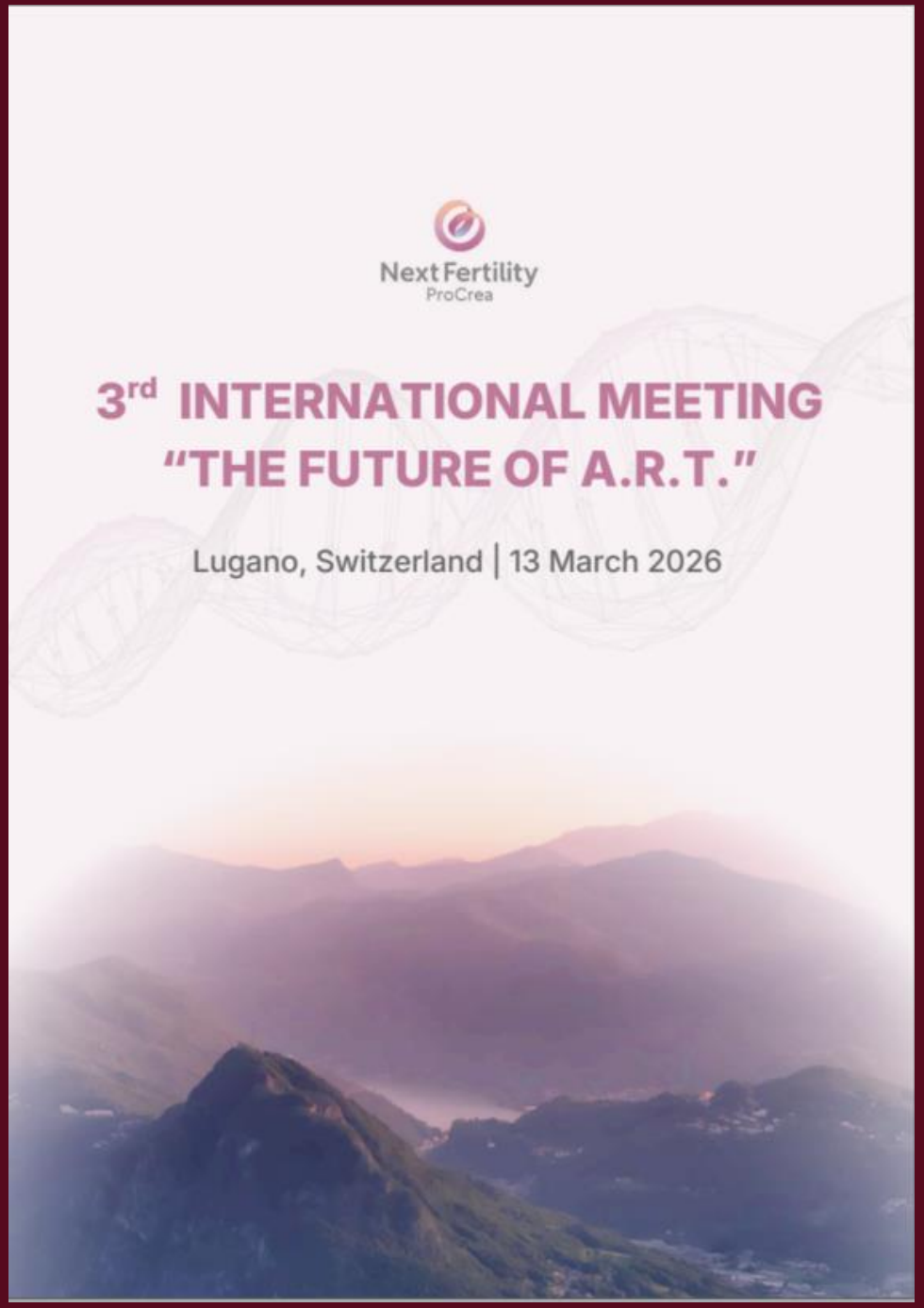



# Fresh versus Frozen MicroTESE/ICSI

**Giovanni Maria Colpi**

Head of Andrology Unit
and Scientific Director
of Next Fertility ProCrea,
Lugano (Switzerland)

DISCLOSURE: no conflicts of interest

- NOA is usually an irreversible and untreatable condition: however, it does not mean sterility because focal sperm production can be found in 30-60% of these men.[1]
- Actually, clinical experience has shown that in just over half of NOA patients, sperm can be retrieved from the testicular parenchyma and used for ICSI.

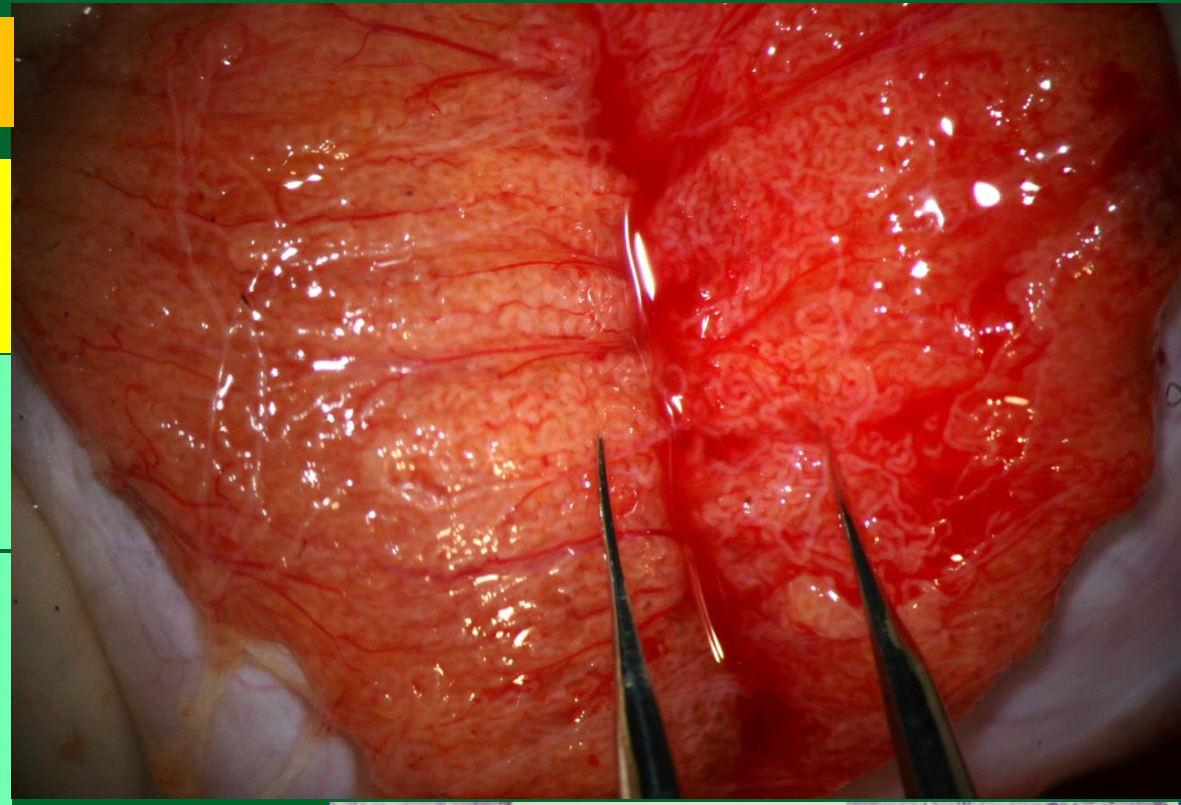
[1]*Achermann APP, Pereira TA, Esteves SC. Microdissection testicular sperm extraction (micro-TESE) in men with infertility due to nonobstructive azoospermia: summary of current literature. Int Urol Nephrol. 2021 Nov;53(11):2193-2210. doi: 10.1007/s11255-021-02979-4. Epub 2021 Aug 19. PMID: 34410586.*

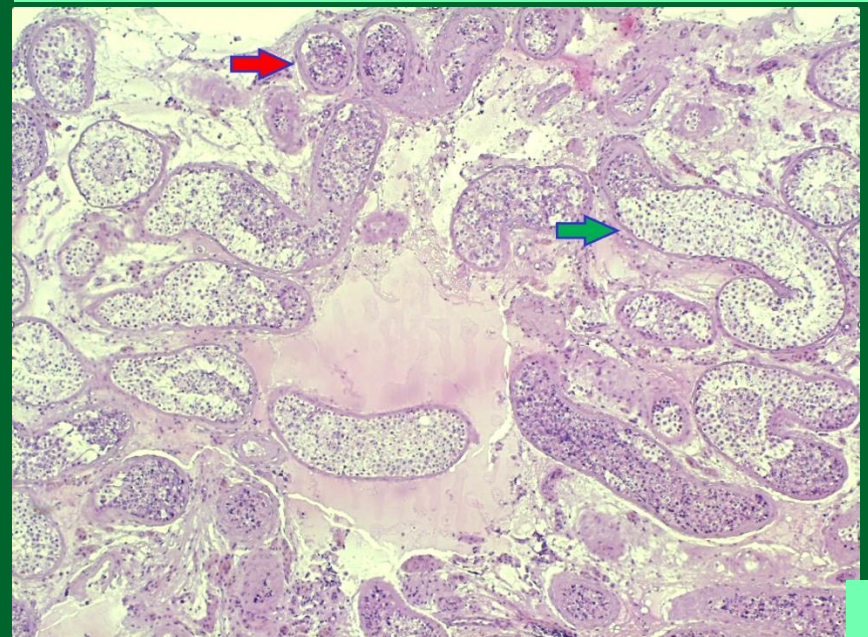
# EVOLUTION IN THE LITERATURE

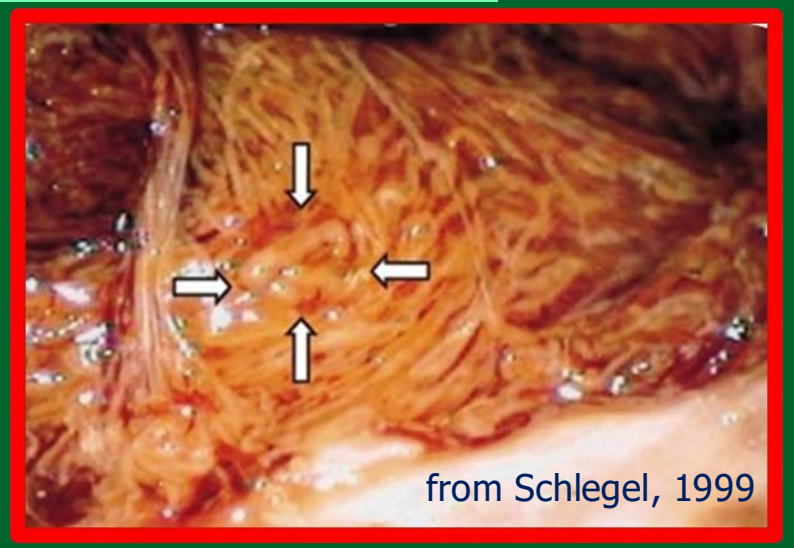
Schlegel in 1998 and 1999 presented (in an International Congress and in a paper, respectively) the MicroDissection TESE (mTESE) as a new effective technique for NOA patients.

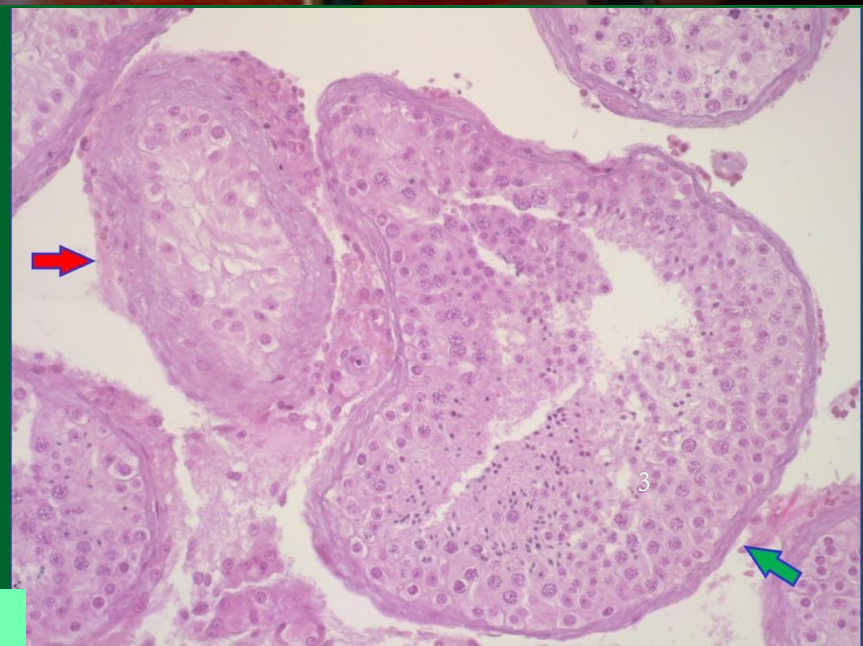
This procedure required a bivalve incision of the testis and the exploration at high magnification of the tissue to search for "dilated" tubules.

Rationale: Histological studies show a "patchy distribution" (Tournaye, 1995; Hauser, 1998) in a high percentage of NOA testes, i.e. the parenchyma is inhomogeneous and may host small isles of tubules with residual spermatogenesis.







without ➡ and with ➡ spermatogenesis

## **Sperm retrieval in NOA** can be performed with different surgical techniques:

***TeFNA*** *(testicular fine-needle aspiration):*

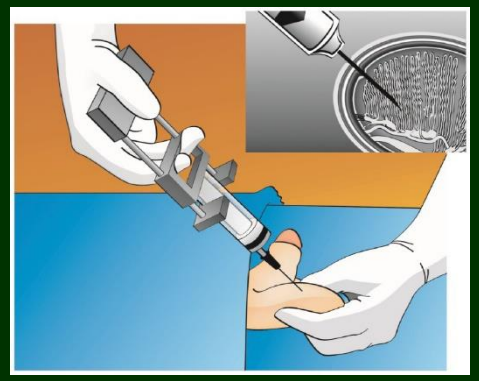
- currently excluded from EAU guidelines !!!

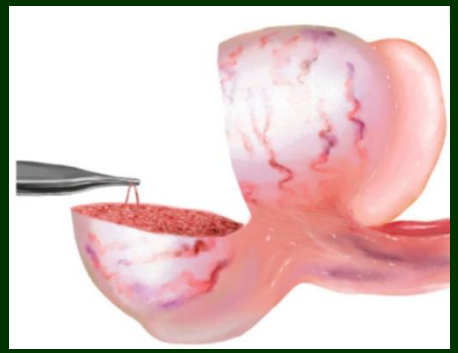
***cTESE*** *(conventional testicular sperm extraction):*

- actually considered a suboptimal procedure, no longer recommended in EAU 2025 and GAF 2025 guidelines

***mTESE*** *(microdissection testicular sperm extraction):*

- considered the **gold standard procedure**





*Esteves SC et al. An update on sperm retrieval techniques for azoospermic males. Clinics (Sao Paulo). 2013*

*Punjani N et al. Two Decades from the Introduction of Microdissection Testicular Sperm Extraction: How This Surgical Technique Has Improved the Management of NOA. J Clin Med. 2021*

# TESE versus MicroTESE

**For two decades, a long dispute was carried on between supporters of cTESE vs. mTESE. This is no longer debatable today** (Esteves et al, 2020).

