higher than in those with Sertoli-cell-only (11.3%, p 0.001 in both groups).
- ✓ Patients with ***late maturation arrest*** had a higher probability of success (45.8%) than those with early maturation arrest (0%, p 0.007).

TEFNA predicts seminal outcome of varicocele treatment

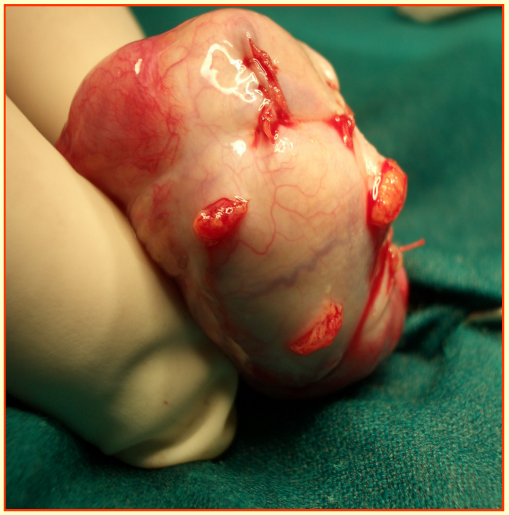
- 70 pts with **late** maturation arrest= impovement of sperm parameters

Bettella, Foresta et al, 2001

SRR of TESE in NOA

TESE SRR in NOA= **51,3%** (range: 28-77%); statistically superior to TEFNA for both amount and quality quality of retrieved sperm ($p<0.001$)

Hauser, 2006



Courtesy of G. Franco

NOTE:

In NOA TESE is MULTIFOCAL, while in OA it is mostly MONOFOCAL

Introduction of operating microscope: the Microdissection-TESE (Micro-TESE)

Why microscope magnification:

"The tubules with likely spermatogenesis, that is those larger and darker or those closest to vessels, are identified"

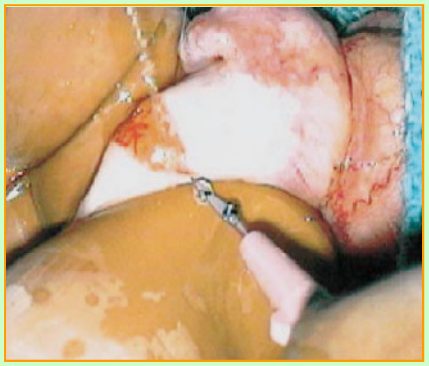
Schlegel & Li 1998

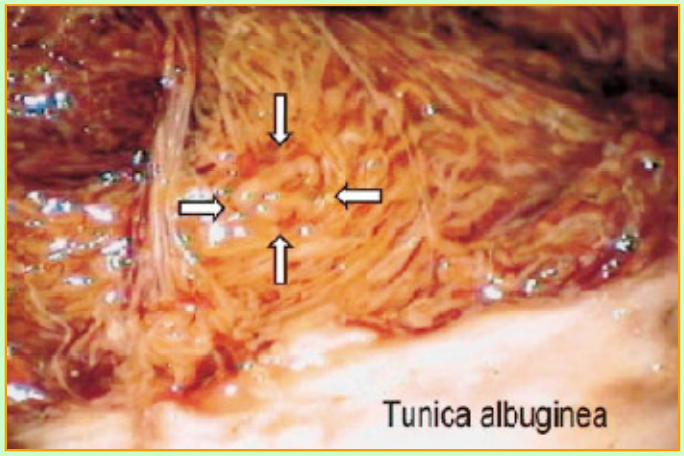
First reports on Micro-TESE

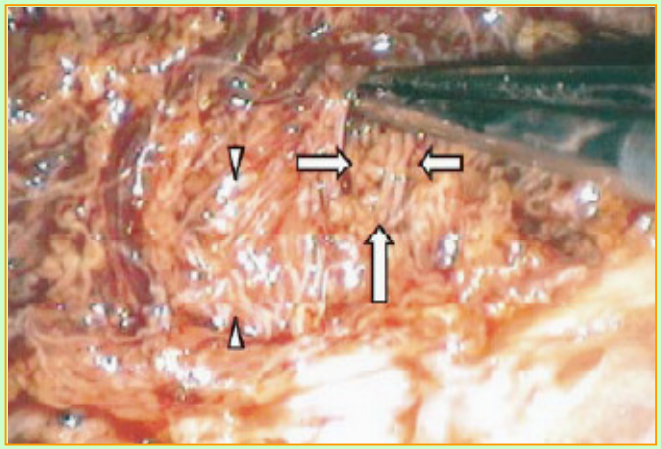
Human Reproduction vol.14 no.1 pp.131–135, 1999

Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision

Peter N.Schlegel[1]



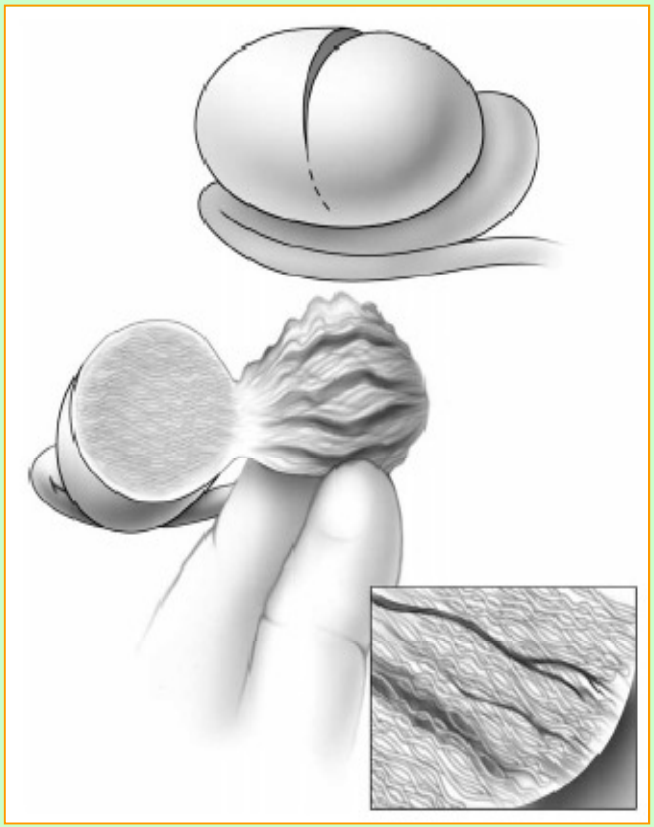




PN Schlegel, P Shihua Li, Hum Repr Update1998, 4: 439

PN Schlegel, Hum Repr 1999, 14: 131-135

Micro-TESE operative technique- 1



- equatorial incision of albuginea
- bipolar cauterization
- magnified (microscope) vision of tubuli: 10-20 X
- samplings from selected areas

Micro-TESE operative technique- 2

From: GM Colpi, ESHRE 2009

MicroTESE goals

- Best SRR in NOA
- Successful also in TESE failures: “Salvage Micro-TESE”

- minimal loss of testicular parenchima
- minimal vascular damages

SRR in NOA pts.: TESE vs. MicroTESE

n TESE/M-TESE	SRR TESE (amount of tissue removed)	SRR M-TESE (amount of tissue removed)	Advantage M-TESE	Reference
37/56	35% (–3 × 50 mg)	43% (≥3 × 10–15 mg)	+8%	[58]
100/100	30% (Unifocal 54 ± 27 mg)	47% (5 ± 3 mg)	+17%	[56]
22/27	45% (250–750 mg)	63% (×2–10 mg)	+18%	[55]
83/460	32% (–500 mg)	57% (×2–10 mg)	+25%	[57]
24/74	17% (×150 mg)	45% (20–100 mg)	+28%	[59]
176/176	17% (Variable 1–4 biopsies)	50% (×2–10 mg)	+33%[a]	[60]
46/46	0% (Failed TESE elsewhere)	46% (×10–15 mg)	+46%[a]	[61]

[a] Repeated sperm retrieval procedure: Advantage of M-TESE has to be corrected by SSR that would have been achieved with repetitive TESE.

