

AZOOSPERMIA NON OSTRUTTIVA: LA RISPOSTA ANDROLOGICA DI MICROTESE ALLA PROPOSTA GINECOLOGICA DI AID: TIP & TRICKS ANDROLOGICI E BIOLOGICI

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**PRIMO CONVEGNO
REGIONALE LOMBARDO
DELLA SOCIETÀ ITALIANA DI
RIPRODUZIONE UMANA
(S.I.R.U.)**

**XXXIX
SABATO DELL'ANDROLOGIA**

**COLLOQUI IN PMA
TRA GINECOLOGI,
BIOLOGI E ANDROLOGI**

**16 FEBBRAIO 2019
PADERNO DUGNANO**
Clinica San Carlo - Via Ospedale, 21
(Auditorium del Nuovo Ospedale)



Non-obstructive azoospermia (NOA) is an unfavorable prognostic condition for male infertility since **spermatogenesis is disrupted**

Men with NOA have **no treatment options other than attempting testicular sperm retrieval** coupled with intracytoplasmic sperm injection

The technique of testicular sperm extraction (TESE) via an open testicular biopsy was described for obstructive azoospermia (OA) by Schoysman et al. as well as Craft et al. and subsequently by Silber et al. and Devroey et al. for NOA.

Ashraf et al. 2013; Schoysman et al. 1993; Craft et al 1993; Silber et al. 1995; Devroey et al 1995

Testicular sperm extraction (TESE)

Testicular biopsy can be part of intracytoplasmic sperm injection (ICSI) treatment in patients with clinical evidence of NOA. Testicular sperm extraction (TESE) is the technique of choice. Spermatogenesis may be focal, which means that in about 50% of men with NOA, spermatozoa can be found and used for ICSI.

There is a good correlation between the histology found upon diagnostic biopsy and the likelihood of finding mature sperm cells during testicular sperm retrieval and ICSI.

However, no threshold value has been found for FSH, inhibin B, or testicular volume and successful sperm harvesting. When there are complete AZFa and AZFb microdeletions, the likelihood of sperm retrieval is virtually zero and therefore TESE procedures are contraindicated.

Microsurgical TESE yields the highest sperm retrieval rates, and multiple TESE is superior to conventional TESE. Microsurgical TESE should be preferred in severe cases of non-obstructive azoospermia.




Recommendations	Strength rating
For men who are candidates for sperm retrieval, we recommend appropriate genetic counselling even when testing for genetic abnormalities was negative.	Strong
Perform multiple testicular biopsies (TESE or micro-TESE) in men with non-obstructive azoospermia, to define spermatogenesis, cryopreserve sperm and diagnose germ cell neoplasia <i>in situ</i> .	Strong




AID

???



Summary of evidence	LE
The WHO laboratory manual proposes reference values based on fertility therefore these reference values do not allow classification of men as infertile.	2a
Impaired spermatogenesis is often associated with elevated FSH concentration.	3
For patients with NOA who have spermatozoa in their testicular biopsy, intracytoplasmic sperm injection (ICSI) with fresh or cryopreserved spermatozoa is the only therapeutic option. Spermatozoa are found by a TESE procedure in about 50% of patients with NOA.	2a
Pregnancies and live births are eventually obtained in 30-50% of couples with NOA, when spermatozoa have been found in the testicular biopsy.	3



Tecniche di Recupero

Table 1 - Sperm Retrieval Techniques and their Indications for Assisted Reproduction.