Human Reproduction Update, pp. 1–3, 2020
doi:10.1093/humupd/dmaa006

human reproduction update | LETTER TO THE EDITOR

## Sperm retrieval rates by micro-TESE versus conventional TESE in men with non-obstructive azoospermia—the assumption of independence in effect sizes might lead to misleading conclusions

Sandro C Esteves[1,2,3], Ranjith Ramasamy[4], Giovanni M Colpi[5,6], José F Carvalho[7], Peter N Schlegel[8]

**Table II Summary statistics of eligible controlled studies on the effectiveness of sperm retrieval in patients with non-obstructive azoospermia included in the meta-analysis of Corona *et al.* (Hum Reprod Update 2019; 25(6):733–757 [see Supplementary Table SI for list of included studies]).**

| | mTESE | cTESE | *P* value† | Relative risk‡ (95% CI) | NNT‡ (95% CI) |
|---|---|---|---|---|---|
| No. of patients (total) | 663 | 268 | | | |
| No. of patients positive SR (%) | 329 (49.6) | 98 (36.5) | 0.0003 | 1.35 (1.14–1.61) | 7.6 (5.0–16.6) |
| No. of patients with histopathology data | 636 | 246 | - | - | - |
| No. of patients positive SR (%) | 312 (49.0) | 88 (35.8) | 0.0004 | 1.37 (1.14–1.65) | 7.5 (4.9–16.6) |
| No. (%) of patients with Sertoli cell-only (SCO)/atrophy histopathology | 399 (62.7) | 135 (54.9) | 0.03 | - | - |
| No. of patients with SCO and positive SR (%) | 144* (36.1) | 18* (13.3) | <0.0001 | 2.70 (1.72–4.24) | 4.4 (3.2–7.1) |

mTESE: microdissection testicular sperm extraction; cTESE: conventional testicular sperm extraction; NNT: number needed to treat; NOA: non-obstructive azoospermia; SR: sperm retrieval; SCO: Sertoli cell-only; CI: confidence interval

†Comparison of proportions by chi-square: Campbell I (2007) chi-squared and Fisher–Irwin tests of two-by-two tables with small sample recommendations. Statistics in Medicine 26:3661–367

Altman DG (1998) Confidence intervals for the number needed to treat. British Medical Journal 317: 1309–1312

*Total number of patients with Sertoli cell-only reported in primary studies.

**In the early following years, an anthology of papers was published, drawing attention to:**

- technical details,

- the **long learning curve** needed for obtaining a good surgical sperm retrieval rate,

- the **long duration of mTESE surgery**,

- its **effectiveness in the different histologic patterns** of NOA,

- the possible **long time required by biologist** for a careful sperm search in the removed tissue sample.

## LEARNING CURVE

| 150 consecutive mTESE by the same surgeon | SSR ($p<0.05$) |
|---|---|
| Group A (first 50) | 32% |
| Group B (middle 50) | 44% |
| Group C (last 50) | 48% |

(Ishikawa et al, 2010)

## SURGERY DURATION

longer than a cTESE (Tsujimura, 2002)

---

mean: 1.8 h (r. 0.5–6.6 h) if positive,
mean: 2.7 h (r. 0.8–7.5 h) if negative.
(Ramasamy, 2011)

## MICROSURGEON EXPERIENCE

Schlegel claims that SRR improves very slowly until microsurgeon experience oversteps a 500 case-threshold, because eyes succeed in seizing even minimal differences in tubular diameter, and consequently surgery results shorter.

(Dabaja & Schlegel, 2013)

## LONG BIOLOGICAL WORK

The chance of finding sperm following chemical digestion when sperm were not initially found is 25%–30% (Aydos, 2005)
A success rate of +7% was reported when effective mechanical preparation of the removed specimen is followed by a multihour, many-technician search for spermatozoa in an experienced Lab. (Ramasamy, 2011)

## EFFECTIVENESS vs HYSTOLOGY

mTESE vs cTESE

**SCOS:**
•Mean SRR 37% vs 14%
**Maturation Arrest:**
•Mean SRR 49% vs 27%
**Hypospermatogenesis:**
•Mean SRR 85% vs 73%
**Overall SSR:**
•Mean SRR **54%** vs 33%
(Deruyver et al, 2014)

The Canadian Guidelines in 2015 stated : **“The testicular sperm extraction procedure should be offered to all men with NOA but should only be undertaken in a Centre with expertise in mTESE and where an ICSI laboratory with expertise in handling these samples is available”.**

Nine years later, these words are worthy of being set in stone, because they express a truth still indisputable today.

As clinicians, we have **the ethical duty to offer the NOA patient the greatest chance of success**, and therefore a careful evaluation of all aspects of a given NOA patient is mandatory.

A basic aspect is the adequate preparation of the NOA patient for mTESE.

PREOPERATIVE MEASURES

# Trying to Increase Successful Testicular Sperm Retrievals

➔ **Avoiding (or limiting) patient exposure to certain harmful physical and chemical agents** for three – six months (one - two spermatogenic cycle/s).

**Semen may be considered a sort of liquid biopsy of the patient's state of health.**
Therefore, in Clinical History, any patient's exposures should be investigated (Pacey, 2010):

to **excessive heat** (Jung and Schuppe, 2007):
- recreational habits: use of saunas or hot baths, prolonged periods of exposure to the sun at the seaside.
- work activities in very hot environments: stokers, glassblowers, workers in metal melting factories, etc.
- lifestyle habits: prolonged car driving, very tight clothing.
- health events: fever episodes.
- anatomical data: e.g. huge obesity with testicles actually encased in a sort of thermal cage.

to **other physical agents**: radioactivity exposure, electromagnetic fields (?), mobile phones (?).

to **chemical agents**: cigarette smoking, ethyl alcohol abuse (EAA Guidelines, 2018), volatile pollutants, some medicines, endocrine disruptors (pesticides, insecticides, phthalates, dioxins, estrogens, heavy metals such as lead, cadmium, mercury)(Bonde and Toppari, 2010), drugs (cannabis, opioids and cocaine alter the hypothalamic-pituitary axis)(Subiran et al, 2011).

# Trying to Increase Successful Testicular Sperm Retrievals

➔ Supplementation therapy : Nutraceuticals ? (Agarwal et al, 2017-2020).
(limited data are available for adjuvant medical therapy)

➔ Although also limited, data for varicocele repair support increased sperm retrieval, and even return of sperm to the ejaculate in rare cases*.
Performing varicocele repair in cases of NOA is a shared decision between the physician and the couple, guided by testicular volume, FSH level, female partner's age, testicular histology if available, and overall fertility status^.

*Flannigan RK, Schlegel PN. Microdissection testicular sperm extraction: preoperative patient optimization, surgical technique, and tissue processing. Fertil Steril. 111(3):420-42, 2019.

^Rambhatla et al. Global practice patterns and variations in the medical and surgical management of NonObstructive Azoospermia: results of a world-wide Survey, Guidelines and Expert Recommendations. World J Mens Health. 2024 Apr 4. doi: 10.5534/wjmh.230339.

NOA patients with normal T levels had a significantly higher chance of SR by mTESE compared to those with subnormal T levels (OR 1.63, 95% CI 1.08–2.45, p = 0.02).

Caroppo and Colpi, 2021

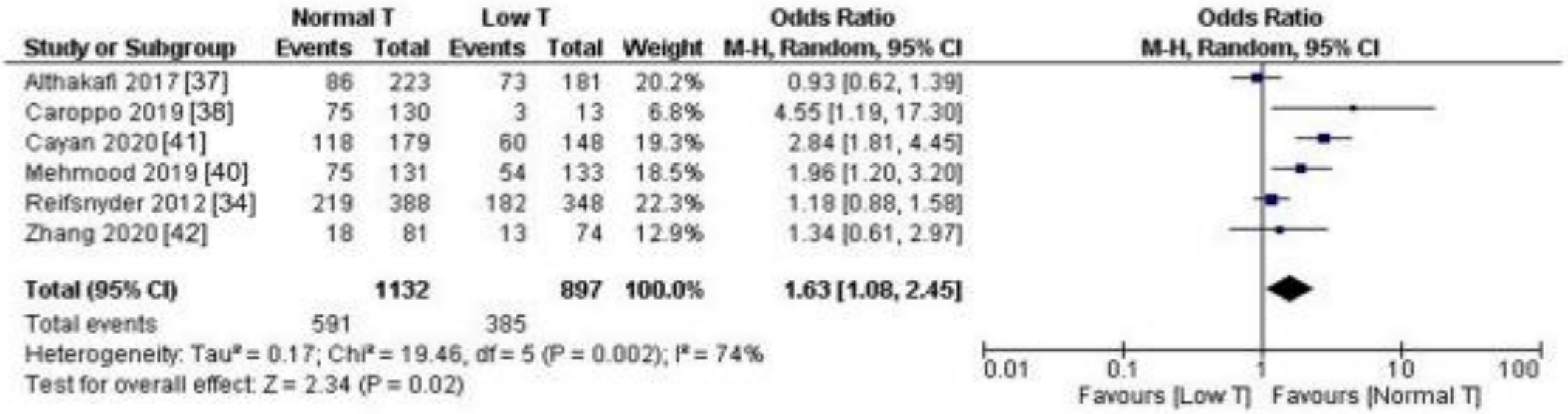



**Figure 1.** Pooled estimation of the sperm retrieval rate in patients with subnormal vs. normal testosterone level.

**Is ITT (Intra-Testicular Testosterone) relevant ?** ITT levels were assessed by TeFNA of testicular fluid.
Serum 17-OHP did not correlate with ITT at baseline, but following hCG treatment, a strong relationship between ITT and 17-OHP was found in men who received 250 or 500 IU hCG (Amory et al, 2008).
Despite serum T level was in the normal range in all men, serum 17-OHP was undetectable in men who received exogenous testosterone replacement therapy compared to the other two groups, and increased after CC and hCG treatment (Lima et al, 2020).

Therefore, during treatment by CC or hCG, 17 OHP can be considered a marker of Intratesticular Testosterone.

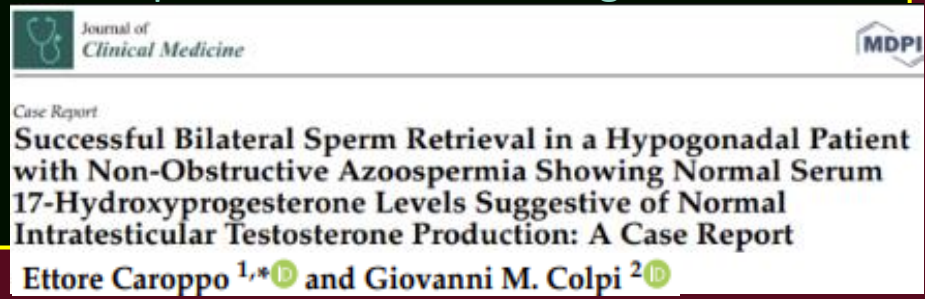
# PROPOSED REGIMENS

Meta-analysis and sistematic review, Guo et al, 2022

| | |
|---|---|
| GONADOTROPINS | hCG 2000 UI x 2/week for 3-6 months<br>FSH 150 UI x 3/ week for 3-6 months<br>(combined or sequential therapy) |
| SERM | CLOMIPHENE CITRATE 25 mg / day or every other day for 3-6 months<br>50 mg / day for 3-6 months<br>TAMOXIPHENE 10 mg twice / day for 3-6 months<br>30 mg / day for 3-6 months |
| AROMATASE INHIBITORS | ANASTROZOLE 1 mg daily for 3-6 months<br>LETROZOLE 2.5 mg daily for 3-6 months |

Hormonal therapy for 3 to 6 months was suggested prior to mTESE by 29.6% and 23.6% of participants for normogonadotropic hypogonadism and hypergonadotropic hypogonadism respectively.
The quality of evidence supporting hormonal therapy before SSR in NOA patients is low. However, it may improve the SSR rates in some NOA patients. Given the limited and poor-quality evidence, it is not routinely recommended but may be considered after adequate counseling. (GAF Survey, Rambhatla et al, 2024)

PERSONAL OPINION: Before performing mTESE we usually treat for 3 to 6 months all NOA patients with T < 350 ng/dL to enhance their T levels (Caroppo, Colpi et al.: Andrology. 2023 Mar;11(3):508-514, 2023, where 33.3% of candidates to Microtese were pretreated by hormones).
In case of lack of Testosterone response, 17-OHP can be used as a marker to proceed to mTESE.

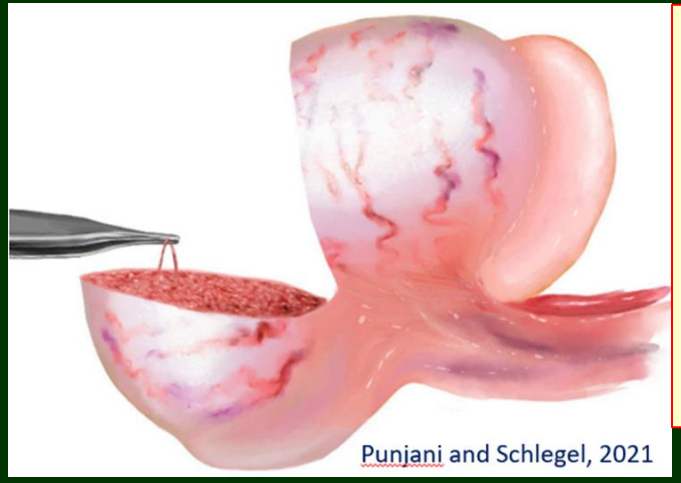

Journal of Clinical Medicine

MDPI

Case Report

Successful Bilateral Sperm Retrieval in a Hypogonadal Patient with Non-Obstructive Azoospermia Showing Normal Serum 17-Hydroxyprogesterone Levels Suggestive of Normal Intratesticular Testosterone Production: A Case Report

Ettore Caroppo [1,*] and Giovanni M. Colpi [2]




**MicroTESE offers the advantage of identifying, by an operative microscope, the seminiferous tubules with still normal calibre, which are more likely to have a complete spermatogenesis.**

**Their selective excision usually results in positive sperm retrieval and minimizes testicular tissue damage and risk of surgical complications.**

**Localization of Sperm During Microdissection Testicular Sperm Extraction in Men with Nonobstructive Azoospermia**

Ranjith Ramasamy, Jennifer E. Reifsnyder, Jad Husseini, Pierre-Alexandre Eid, Campbell Bryson, and Peter N. Schlegel J Urol. 189: 643, 2013

**MicroTESE, thanks to its extensive exploration of testis parenchyma, can increase the sperm retrieval rate in NOA. Ramasamy et al.** (2014) report data by a **«bivalve testis opening»** on their positive sperm retrievals: 65% from the superficial parenchyma plus additional 35% from the deeper explored tissue when needed. **Deep microdissection resulted significantly more effective in case of SCO, which represents 54.9% of their NOA** cases (p=0.0002).

**Colpi et al.** (2023), applying a **MicroTESE surgical variant** and considering as **«positive a retrieval good enough for at least one ICSI cycle»**, showed that a positive retrieval was obtained from the superficial vs. deep tissue in 26.4% vs. 73.6% of testes, respectively.

In SCO testes, from deep tissue in 82.9% of cases.