Reproduced from P Pantke, 2008

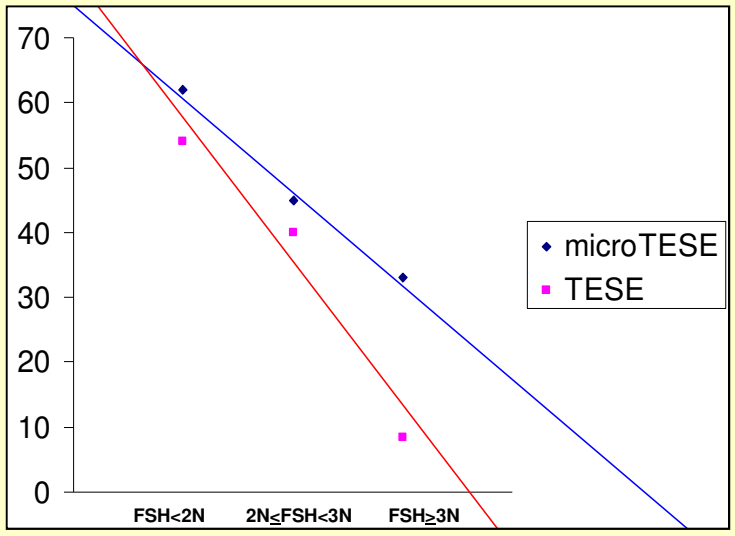
[55] Schlegel PN. Hum Reprod 1999;14:131–5
[56] Amer M. Hum Reprod 2000;15:653–6
[57] Ramasamy R. Urology 2005;65:1190–4
[58] Tsujimura A. Hum Reprod 2002;17:2924–9
[59] Okada H. J Urol 2002;168:1063–7
[60] Ramasamy R. J Urol 2007;177:1447–9
[61] Tsujimura A. J Urol 2006;175:1446–9

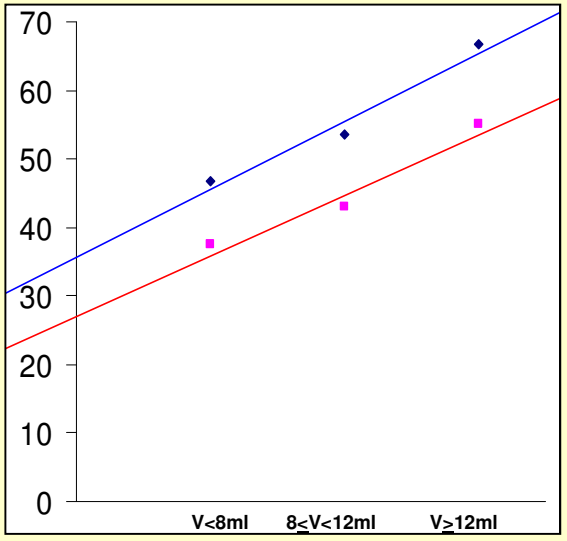
Micro-TESE versus conventional TESE for ICSI in NOA: a randomized controlled study

Colpi GM, Colpi EM, Piediferro G, Giacchetta D, Gazzano G, Castiglioni FM, Magli MC, Gianaroli L

Repr Med Online, 2009

- ✓ **FSH value** and the **surgical procedure (TESE vs M-TESE)** were the two variables that could significantly predict a positive sperm retrieval ($p<0.05$).
- ✓ **Testis volume** and **histology** were shown to play a less important role.





Technique of Binary Logistic Regression with statistical significance set at $p<0.05$.

The higher SRR of MicroTESE vs TESE could be due to:

- ✓ selecting larger tubules (110μm is the smaller tubule diameter permitting successful sperm retrieval) - Amer, 2007,
- ✓ taking biopsies from the more vascularized areas (Schlegel, 1999), since this strategy is not possible with TESE;
- ✓ recovering testicular microfragments in different areas of the testicle in a sort of testicular mapping, while TESE consists of a large monofocal biopsy Colpi, 2008, personal commun.