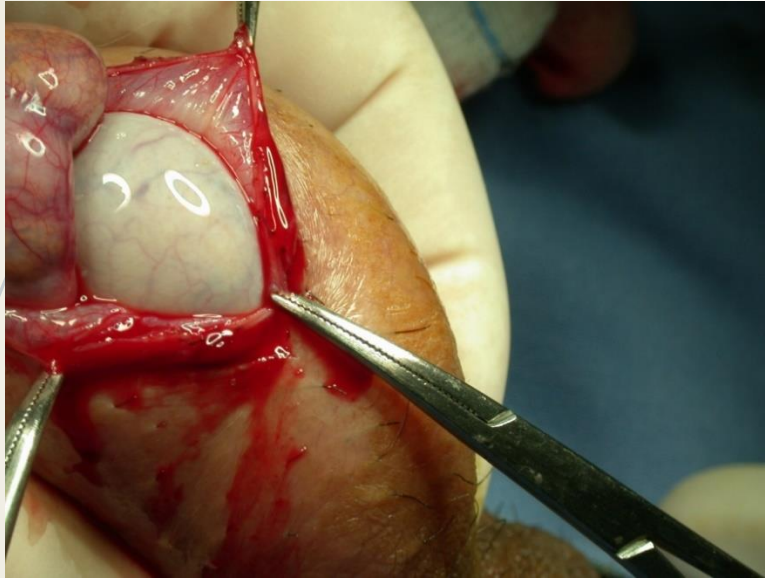
Technique	Acronym	Indications
Percutaneous epididymal sperm aspiration	PESA	OA cases only
Microsurgical epididymal sperm aspiration	MESA	OA cases only
Testicular sperm aspiration	TESA; TEFNA ¹	Failed PESA in OA Epididymal agenesis in CAVD cases Favorable testicular histopathology ² in NOA Previous successful TESA attempt in NOA
Testicular sperm extraction (single or multiple biopsies)	TESE	Failed PESA or TESA in OA NOA cases
Microsurgical testicular sperm extraction	Micro-TESE	NOA cases only

OA: obstructive azoospermia; NOA: nonobstructive azoospermia

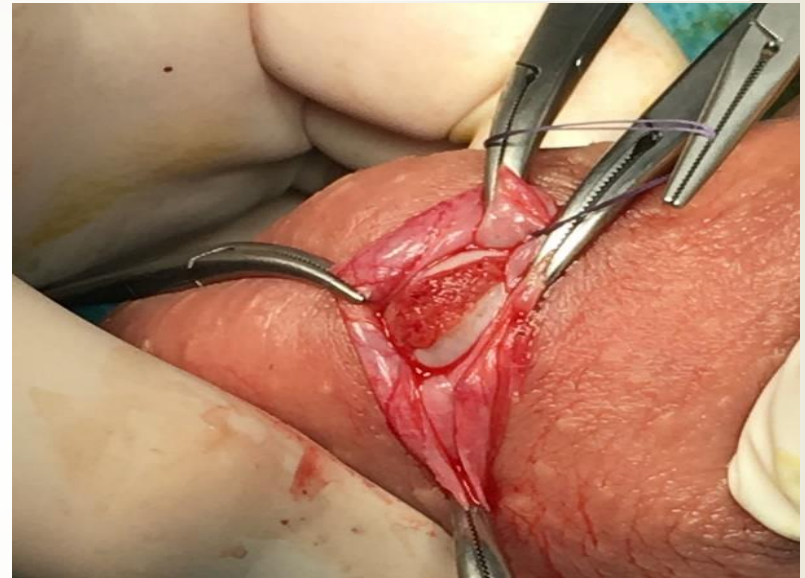
1 - Testicular fine-needle aspiration (TEFNA) is a technical variation of TESA; 2 - Hypospermatogenesis

Altre Indicazioni: Anejaculazione - Necrozoospermia - Azoospermia virtuale - Criptozoospermia (?)

La **cTESE** (open single biopsy) è una procedura molto usata sia per le OA che per le NOA: in quest'ultimo caso si basa sull'assunto di una distribuzione intratesticolare multifocale e/o omogenea della spermatogenesi residua.



OA



NOA

Probabilità di recupero positivo (SRR):
nelle OA: **98% (→100%)**
nelle NOA: **35% (30-40%).**

Nelle NOA, Prelievi Multipli (multiple TESE) possono migliorare il tasso di recupero

MicroTESE was described by Schlegel in 1999 as a combination of Simple TESE and Multiple TESE, but assisted with an operative microscope.

In many cases of NOA, a “patchy distribution” of areas with residual spermatogenesis was shown. (Tournaye, 1995; Hauser, 1998).