**SCO cases represented 65.7% of our NOA series.**

**Our data indirectly support the superiority of MicroTESE vs conventional TESE, in which the tissue fragment is ≤ 5 mm. thick.**

**A complete dissection of the whole testicular parenchyma is required in most patients with nonobstructive azoospermia to obtain enough good quality testicular spermatozoa for ICSI**

Ettore Caroppo[1] | Fabrizio Castiglioni[2] | Franco Nerva[3] | Elisabetta Maria Colpi[2] | Giacomo Gazzano[4] | Giovanni Maria Colpi[2] Andrology. 11:508, 2023

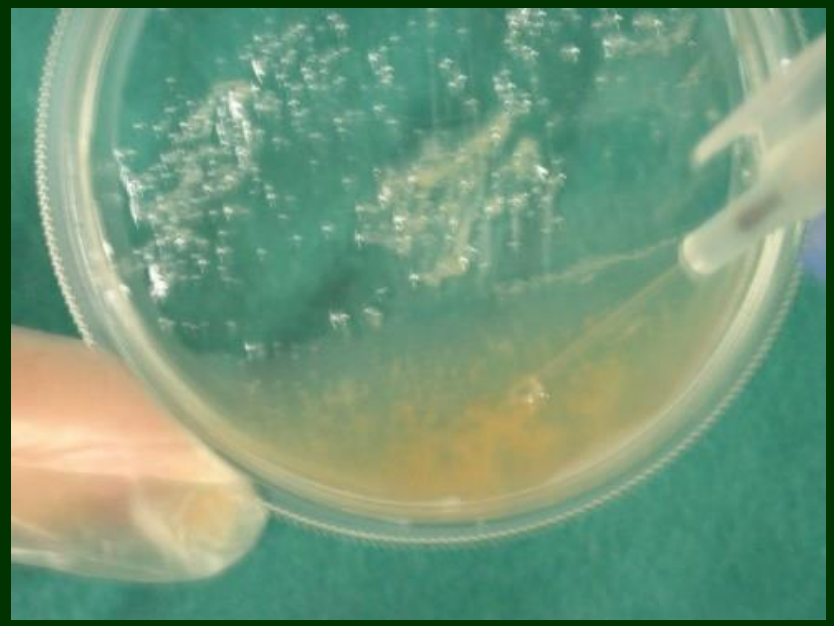
- The surgical procedure is time-consuming, even in experienced hands.

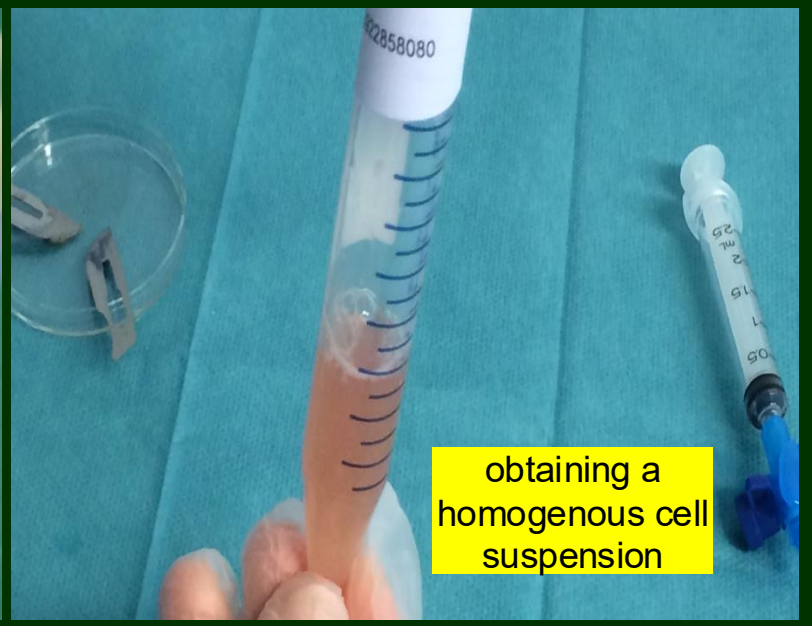
- For this reason, mTESE is usually scheduled on a day convenient for the team, and in case of successful retrieval, spermatozoa are frozen and ICSI cycles are performed on a later date using thawed spermatozoa.

# ACQUIRING BIOLOGICAL EXPERTISE IN MICROTESE

shredding the tubules

use of angiocatheter to obtain a fine cell suspension





obtaining a homogenous cell suspension

SPERM PROCESSING AFTER SURGERY :

traditional biological steps developed by Schlegel.

"Our laboratories reported a success rate of +7% when effective mechanical preparation of the removed specimen is followed by a multihour, many-technician search for spermatozoa in an experienced laboratory"

(Ramasamy et al, 2011).

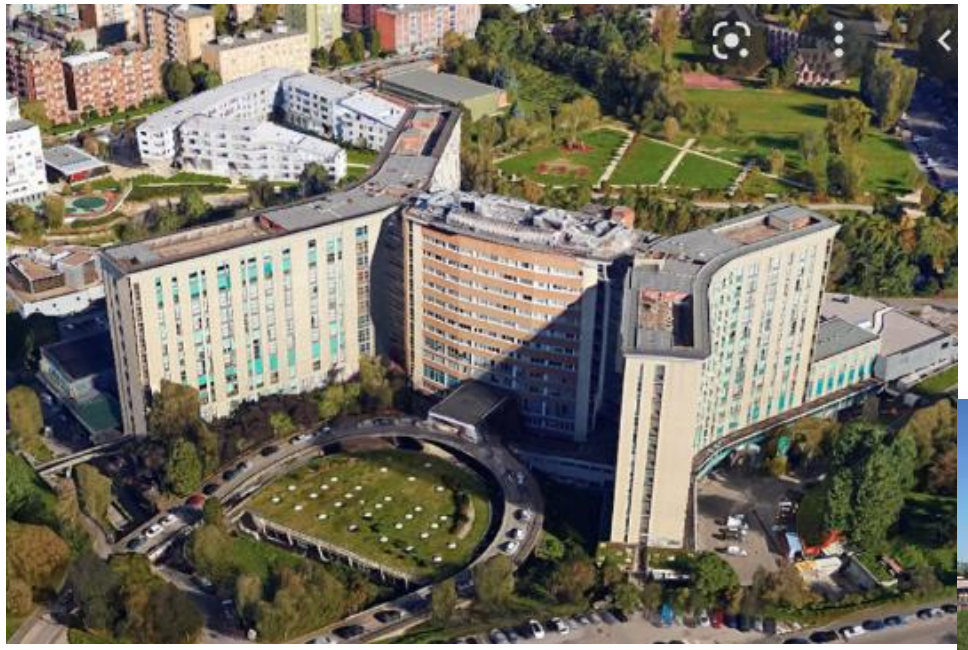
A biological sperm search lasting from ≥ 1 hour to 4 hours was able to increase SRR ($p<0.01$).

(Piediferro G et al: Giorn It Androl.1:157, 2004; Colpi GM et al.: 6th Congress of the Hellenic Andrologic Society, Thessaloniki, November 5-6, 2004)

# EVOLUTION OF MICROTESE TECHNIQUE IN OUR SCHOOL

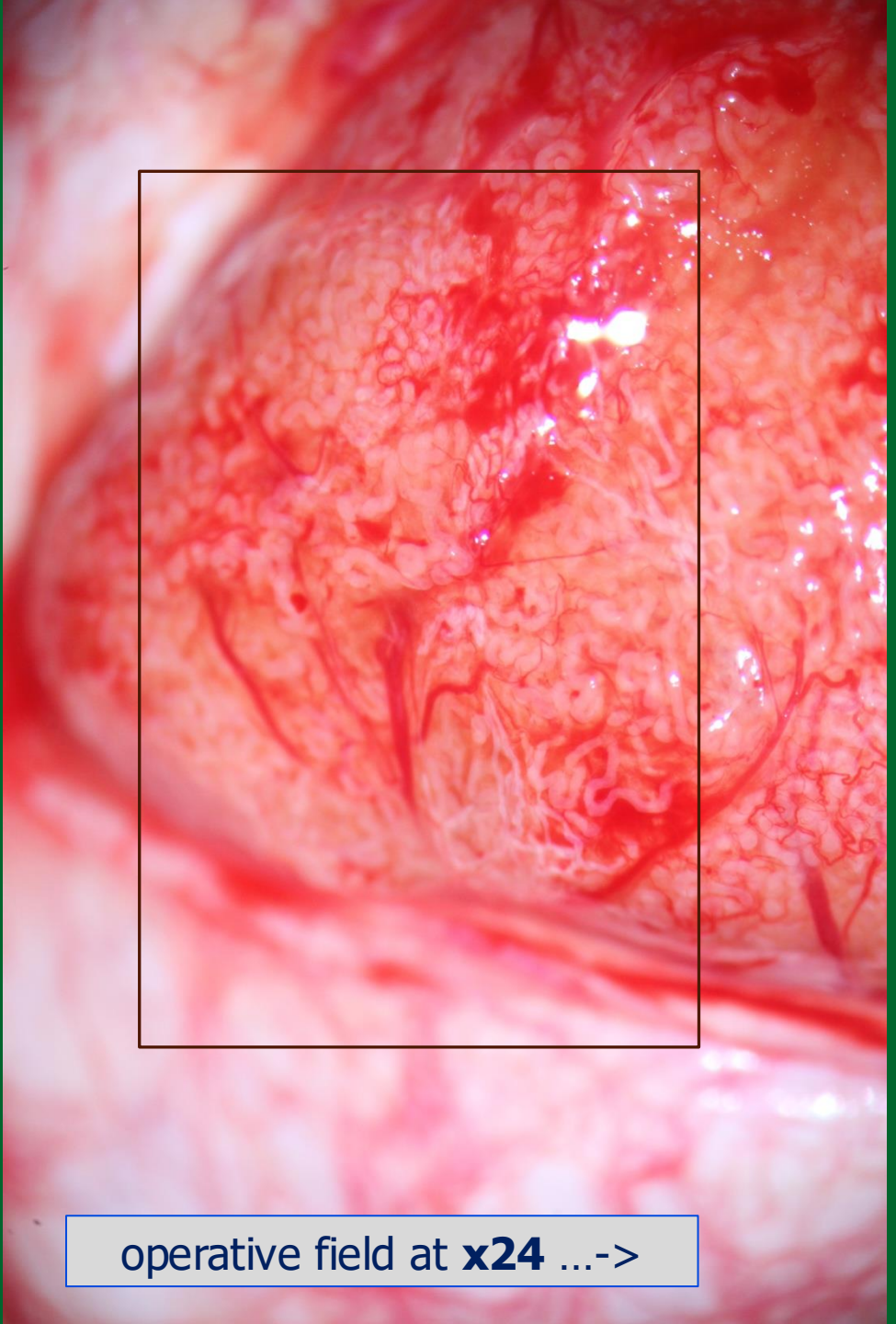
( > 2200 MicroTESE performed by the same urologist)