Micro-TESE after failed TESE: *"Salvage Micro-TESE"*

A. Tsujimura, 2006

- Aim: comparison of SRR betw. conventional micro-TESE (n=134), and salvage micro-TESE (n= 46)
- Sperm retrieval rate: **salvage Micro-TESE= 45.7%;** conventional M-TESE= 44.0% (p NS), regardless testicular histology

"There is no threshold of prior negative biopsies that precludes the success of sperm retrieval (in NOA pts) using micro-TESE" R Ramasamy, 2007

Testis health: TESE vs MicroTESE - 1

Amount of testicular parenchima removed

n TESE/M-TESE	SRR TESE (amount of tissue removed)	SRR M-TESE (amount of tissue removed)	Advantage M-TESE	Reference
37/56	35% (~3 x 50 mg)	43% (≥3 x 10–15 mg)	+8%	[58]
100/100	2[illegible]% (Unifocal 54 ± 27 mg)	[illegible]7% (5 ± 3 mg)	+17%	[56]
22/27	45% (250–750 mg)	63% (×2–10 mg)	+18%	[55]
83/460	32% (~500 mg)	57% (×2–10 mg)	+25%	[57]
24/74	[illegible]7% (×150 mg)	45% (20–100 mg)	+28%	[59]
176/176	[illegible]% (Variable 1–4 biopsies)	[illegible]0% (×2–10 mg)	+33%[a]	[60]
46/46	0% (Failed TESE elsewhere)	46% (×10–15 mg)	+46%[a]	[61]

[a] Repeated sperm retrieval procedure: Advantage of M-TESE has to be corrected by SSR that would have been achieved with repetitive TESE.

Reproduced from P Pantke, 2008

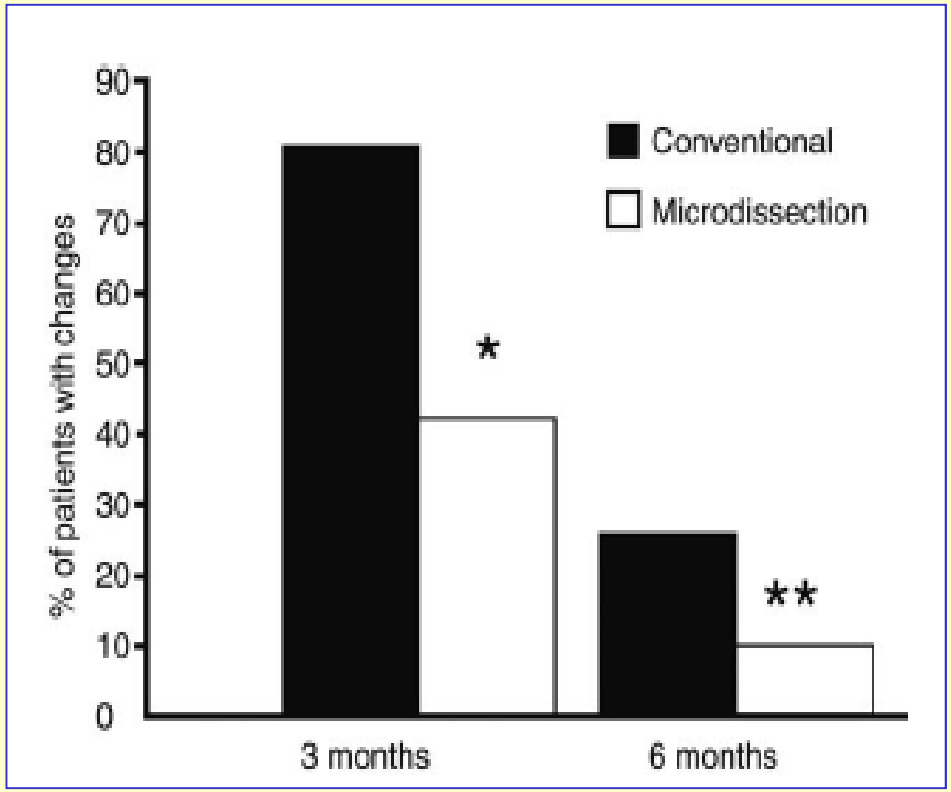
NOTE: *in NOA pts TESE is a MULTIFOCAL procedure*

Testis health: TESE vs MicroTESE - 2

Structural & functional changes to the testis
R Ramasamy, 2005

- **Hormonal changes (T, FSH, LH)**= similar in the 2 groups

- **Ultrasound findings**: fewer acute and chronic changes in micro-TESE than in TESE ($P < 0.05$)



Modifications of classic MicroTESE - 1

Single tubule biopsy:

a new objective microsurgical advancement for testicular sperm retrieval in NOA

Micro-TESE using a micrometer fixed to the operating microscope

- During micro-TESE, the best cutoff level of the ST diameter for harvesting testicular spermatoza is **110 µm** with sensitivity 86.0% and specificity 74.4%.