Therefore, very soon MicroTESE has been reported to offer a higher SRR than Single TESE and Multiple random TESE. (Okada, 2002; Tsujimura, 2002).



“MicroTESE is based on the principle of identifying the most advanced pattern, though not necessarily the predominant pattern, of spermatogenesis in the testis” (Schlegel, 1999).

MAJOR ADVANTAGES:

- 1) Identification of individual clumps of tubules with better spermatogenesis, so maximizing sperm recovery
- 2) Excision of single tubules, so providing the biologist with less testicular tissue to dissect in order to find sperms
- 3) The optimal visualization of the intratesticular and the subalbugineal terminal vessels allows the preservation of blood supply with minimal damage to the testis.

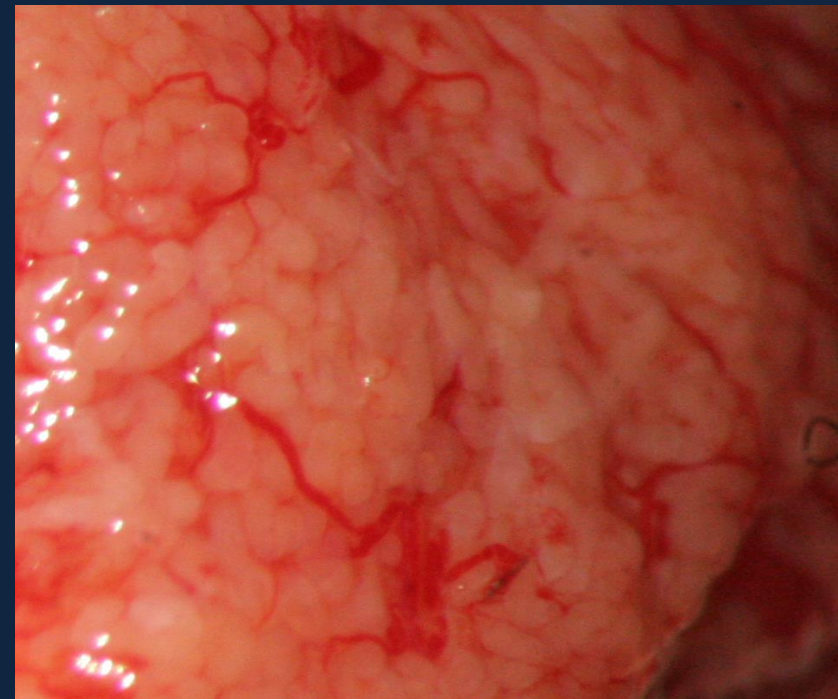
MICROTESE: Surgical Technique

The testis is widely opened (for 2/3 or 3/4 of its circumference) along an equatorial or para-equatorial plane, avoiding any parenchymal stretching. A vast exposition of the seminiferous tubules is obtained, following the physiologic distribution of the intratesticular vessels (5->15 x).

Some Authors use a longitudinal incision, which nevertheless makes more difficult to respect the fine internal arterial supply of the testis, mainly that involving the posterior and inferior gonadal portions.