San Paolo Hospital – University of Milan, Milan
1998-2013

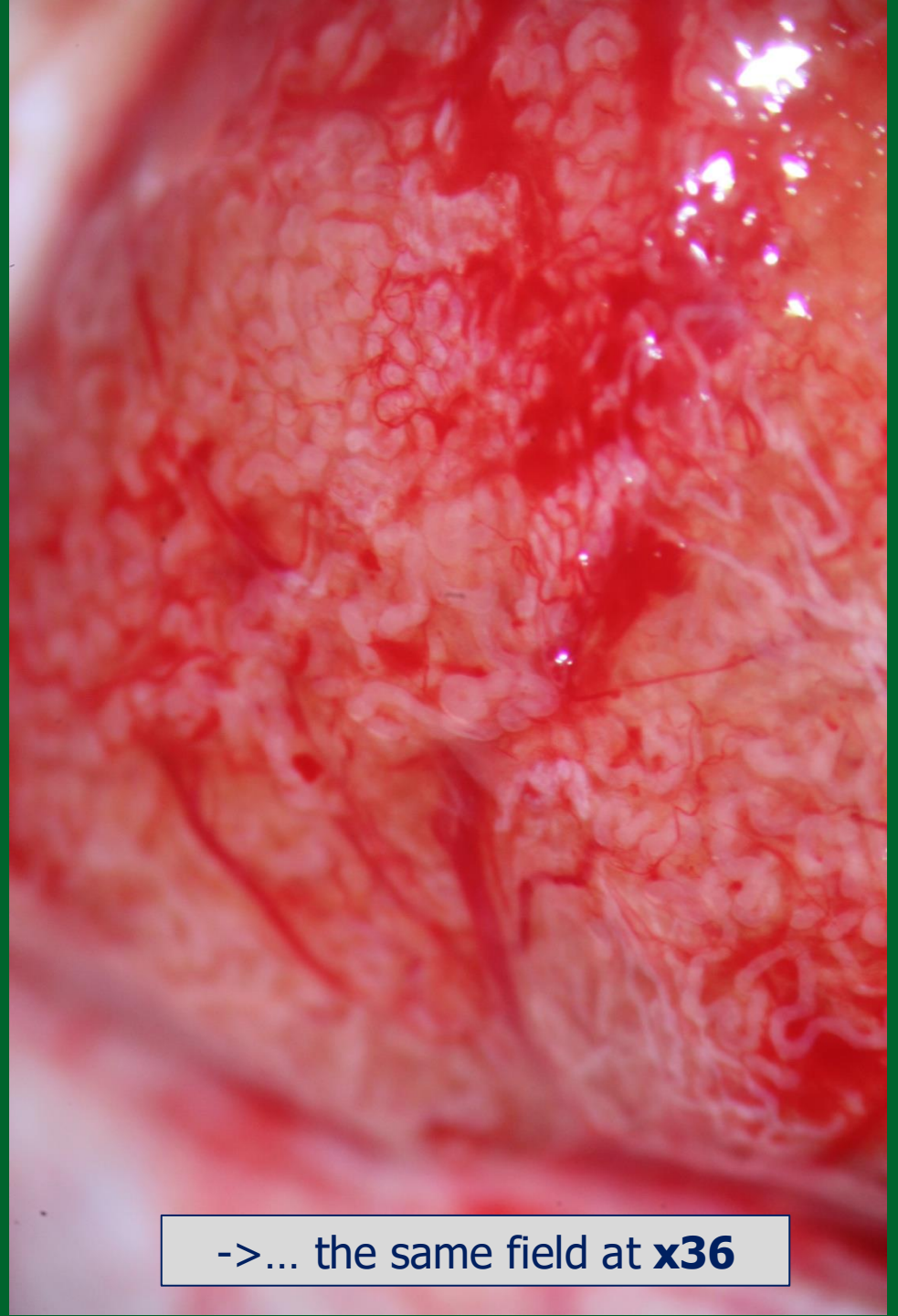


San Carlo Clinic, Paderno Dugnano / Milan
2014-2019

Next Fertility Procrea, Lugano
2013-2025



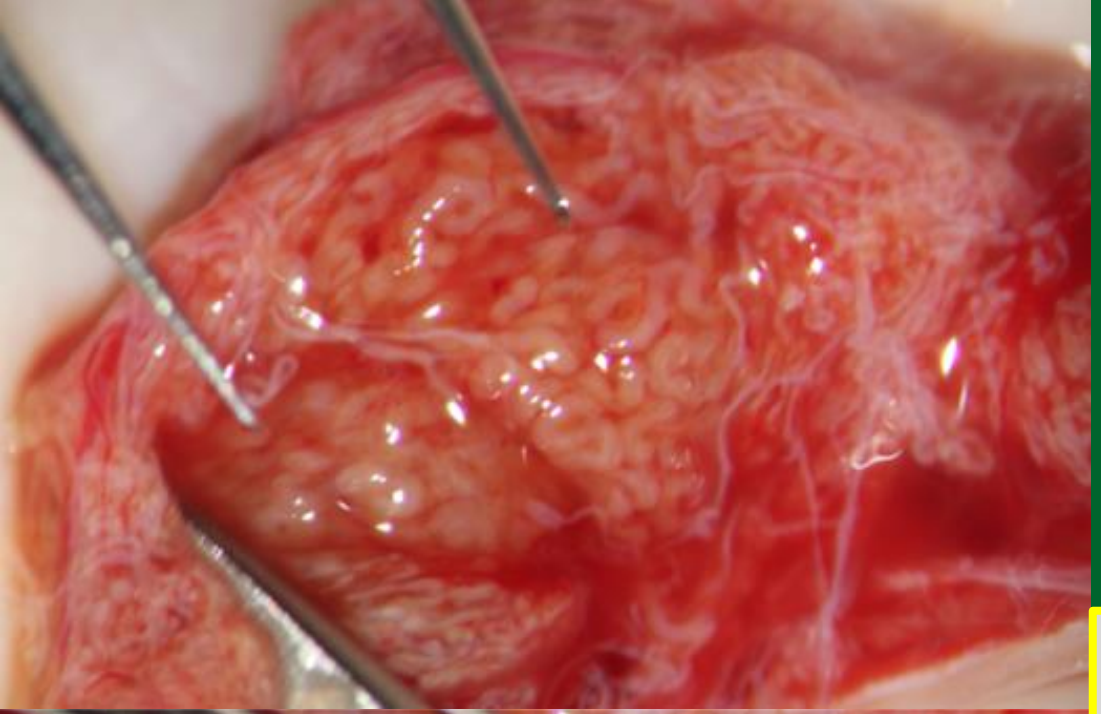
operative field at **x24** …->

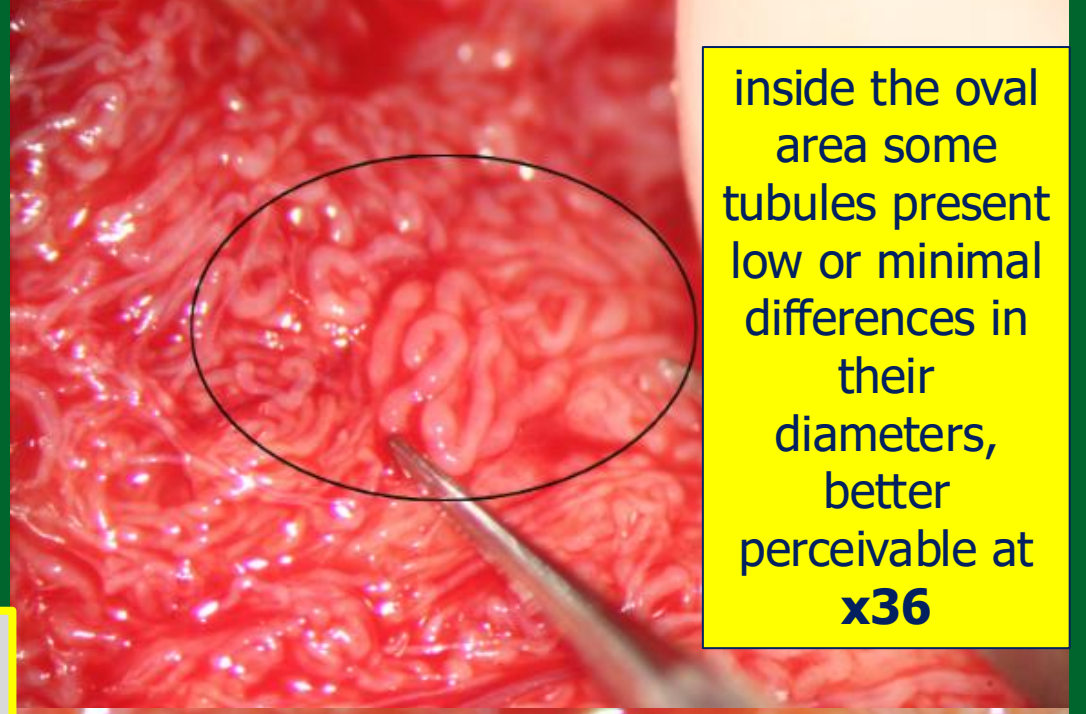


->… the same field at **x36**

## PERSONAL VARIANTS

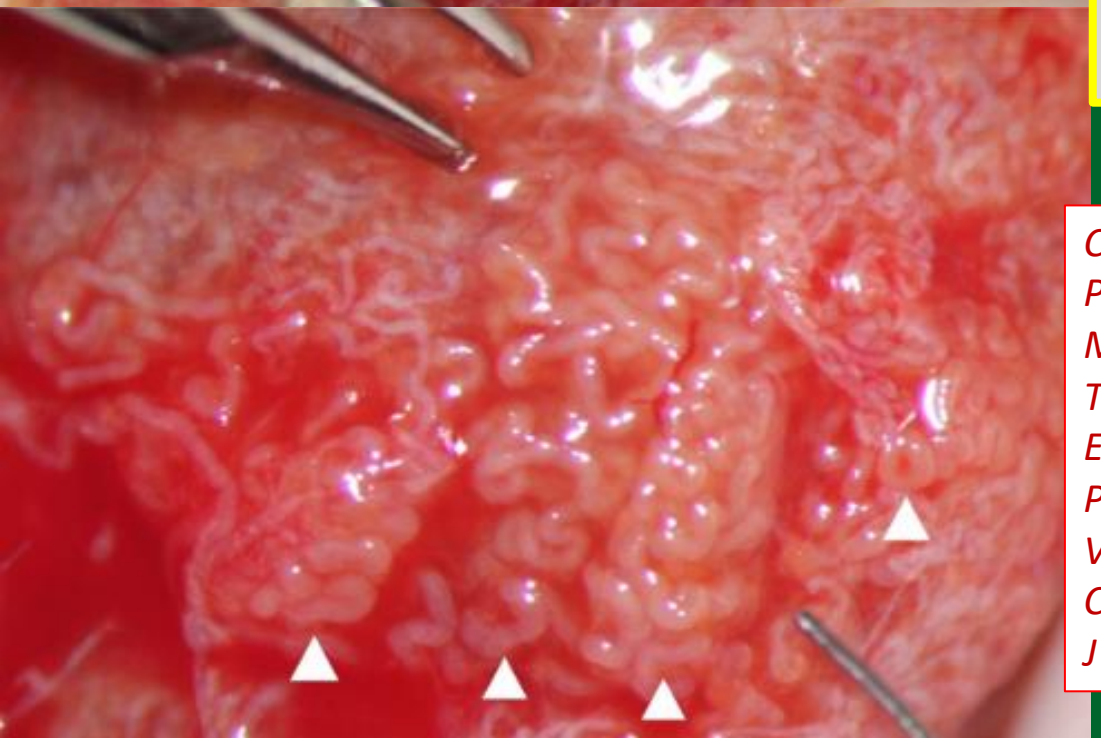
(2016) Operative high-magnification was increased from x24 to x36, helping discriminate between tubules that, at a lower magnification, may appear of comparable size.

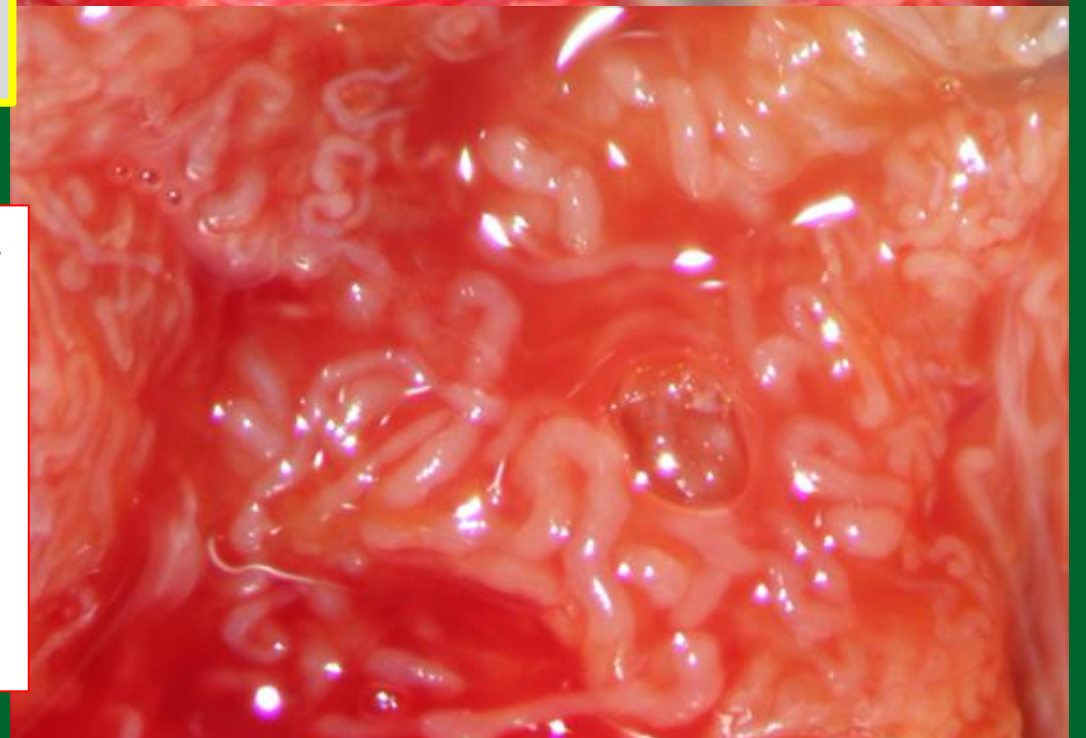




inside the oval area some tubules present low or minimal differences in their diameters, better perceivable at **x36**

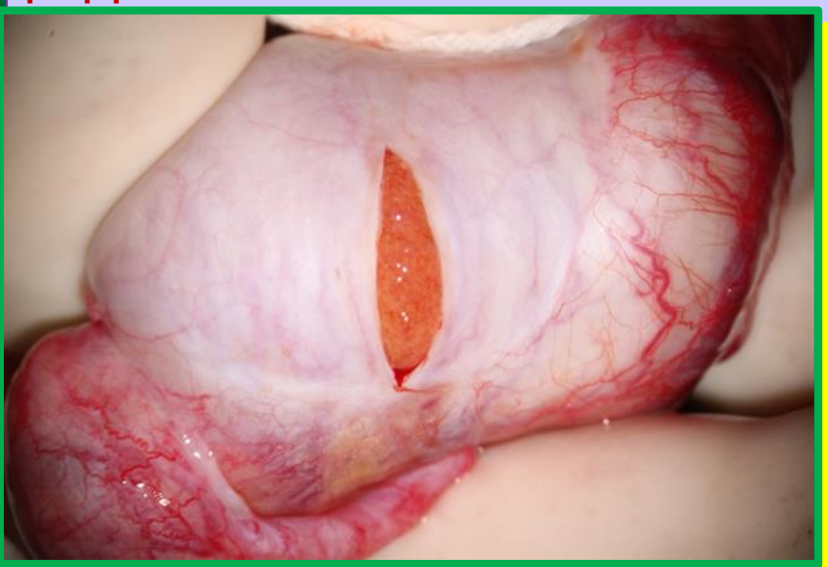
original photographs at **x36**

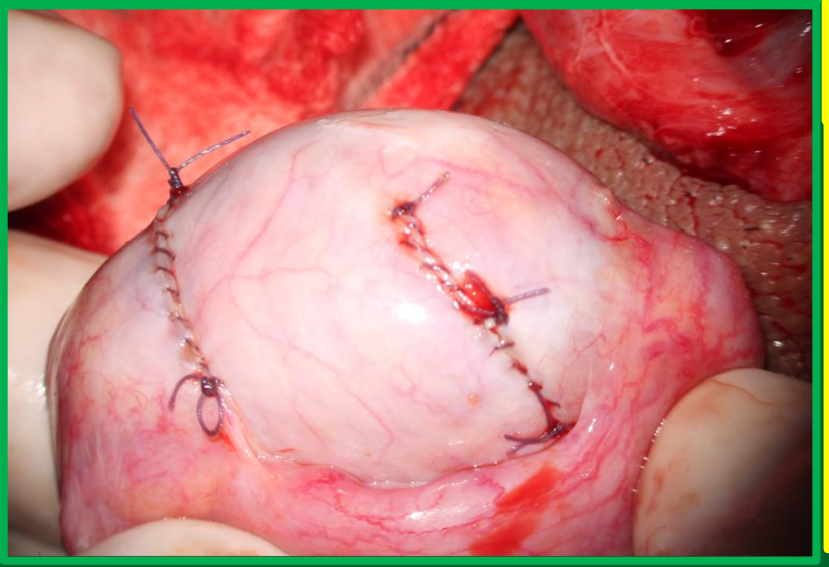


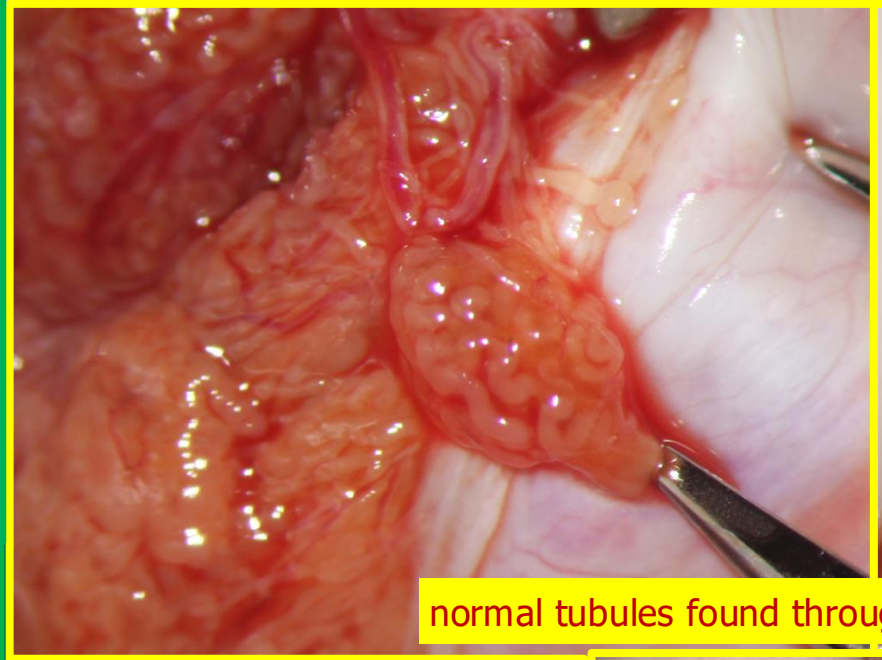


*Colpi GM & Caroppo E. Performing Microdissection Testicular Sperm Extraction: Surgical Pearls from a High-Volume Infertility Center. J Clin Med. 2021*

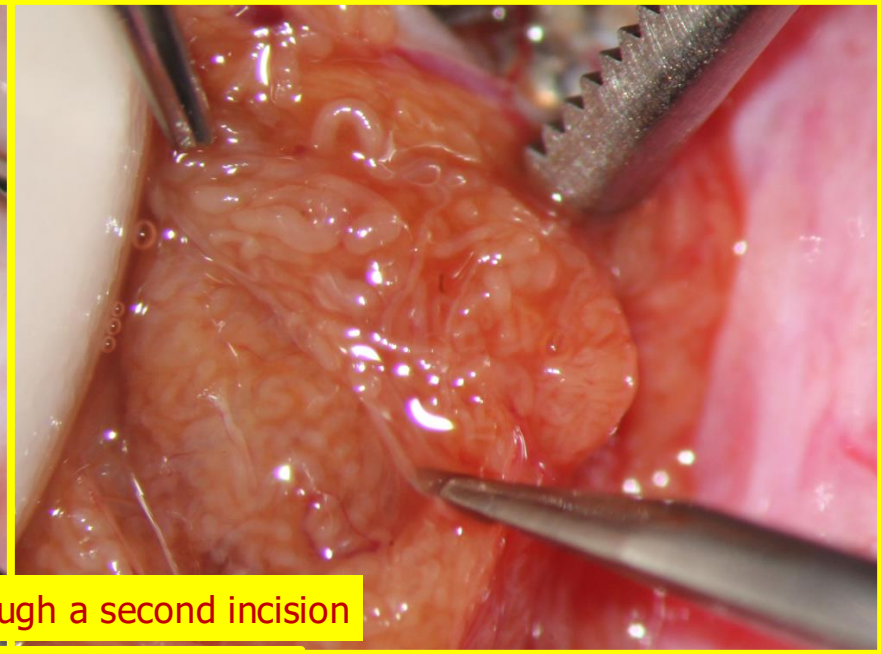
**(2018) More SAFETY and EFFECTIVENESS :** The equatorial 180-270° incision can be abandoned due to its sometime difficult closure and replaced by a stepwise incision of 1.5 cm, possibly extendable if necessary. If needed, a second (and third) stepwise incision can be performed to explore the parenchyma more extensively and in depth, far from the first.