- When ST diameter is **300 µm** or more **a single tubule biopsy** is usually sufficient to harvest enough testicular spermatozoa for intracytoplasmic sperm injection or sperm freezing with minimal tissue excision.

M Amer, 2008

Modifications of classic MicroTESE - 2

Stepwise approach

1) small equatorial incision of albuginea and single TESE;
2) If negative SR: equatorial extension of the incision to perform microTESE;
3) If still negative SR: multiple traditional biopsies on the contralateral testicle.

G Franco, **2009**

1. Conventional TESE with 3 incisions on 3 testis quadrants;
2. With negative SR: microTESE by enlarging the middle incision vertically

T Turunc, in press **2010**

Goal: optimize SR reducing MicroTESE only to cases which may benefit from it.

Should non-mosaic Klinefelter syndrome men be labelled as infertile in 2009?

Fullerton, **2010**

✓ METHODS Medline and EMBASE (1980-2009) were searched independently.

✓ RESULTS The overall success rate for **sperm retrieval** was **44%,** with a higher rate of success using micro-dissection testicular sperm aspiration **(micro-TESE) (55%).** This, along with ICSI, has led to the birth of **101 children**.

✓ There are **no known predictors** for successful sperm retrieval.

Agenda

- ✓ Definition and Epidemiology of azoospermia
- ✓ Azoospermia: Obstructive (OA) vs. Non-Obstructive (NOA)
- ✓ Sperm retrieval in OA
- ✓ Sperm retrieval in NOA (80-85%)
- ✓ **A glimpse on how retrieved sperm does in ICSI**

Reproductive capacity of spermatozoa from men with testicular failure

Human Reproduction vol.14 no.11 pp.2796–2800, 1999

Filippo Ubaldi[1,5], Zsolt Peter Nagy[1], Laura Rienzi[1], Jan Tesarik[2], Reno Anniballo[1], Giorgio Franco[3], Fabrizio Menchini-Fabris[4] and Ermanno Greco[1]

- ✓ICSI treatment cycles with testicular spermatozoa from OA-NOA men were compared with ICSI ejaculated sperm cycles.
- ✓The fertilization, cleavage, pregnancy and abortion rates were similar in matched groups irrespective of the type of azoospermia.
- ✓The **implantation rate** in the **NOA** patient group was significantly **lower** than that in the matched ejaculated sperm group (13.4% versus 26%). *(No impairment of the implantation rate was observed in the **OA** patient group).*

ICSI outcomes: OA vs NOA sperm

The results of ICSI are worse when using sperm retrieved in men with NOA as compared to OA (Monzo, 2001; Vernaeve, 2003; Siber, 2003):

- **birth rates** of 19% in NOA versus 28% in OA (Schwarzer, 2003),
- significantly lower **fertilization** and **implantation rates** (Ghanem, 2005)
- higher **miscarriage rates** (11.5% vs. 2.5%) (Borges, 2002).

SR in Azoospermia: Andrologic take-home messages

- ✓ **OA**: whenever possible offer microsurgical reconstruction along with concomitant sperm retrieval
- ✓ **OA**: if reconsruction is not feasible, give preference to controlled procedures (MESA, TESE) vs blind ones (PESA, TESA/TEFNA) to minimize risks of iatrogenic epididymal obstructions / testicular hematomas
- ✓ **NOA**: percutaneous techniques are not effective; TESE is more morbid (repetitive!) and less effective than Micro-TESE: GO DIRECTLY FOR Micro-TESE!!! (alternatively: stepwise approach)
- ✓ **NOA**: follow-up the male patient for possible iatrogenic **hypogonadism** up to 1 year