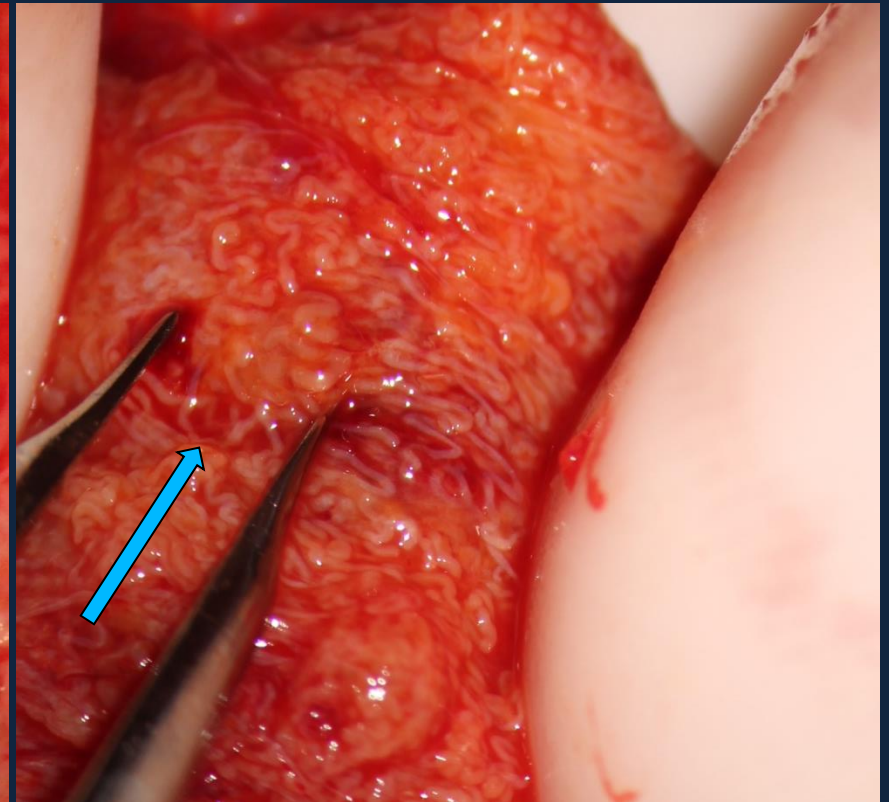
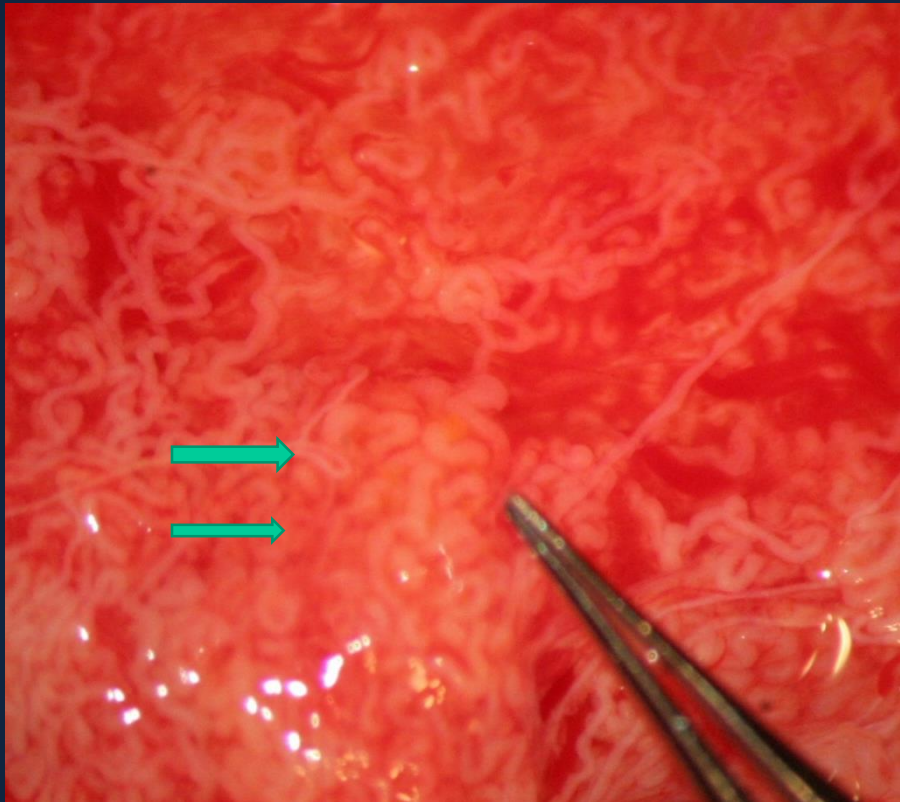
Seminiferous tubules are curled-up inside lobules, which are separated by thin septa; the subtle vessels run parallel to lobules and septa.

A careful and fine microdissection allows to access to the deeper sections of tubules of a lobule, and to many of more internal lobules. Respecting blood supply of lobules, and avoiding any rough disconnection of tubules from tunica albuginea (rich of easily bleeding vessels) are mandatory.