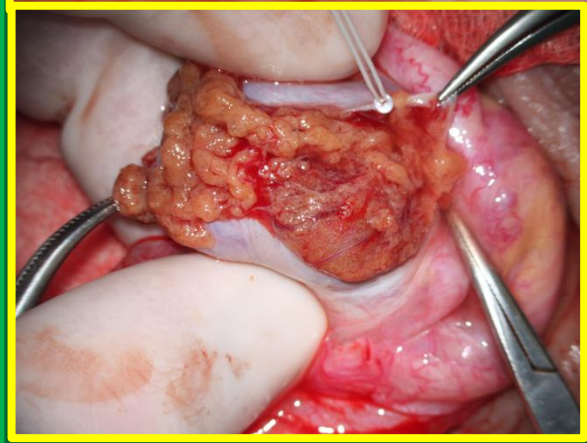


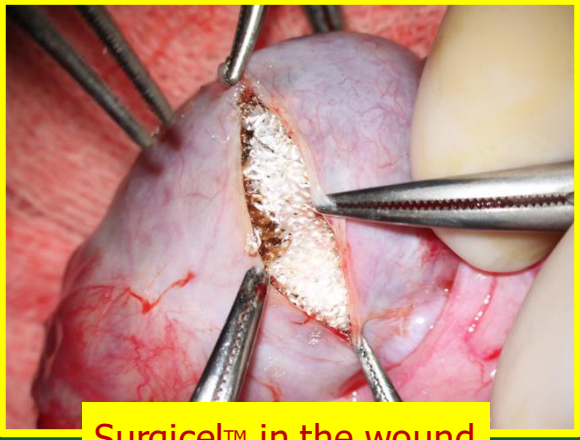




normal tubules found through a second incision



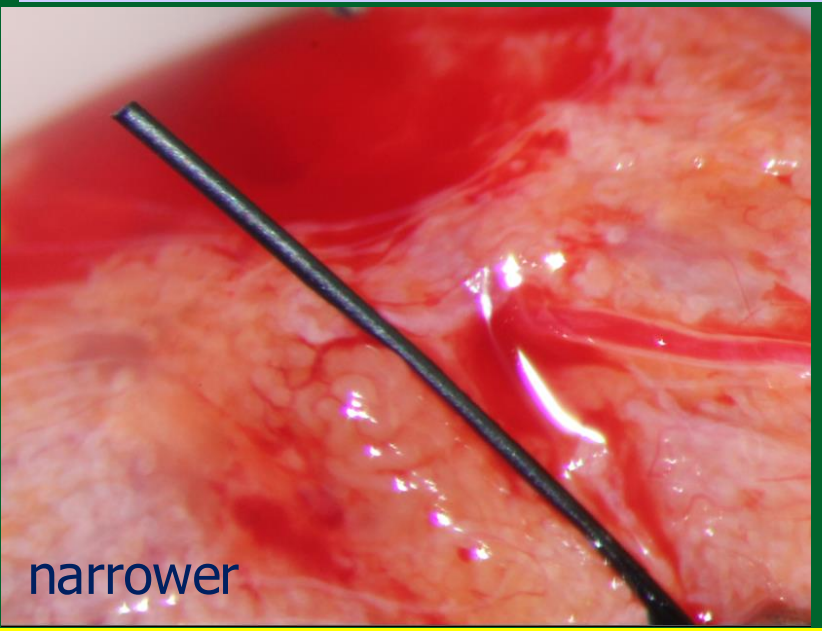


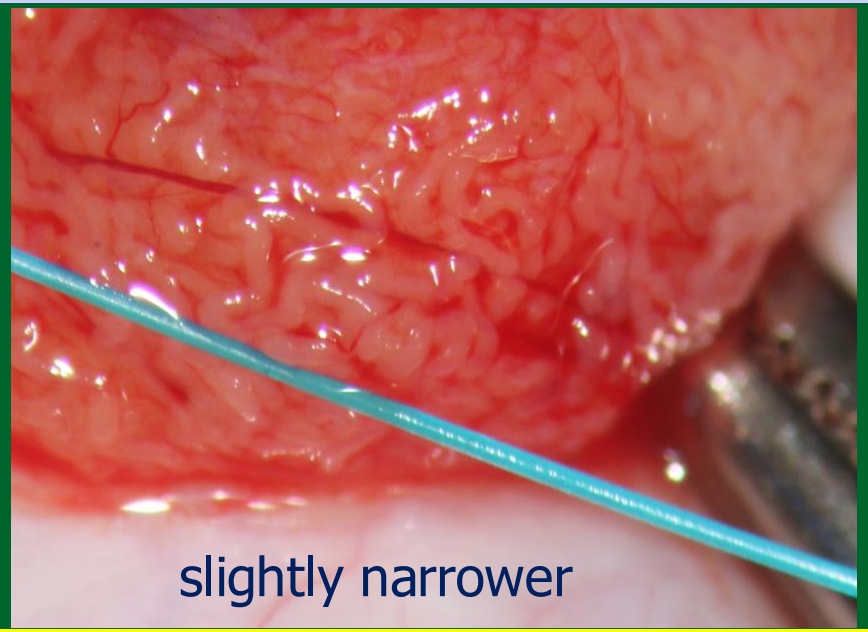


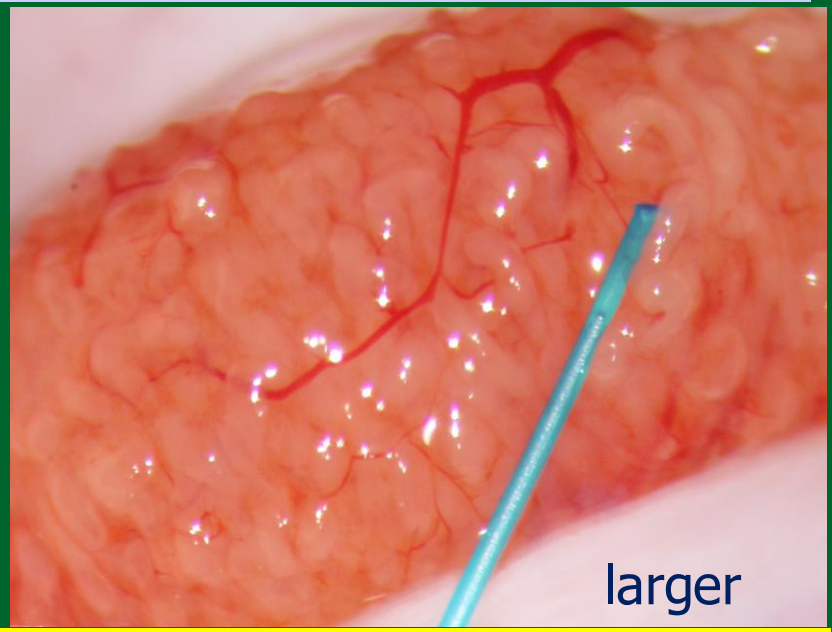
Surgicel™ in the wound

# STEPWISE MICROTESE

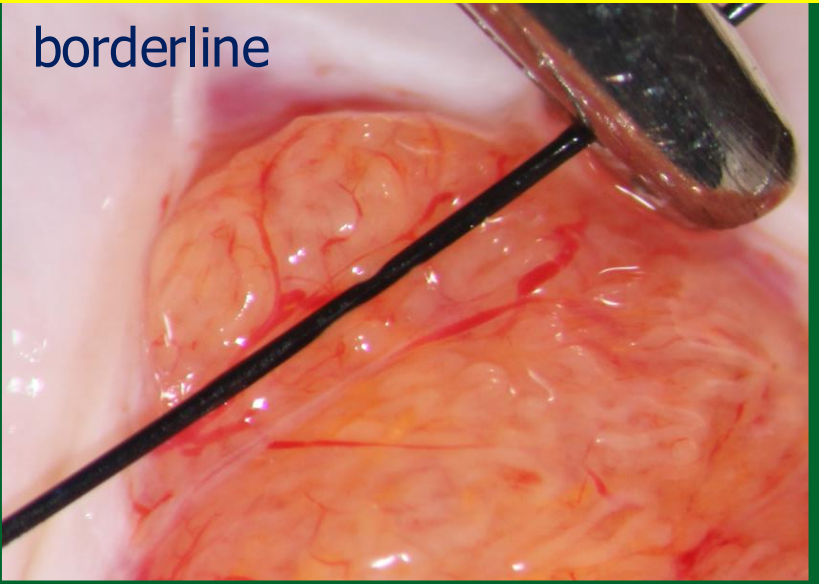
(2019) Our studies on the caliber of the tubules in the different histologic patterns has led us to use a **caliper** to identify more easily those tubules with a higher probability of containing sperm.

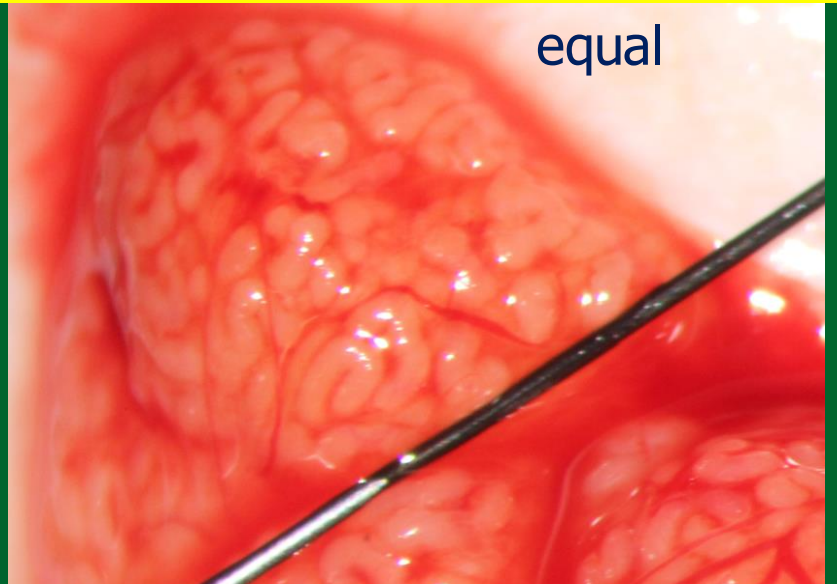


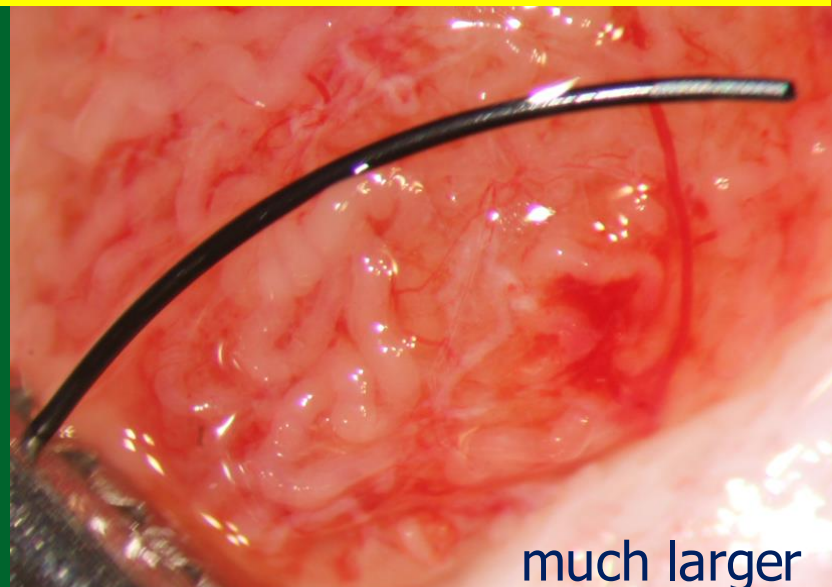




Caliper is an objective method to estimate the real caliber of the "better" tubules in the operative field.



# Data presented at NAFA Annual Meeting, Aarhus, 11-12 September 2009

## CLINICAL COMPLICATIONS in our experience on the first 360 cases

| COMPLICATIONS | |
|---|---|
| Haematoma * (US after 3 and 6 months) | 0 |
| Local infection § | 0 |
| Chronic post-operative testicular pain # | 0 |
| «Acute» post-operative testicular pain # | Mild and very rare |

### TIPS and TRICKS

- * Hemostasis is mainly performed at normalized blood pressure by a gentle compression of testicular pulp with gauze followed by very limited and careful bipolar microcoagulation.
- § Just after concluding the microsurgical retrieval, the surgical field was washed by gentamycine solution (80 mg/100 ml).
- # In both procedures, while closing the tunica vaginalis, bethametasone (1.5 mg) was instilled into the vaginalis cavity to prevent pain and adhesions.

# IMPROVING BIOLOGICAL EXPERTISE IN HANDLING MICROTESE

A Panorama of the Biological Procedures to Improve Quantity and Quality of Sperm Retrievals

## Processing and Selection of Surgically Retrieved Sperm for ICSI

- Mechanical Processing of Testicular Tissue, by shredding, mincing [1,2,3], tubule squeezing [4,5], then swim-up, or immediate or delayed [6] density gradient centrifugation method [7,8]
- Use of Erythrocyte-Lysing Buffer [9,10,11,12]
- Enzymatic Digestion by Collagenase AI earlier [13], Collagenase IV later [14], plus addition of DNAase [14]
- Motility Enhancers, like Pentoxifylline [15,16], Theophylline [17], used both for post-freezing and fresh sperm
- Use of sperm after short-term culture or for simultaneous ICSI.

  [Karacan et al (2013), comparing ICSI outcomes using testicular sperm freshly obtained on the day of or the day before oocyte retrieval or after a freeze/thaw cycle, demonstrated that in the presence of motile sperm, neither the timing of TESE nor the use of post-freezing sperm affected ICSI results].

  Warning: culturing of testicular sperm for up to 24 h increases DNA fragmentation [18]
- Motile Sperm Identification with HOST [19]
- Sperm Tail Flexibility Test to Check Sperm Viability [20]

1) Silber et al, 1995; 2) Edirisinghe et al, 1996; 3) Liu et al, 1997; 4) Allan & Cotsman, 1997; 5) Papal & Nagy, 2013; 6) Crabbé et al, 1999; 7) Pousette et al, 1986; 8) Verheyen et al, 1995; 9) Verheyen et al, 1997; 10) Soygur et al, 2018; 11) Tanaka et al, 2015; 12) Goswami et al, 2015; 13) Salzbrunn et al, 1996; 14) Crabbé et al, 1997; 15) Mangoli et al, 2011; 16) Kovacic et al, 2006; 17) Ebner et al, 2011; 18) Dalzell et al, 2004; 19) Jeyendran et al, 1984; 20) Soares et al, 2003.

**Review** from: Aydos & Aydos: Sperm Selection Procedures for Optimizing the Outcome of ICSI in Patients with NOA. J Clin Med. 2021:Jun 18;10(12):2687.

# IMPROVING MICROSURGICAL EXPERTISE IN MICROTESE (1999 -> today)

(no prognostic previous selection of NOA patients)

**Table 1.** Comparison of the mTESE outcome performed by the same urologist (GMC) in two cohorts of patients with NOA.

| | San Paolo Cohort | Procrea Cohort |
|---|---|---|
| Years | 2004–2009 | 2015–2017 |
| Number of patients | 202 | 143 |
| Overall sperm retrieval rate (SRR) | 80/202 (39.6%) | 79/143 (55.2%) |
| SRR per testis histology subcategories | | |
| Sertoli cells only syndrome | 28/125 (22.4%) | 45/143 (31.5%) |
| Maturation arrest | 6/16 (37.5%) | 11/29 (37.9%) |
| Hypospermatogenesis | 20/26 (76.9%) | 27/28 (96.4%) |
| Focal Sertoli cells only syndrome | 26/35 (74.2%) | 9/9 (100%) |
| Hyalinosis | / | 2/9 (22.2%) |
| Intraepithelial neoplasia | / | 1/2 (50%) |

*Colpi GM et al. Performing Microdissection Testicular Sperm Extraction: Surgical Pearls from a High-Volume Infertility Center. J Clin Med. 2021*

**Procrea Cohort**

2023-Sept 14, 2025

183

104/183 (56.83%)

**Naïve vs Salvage**

Naïve 76/136 (55.88%)

Salvage 28/47 (59.57%)

**Mean Operative Time on 100 consecutive Microtese**

| | |
|---|---|
| monolateral | 1h 17 min |
| bilateral | 1h 54 min |

(Colpi, 2024)

# IMPROVING BIOLOGICAL EXPERTISE IN MICROTESE (2021*->today)

## Our Biological Classification of Sperm Retrievals

- **Poor:** 1/10 µl - 10/10 µl *
- **Moderate:** 10/10 µl - 100/µl
- **Good:** 100/µl - 500/µl
- **Excellent:** > 500/µl

* *Note: in these case sperm concentration does not correspond exactly to their total number, since when the sperm cannot be counted in a counting chamber the final sample volume is brought to 0.3 ml. (except in cases of maturation arrest).*
*In addition, a semi-quantitative estimate is also carried out by calculating the average number of spermatozoa per drop of 10 µl.*

Total volume after centrifugation: 0.3 ml

* Following a first methodological improvement suggested by prof. A. Argyriou

# FROZEN/THAWED SPERM QUALITY FOR ICSI

- **A meta-analysis** (Ohlander et al, 2014) **on only NOA patients comparing fresh vs frozen sperm showed that both FRs** (52.9% vs 54.0%) **and CPR** (28.7% and 28.1%) **were similar;** data confirmed by a subsequent meta-analysis (Yu et al, 2018) on 1261 ICSI cycles .
- However, the cryopreservation methods vary among the laboratories (Karacan, 2013; Schachter-Safrai, 2017), and cryopreservation/thawing can induce sperm damage *
- **A potential risk to be considered is testicular sperm loss after freezing and thawing, as survival of frozen–thawed samples is not uniform in all Centres: (20–90%)** (Chen et al, 2019), **(33%)** (Flannigan et al, 2017).
- The percentage of sperm usable for ICSI from NOA patients is higher if fresh (85.7%) versus cryopreserved/thawed sperm (52.7%) **(-38.5%)**. (Aust NZJ Guidelines 2025)

Chen X et al. (2019) observed that:

- Sperm of poor quality is eliminated by freezing-thaw procedure.
- When too few sperms are acquired, they may be lost during the thawing procedure.
- **Using fresh sperm for ICSI would be an appropriate choice in such condition.**

**Table 2: Advantages and disadvantages of the use of fresh or frozen testicular sperm for men with nonobstructive azoospermia**[79,87,92,93]

| *Category* | *Fresh sperm* | | *Frozen sperm* | |
|---|---|---|---|---|
| | *Advantage* | *Disadvantage* | *Advantage* | *Disadvantage* |
| Availability at the time of ICSI procedure | Immediate use possible; suitable for rare, delicate sperm | Risk of not finding viable sperm at the time of retrieval; potential for cancelled cycles | Ensures availability before ICSI; acts as a backup if fresh retrieval fails | Potential damage during freezing–thawing process |
| Sperm quality | Avoids freezing–thawing damage, generally higher motility and viability, no fear of losing motility | Limited by sperm quality at retrieval | Allows pre-evaluation of sperm quality; allows embryologists to identify viable sperm | Freezing can reduce motility and viability |
| Procedure timing | Immediate ICSI if sperm is found, suitable for synchronized cycles | Scheduling challenges if coordination is needed | Allows better scheduling and planning; avoids scheduling conflicts (*e.g.*, operating room and urologist availability) | Requires precise planning for cryopreservation and thawing |
| Stress and logistics | Reduces need for multiple procedures; less stress if sperm is found and used immediately | Stress and uncertainty if no viable sperm is found (even if female partner has been prepared/stimulated) | Confirms sperm availability, reducing stress; practical for busy centers; avoids the male partner having surgery on the same day as oocyte retrieval, avoiding the challenge of postcare for both partners | Additional steps for freezing, storage, and thawing |
| Cost and efficiency | Lower costs if successful on first attempt | Financial loss if no viable sperm is found, leading to cancelled procedures | Avoids multiple retrievals, practical for busy IVF centers | Costs associated with freezing, storage, and potential additional procedures |
| Clinical outcomes | Often better in cases with high motility and viability; higher reported motility and viability | Variability in clinical outcomes; risk of no viable sperm | Provides a backup in case fresh retrieval fails, comparable pregnancy rates in many cases; avoids postmaturity oocyte damage | Some studies report lower pregnancy rates and higher miscarriage rates |

Mantravadi et al, 2025

# FRESH or FROZEN/THAWED MICROTESE/ICSI?

Although multiple studies and meta-analyses report no significant differences in FR, CPR or LBR between fresh and cryopreserved testicular sperm in NOA, the question remains clinically relevant for both patients and professionals involved in the ICSI process (Amer et al, 2021).

Several considerations contribute to this debate. The number of sperm retrieved after MicroTESE may be insufficient for standard cryopreservation, and in such cases, specific freezing protocols have been developed (Mangoli et al, 2025).
However, there are no reports on the long-term efficacy of such methods, and these protocols may not be available in all ICSI settings.

Moreover, using fresh sperm avoids the risk of complete post-thaw sperm loss, reported at ~6% to 9% of patients in a series of very low/rare sperm, which is particularly critical in NOA, where often only a very low number of sperm can be retrieved (Huang et al, 2022; Kathrins et al, 2017).

Therefore, proper counselling of the couple should be done before choosing to use fresh or cryopreserved sperm.

Ryzkhov et al, 2025

# FROZEN/THAWED SPERM QUALITY FOR ICSI

No significant differences were found in obstetrical outcomes when comparing ET cycles using fresh or frozen thawed testicular sperm retrieved from microTESE (Nagawkar Perlov et al, 2024).

No differences were observed with fertilization or quality embryo rates between fresh and frozen sperm from Microtese for ICSI. However, the rates of clinical pregnancy and live birth were both higher for fresh than frozen sperm (Zhang et al, 2021).

Frozen-thawed samples have a lower usability rate at 45.3% (vs 67.7%), reflecting a substantial loss in quality postthaw (Sawaid Kaiyal et al, 2024). Additionally, frozen sperm generally exhibits higher DNA fragmentation compared to fresh sperm (Asanaid et al, 2021).

Poor-quality sperm samples may be more susceptible to damage during the freezing process, leading to lower survival rates after thawing. Cryopreservation significantly decreases sperm motility, viability, and morphology. (Stanic et al, 2000; Ozkavukcu et al, 2008; Nur Karakus et al, 2021).

Ozkavukcu et al. (2008) found a strong link between reduced motility and decreased viability, and cryopreservation-induced DNA damage suggests correlations with reduced FR, increased pregnancy loss, and other negative reproductive outcomes (Li et al, 2006; Simon et al, 2011; Rappa et al, 2016).

In such cases, a fresh micro-TESE may be necessary to obtain enough sperm for ICSI.

Mantravadi et al, 2025

# Our Centre's data

Next Fertility ProCrea

## mTESE : fresh sperm for ICSI cycles

Update: January 31st 2026

| | |
|---|---|
| N° oocyte pick-up: | 145 (127 couples) |
| Woman age (X,M,r): | 33.42, 33, 21-45 yrs. |
| N° oocytes in metaphase II: | 1298 |
| N° oocytes fertilized: | 548 |
| **Fertilization rate:** | **42.2%** |
| N° blastocysts: | 258 |
| **Blastulation rate:** | **47.1%** |
| N° single blastocyst transfers: | 100 |
| N° clinical pregnancies: | 44 |
| **Clinical pregnancy rate/transfer:** | **44.0%** |
| N° miscarriages: | 7 |
| N° live births: | 20 + 17 ongoing |
| **Live birth rate/transfer:** | **~34.3%** |

(11 cycles: 0 fertiliz.)
(29 cycles: 0 blasto.)

(1.78/cycle; M 1, r 0-7)

From 115/145 (79.3%) patients the sperm surplus could be cryopreserved

Remaining: 158 blastocysts cryopreserved; per Couple: X 1.24; M 1; r 0-7

Man age (X,M,r): 37.12 37, 27-58 yrs.

**Present** Quality of Sperm Retrieval (# fresh spz)

| | |
|---|---|
| Poor (<10spz/10µl): | 82 ← 56.5% |
| Moderate/Good (>10spz/10µl – 500spz/µl): | 47 |
| Excellent (>500spz/µl): | 16 |

### Testis Histology (142+3 ongoing)

| | |
|---|---|
| complete Hyalinosis: | 9 |
| incomplete Hyalinosis: | 5 |
| complete SCO: | 48 |
| incomplete SCO: | 12 |
| complete Maturation Arrest: | 7 |
| incomplete Maturation Arrest: | 16 |
| Hypospermatogenesis: | 38 |
| Mixed patterns: | 6 |
| Hyperplasia Leydig cells | 1 |

**Poor Retrievals only**:
38 single blastocyst transfers
15 clinical pregnancies
9 live births + 6 ongoing
CPR/transfer ~ **39.47%**

Remaining: 497 sperm cryopreserved vials per Patient : X 4.32; M 3; r 0-13

# Our Centre's data

Next Fertility ProCrea

## mTESE : frozen sperm for ICSI cycles

From 01.09.2021
Update: January 31st 2026

| | |
|---|---|
| N° oocyte pick-up: | 101 (64 couples) |
| Woman age (X,M,r): | 36.29, 35, 22-43 yrs |
| N° oocytes in metaphase II: | 731 |
| N° oocytes fertilized: | 278 |
| **Fertilization rate:** | **38.00%** |
| N° blastocysts: | 103 (1.01cycle; M 1; r 1-6) |
| **Blastocyst rate:** | **37.05%** |
| N° single blastocyst transfers: | 96 |
| N° clinical pregnancies: | 36 |
| **Clinical pregnancy rate/transfer:** | **33.3%** |
| N° miscarriages: | 9 |
| N° live births: | 16 + 11 ongoing |
| **Live birth rate/transfer:** | **~26.04%** |

(11 cycles: 0 fertiliz.)
(30 cycles: 0 blasto.)

Man age (X,M,r): 38.18, 38, 27-63 yrs.

Previous Quality of Sperm Retrieval (# fresh spz)

| | |
|---|---|
| Poor (<10spz/10µl): | 34 |
| Moderate/good (>10spz/10µl – 1000spz/µl): | 47 |
| Excellent (>1000spz/µl): | 20 |

66.3%

**Testis Histology** (101)

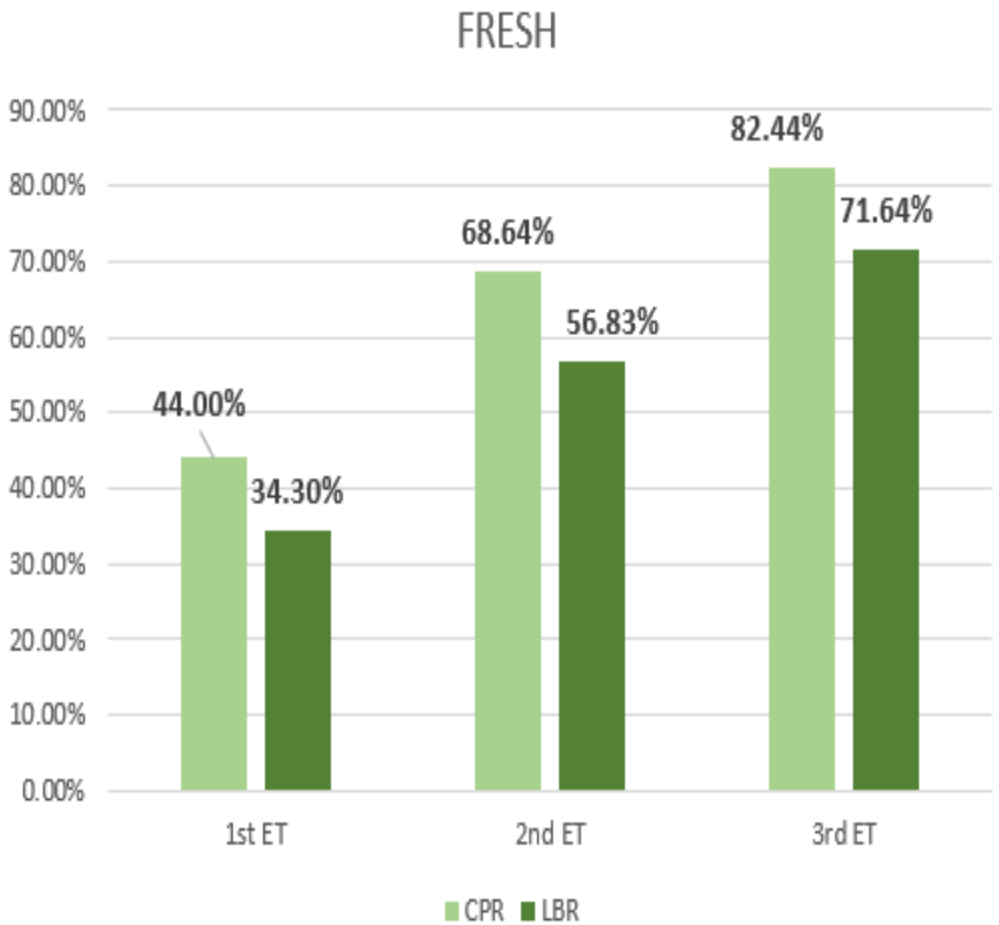
| | |
|---|---|
| complete Hyalinosis: | 4 |
| incomplete Hyalinosis: | 1 |
| complete SCO: | 26 |
| incomplete SCO: | 11 |
| complete Maturation Arrest: | 2 |
| incomplete Maturation Arrest: | 7 |
| Hypospermatogenesis: | 33 |
| Mixed patterns: | 10 |
| not available: | 7 |

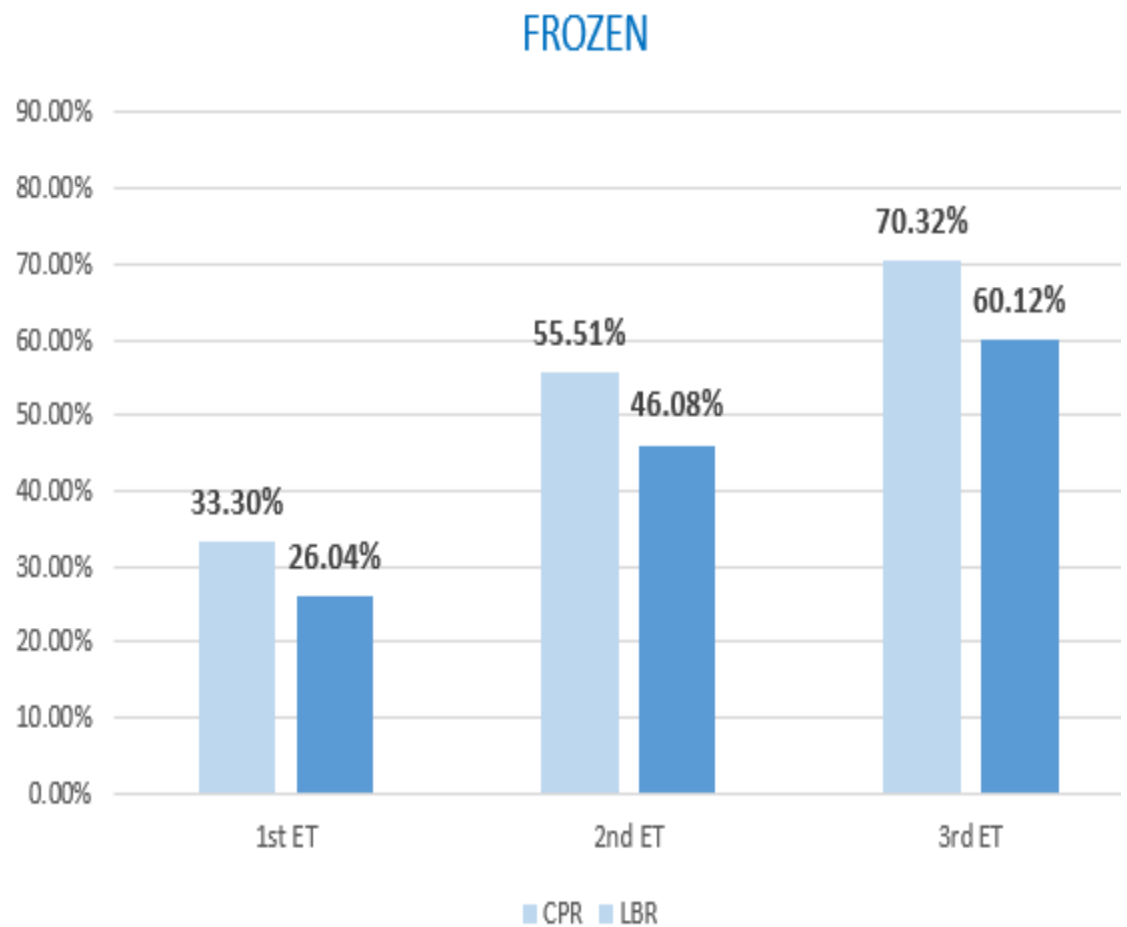
42.6%

79 blastocysts cryopreserved; per Couple: X 1.23; M 1; r 0-6

Remaining: 400 sperm cryopreserved vials; per Patient: X 6.25; M 6; r. 0-13

Micro-TESE CUMULATIVE **EVOLUTIVE PREGNANCY (FHB) RATES** AND **LIFE BIRTH RATES** THEORETICALLY EXPECTED AFTER SERIAL BLASTOCYST TRANSFERS



Next Fertility
ProCrea

ANDROLOGY & I.V.F. CENTER

*Medical Director*
**Dr.med. Marina Bellavia**

*Scientific Director*
**Dr.med. Giovanni M. Colpi**

# THANKS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# FRESH or FROZEN/THAWED MICROTESE/ICSI?

Therefore:

Should we use fresh or frozen spermatozoa from mTESE for ICSI cycles in NOA patients?

- The literature remains poor and controversial on the ICSI outcomes
- Studies are often conducted on a small population
- Not always the same outcomes are measured and compared
- Important parameters in the evaluation of the outcomes are often missing (day of embryo transfer, maternal age/factors, etc)

# «CURIOUS» FINDINGS IN RECENT LITERATURE

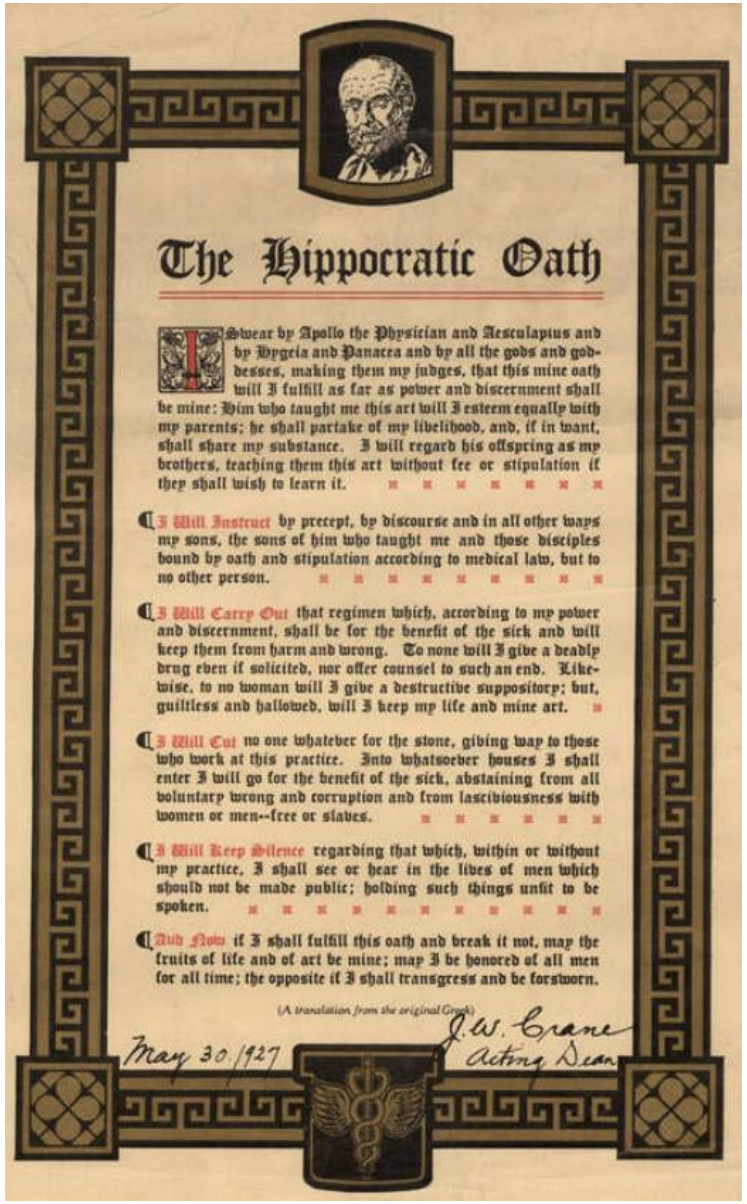
| About our topic a few recent papers report higher LBR than the previously: | | | | | | |
|---|---|---|---|---|---|---|
| AUTHORS | SSR | Fresh/Frozen | FR | CPR | LBR | note |
| Erdem et al. 2017 | 52.4% | fresh | | 27.1% | 23.7% | |
| **Corona et al. 2019** | | | | **25-32%** | **20-28%** | **SR-MA** |
| **Achermann et al. 2021** | **46.6%** | | | **39.0%** | **24.0%** | **review** |
| Lantsberbet et al. 2022 | 61.2% | | 42.4% | 40.4% | 34.0% | |
| | | | | | | |
| Chen et al. 2019 | 33.8% | fresh | 60.3% | 55.8% | | |
| « « « | « usable» | frozen | 53.9% | 50.9% | | |
| Lan et al. 2022 | 44.6% | frozen | 70.1% | 45.9% | 44.0% | |
| Gao et al. 2022 | 40.9% | frozen | 72.2% | 66.7% | 66.7% | |
| Liu et al. 2024 | 39.4% | | 77.1% | 44.2% | | |
| Xu et al. 2024 | ??? | fresh | 64.6% | 58.4% | 43.8% | |
| « « | | frozen | 57.6% | 44.4% | 36.9% | |

# Reaffirming Ethical Values

We live in an era dominated by marketing-driven healthcare, where the quantity of services matters more than patient outcomes.

Many IVF centers prioritize cost efficiency to benefit owners, often at the expense of patients.

As medical professionals, we must monitor or reject this approach.
We are not salespeople - we are doctors committed to ethical practice.



The Hippocratic Oath

I Swear by Apollo the Physician and Aesculapius and by Hygeia and Panacea and by all the gods and goddesses, making them my judges, that this mine oath will I fulfill as far as power and discernment shall be mine: Him who taught me this art will I esteem equally with my parents; he shall partake of my livelihood, and, if in want, shall share my substance. I will regard his offspring as my brothers, teaching them this art without fee or stipulation if they shall wish to learn it.

I Will Instruct by precept, by discourse and in all other ways my sons, the sons of him who taught me and those disciples bound by oath and stipulation according to medical law, but to no other person.

I Will Carry Out that regimen which, according to my power and discernment, shall be for the benefit of the sick and will keep them from harm and wrong. To none will I give a deadly drug even if solicited, nor offer counsel to such an end. Likewise, to no woman will I give a destructive suppository; but, guiltless and hallowed, will I keep my life and mine art.

I Will Cut no one whatever for the stone, giving way to those who work at this practice. Into whatsoever houses I shall enter I will go for the benefit of the sick, abstaining from all voluntary wrong and corruption and from lascivousness with women or men--free or slaves.

I Will Keep Silence regarding that which, within or without my practice, I shall see or hear in the lives of men which should not be made public; holding such things unfit to be spoken.

And Now if I shall fulfill this oath and break it not, may the fruits of life and of art be mine; may I be honored of all men for all time; the opposite if I shall transgress and be forsworn.

(A translation from the original Greek)

May 30/1927 J.W. Crane Acting Dean

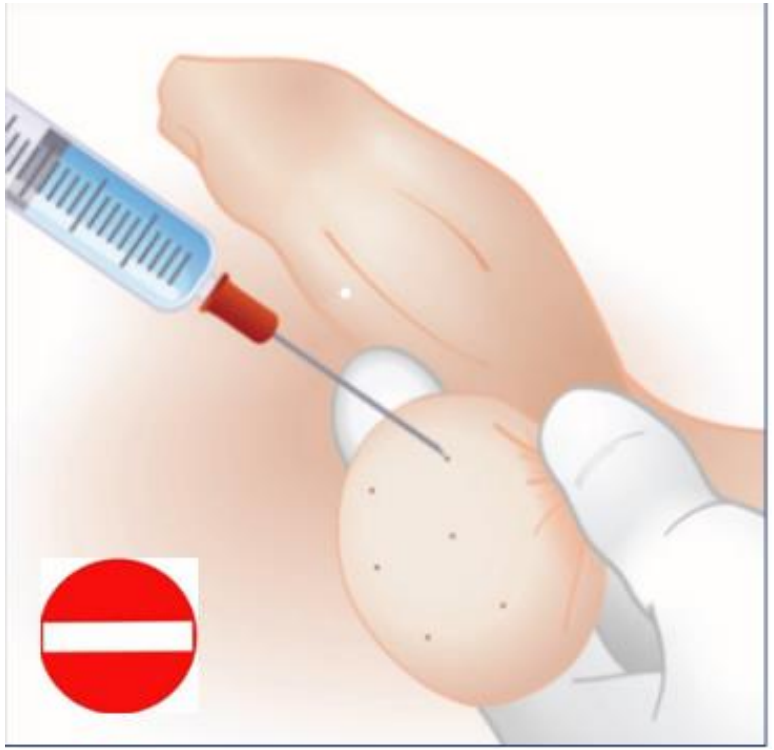
# ETHICALLY QUESTIONABLE PRACTICES : TEFNA AND TESA

In NOA, **TeFNA / TESA** is entirely inappropriate.

It is unreliable as a surgical recovery method, because sperm recovery is typically limited, including a significant risk of dead sperm after freezing and thawing (removed from the EAU Guidelines in 2008 !).

It is also unreliable as a diagnostic pre-surgery tool.

Over the years, I have witnessed many successful recoveries via Micro-TESE (mTESE) in patients previously submitted to TeFNA or TESA and diagnosed with SCOS or suspected tubular hyalinosis, leading to live births.



| | | |
|---|---|---|
| **Recommendations** | Do not perform fine needle aspiration (FNA) and testicular sperm aspiration (TESA) in patients with NOA. | Strong |

**EAU Guidelines 2025**

| | |
|---|---|
| **Summary of evidence** | Fine needle aspiration and testicular sperm aspiration (TESA) should not be considered the treatments of choice in patients with NOA, given the lower probability of positive sperm retrieval compared to cTESE and mTESE. |

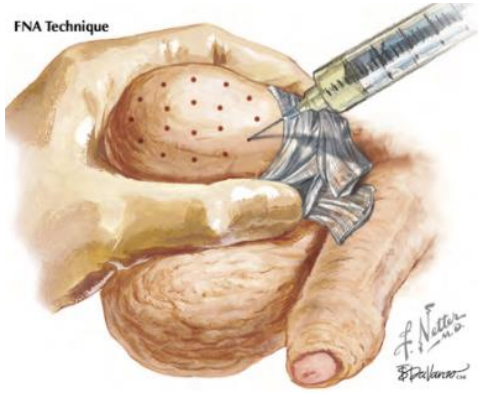
# ETHICALLY QUESTIONABLE PRACTICES : MULTIPLE TEFNA

Multiple TeFNA attempts are equally unreliable and can cause severe damage to testicular parenchyma.

Animal studies (Shufaro et al, 2002; Raviv et al, 2004) and my personal observations during mTESE surgeries have confirmed this damage.

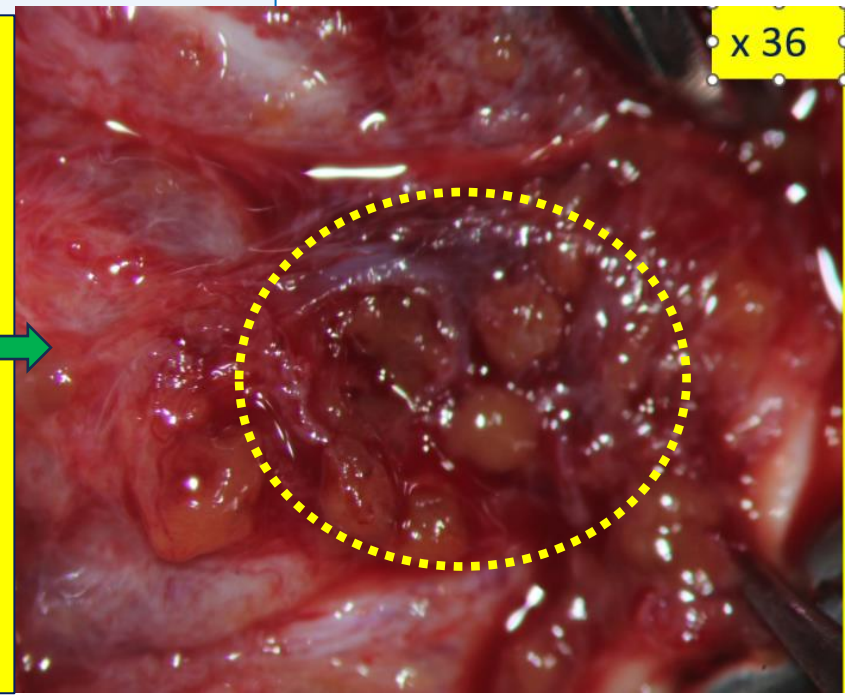
The more punctures performed, the higher the likelihood of bleeding, which can progress to intratesticular fibrosis.

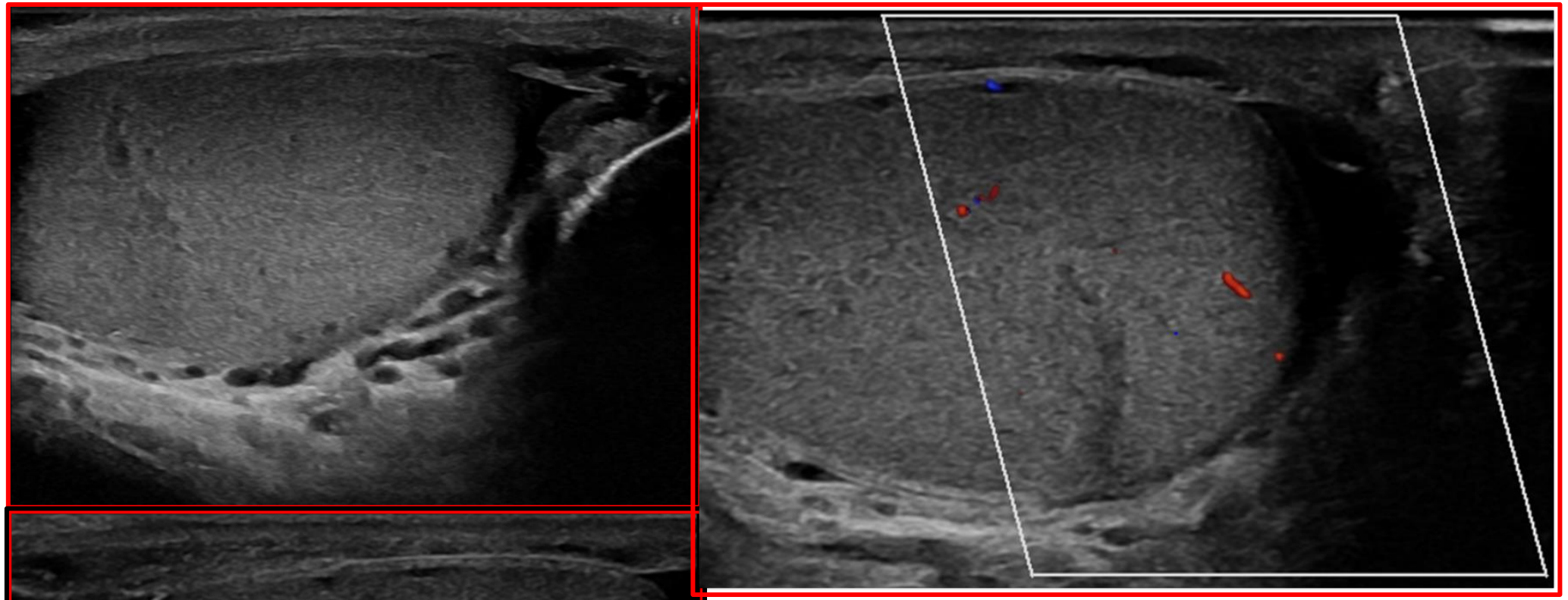
Practitioners of TeFNA tend to operate under the motto: "OUT OF SIGHT, OUT OF MIND."




Compound Mapping may exacerbate damage, although I've never encountered a patient subjected to it; I suspect most who undergo it never seek andrological care again.

NOA patient submitted to 10 TeFNA (each testis): parenchyma results made of hyalinotic lobules with abundant fibrotic tissue and areas probably due to small hematomas, seminiferous tubules actually undetectable everywhere, except for few intact ones at the lower pole of each testis. Histology: rare involuted and hyalinized seminiferous tubules with Leydig cell proliferation.





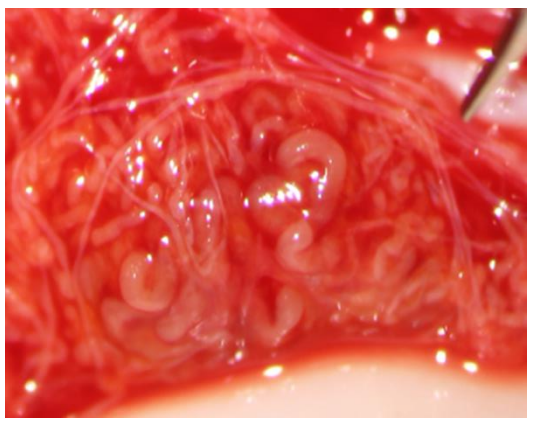
Residual lesion in the testicular parenchyma after a single TESA: testis sonography five months later.

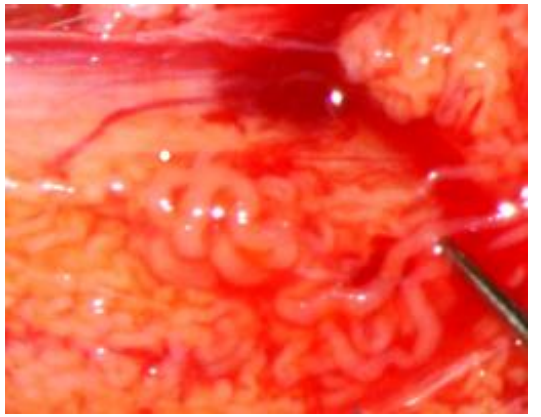
Question: what results following many needle passes ?

# ETHICALLY QUESTIONABLE PRACTICES : (MULTIPLE) cTESE

Even **multiple cTESE** may achieve higher SRRs than single cTESE, it must be emphasized that this procedure is more destructive, particularly for NOA patients with small testicles, posing increased risks of bleeding and testosterone depletion.

| EAU Guidelines 2025 | Summary of evidence | |
|---|---|---|
| | Microdissection TESE has been associated with higher rates of sperm retrieval and lower complications than conventional TESE. | 2a |
| **Recommendations** | Use microdissection TESE as the treatment of choice to retrieve sperm in patients with NOA. | Weak |





# ETHICALLY QUESTIONABLE PRACTICES : FAKE MTESE

I wish I no longer had to read papers like this one:

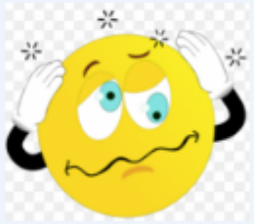
**Preliminary Results of Microsurgical Sperm Retrieval in Azoospermic Patients: A Randomized Controlled Trial Comparing Operating Microscope vs. Surgical Loupes**

which shamelessly claims:

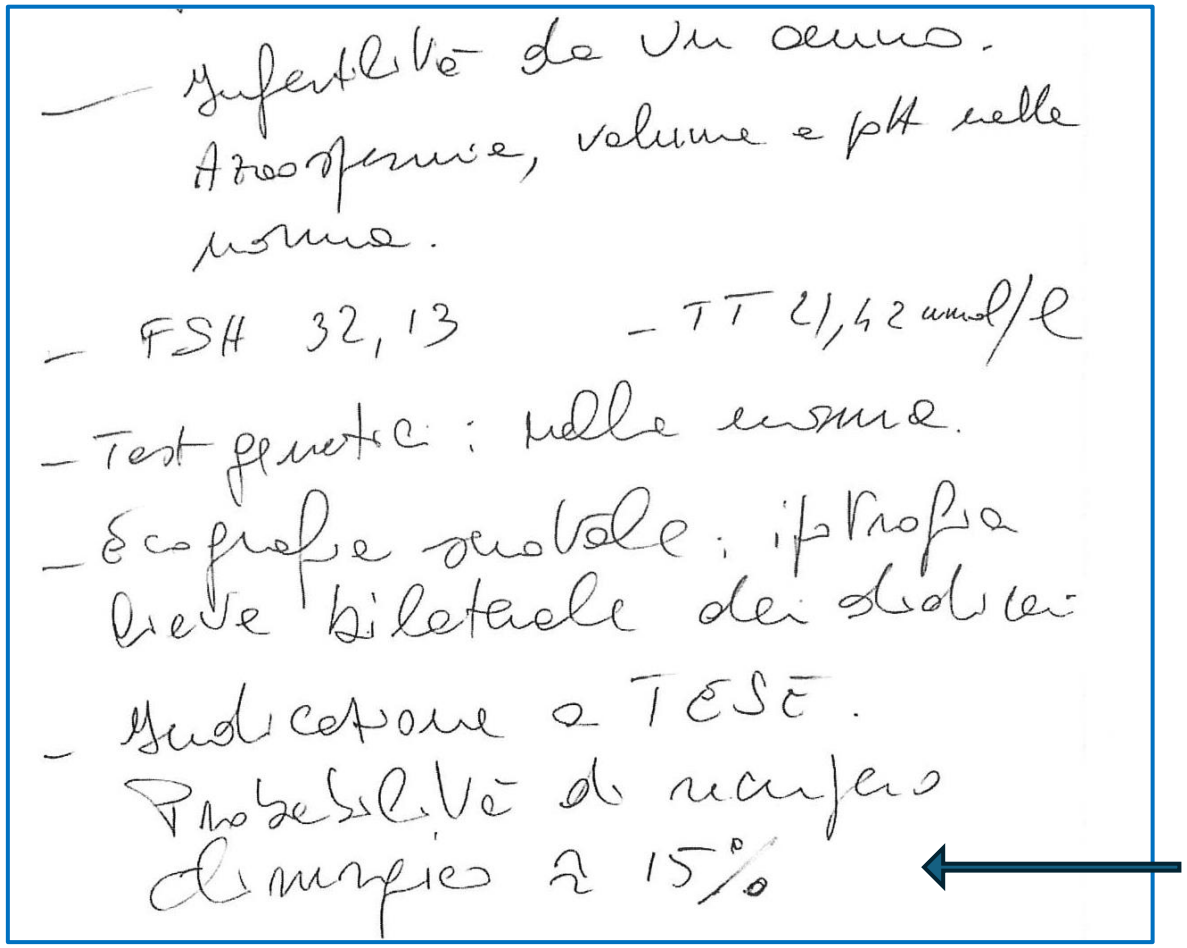
(a) mTESE performed with loupes (3.5–5x magnification)
(b) the equivalence of loupes mTESE results to those of Trifocal TESE
(c) a SRR of 22.6% (!!!), a disturbingly low outcome comparable to that of TefNA

thereby **effectively legitimizing malpractice.**

The average diameter of a seminiferous tubule with preserved spermatogenesis is 180-200 µ.
A difference of ±10-20 µ more or less can make a difference.
How can one expect to discern these diameter differences at 3.5-5 magnifications ?



# Ethically Questionable Practices : Misleading Information



— Infertilità da Un anno.
Azoospermia, volume e pH nella norma.

- FSH 32,13 - TT 21,42 nmol/l
- Test genetici: nella norma.
- Ecografia scrotale: ipotrofia lieve bilaterale dei testicoli
- Indicazione a TESE.
Probabilità di successo di impianto ≥ 15%

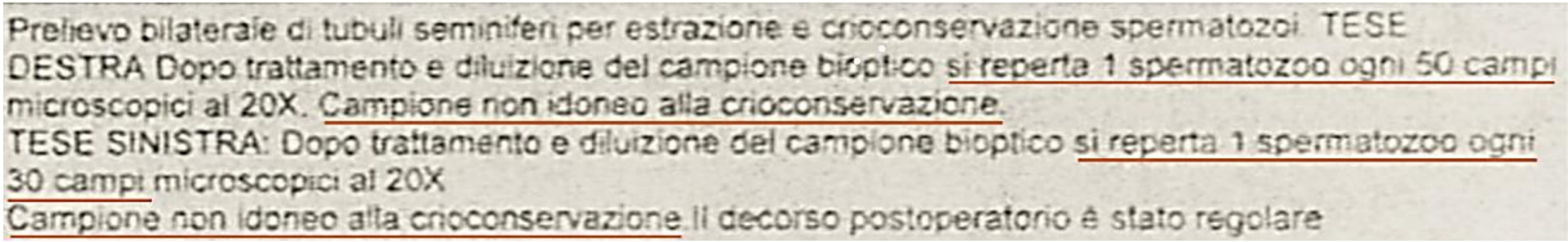
# ETHICALLY QUESTIONABLE PRACTICES : DEFICIENT BIOLOGICAL COMPETENCE

I sincerely hope to stop encountering reports - unfortunately a weekly occurrence ! - that state:

**'rare non-cryopreservable spermatozoa were observed'** (sic!)

or

**'rare unusable spermatozoa were found'** (sic!).



Prelievo bilaterale di tubuli seminiferi per estrazione e crioconservazione spermatozoi. TESE DESTRA Dopo trattamento e diluizione del campione bioptico si reperta 1 spermatozoo ogni 50 campi microscopici al 20X. Campione non idoneo alla crioconservazione.
TESE SINISTRA: Dopo trattamento e diluizione del campione bioptico si reperta 1 spermatozoo ogni 30 campi microscopici al 20X
Campione non idoneo alla crioconservazione Il decorso postoperatorio è stato regolare

# Ethically Questionable Practices : Deficient Biological Competence

- **The higher is your mTESE SRR, the more frequent are your "poor sperm recoveries"** (over one third in our experience).

Specific devices to cryopreserve rare sperm are available.

In any case **fresh rare sperm, if handled correctly, can achieve good fertilization and blastulation rates**, as confirmed by our experience.

- If by 'not usable' we mean that they have acrosome defects, or are slightly malformed, Schlegel demonstrated that **no specific defect, including the use of nonmotile testicular spermatozoa, fully precluded a chance of clinical pregnancy**, albeit with low fertilization rate (FR) and clinical pregnancy rate (CPR) but resulting in normal offspring.

Our own experience has also confirmed this finding.

**Testicular sperm characteristics in men with nonobstructive azoospermia and their impact on intracytoplasmic sperm injection outcome**

Ahmad Aboukhshaba, M.D.,[a] Nahid Punjani, M.D., M.P.H.,[a] Sofia Doukakis,[a] Nikica Zaninovic, Ph.D.,[b] Gianpiero Palermo, M.D.,[b] and Peter N. Schlegel, M.D.[a]

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Fertil Steril. 2022 Mar;117(3):522-527. doi: 10.1016/j.fertnstert.2021.09.024.

PMID: 34674828 Free article.

Review > Int Urol Nephrol. 2021 Nov;53(11):2193-2210. doi: 10.1007/s11255-021-02979-4. Epub 2021 Aug 19.

# Microdissection testicular sperm extraction (micro-TESE) in men with infertility due to nonobstructive azoospermia: summary of current literature

Arnold P P Achermann [1 2 3], Thairo A Pereira [2], Sandro C Esteves [4 5 6]

**THE STATE OF THE ART in 2021**
**Review on 116 articles, including 70 original papers, 32 review articles, and 14 systematic reviews. The evidence accounted for 4895 patients.** (Achermann et al, 2021)

- **MicroTESE** retrieved sperm in **46.6%** of **NOA** pts., but SRRs varied considerably (18.4-70.8%) and were mainly related to the treated population characteristics.
- **In naïve population, the SRR by MicroTESE was 46.8%.**
- In men who had failed TeFNA or cTESE, the **SRR of salvage-MicroTESE was 39.1%.**
- MicroTESE was associated with low (~ 3%) short-term postoperative complication rates.
- The fertilizing ability of testicular sperm retrieved by MicroTESE in ICSI was adequate (~ 57%), whereas: **clinical pregnancy rate** was: **39% per embryo transfer.**
- and **live birth rate** was: **24% per embryo transfer.**
- The health of the resulting children seems reassuring, but the evidence is limited.

Newborn Gender: Male 34.8-42.4 % vs Female 65.2-57.6 (Lan et al, 2022)