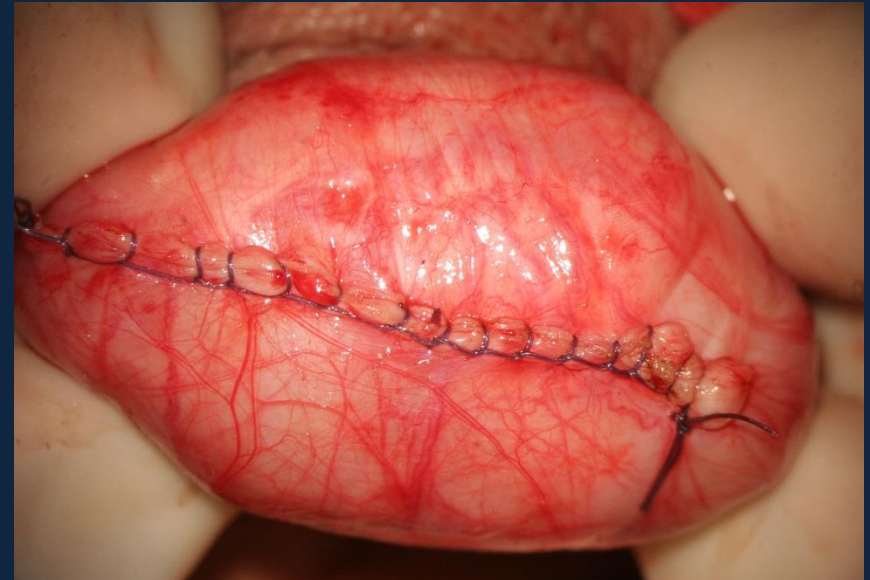
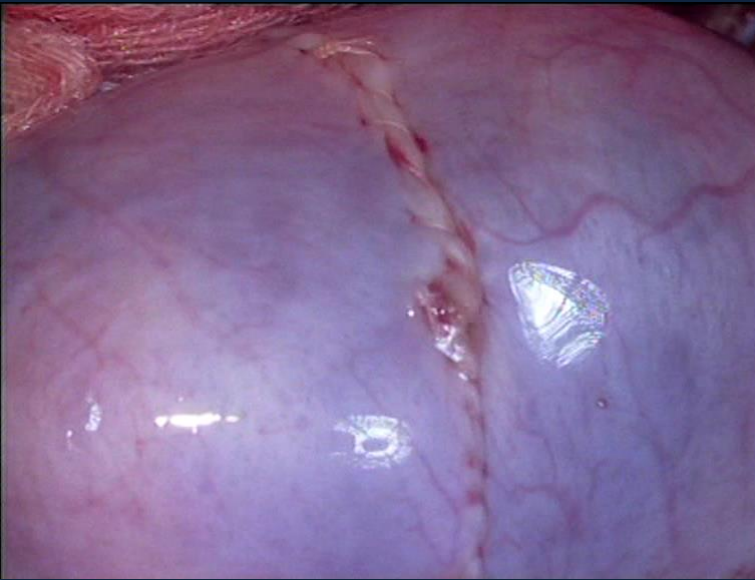
MICROTESE: Surgical Technique

In many NOA testes, foci with residual spermatogenesis are heterogeneously distributed; therefore, microdissection must be extremely exploratory. Operating with a microscope at 15x – 24x(36x) magnification allows to identify the more opaque and larger tubules, more probably those hosting mature spermatogenic cells. These tubules are removed and passed to the biologist in the theatre, for a meticulous sperm search.



MICROTESE: Surgical Technique

At the end, testicular tissue is irrigated with Ringer solution plus gentamycin. Haemostasis is performed by gently pressing parenchyma for 3-4' by a gauze wet with antibiotic and, if necessary, using the bipolar thermal device (0.3 mm tip). Albugineal incision is repaired with continuous suture (Vicryl™ 5-0 or 4-0), followed by closure of tunica vaginalis, **infusion of betamethasone solution into vaginal cavity (Colpi, 2010)** to prevent pain and adhesions, and closure of dartos and skin.



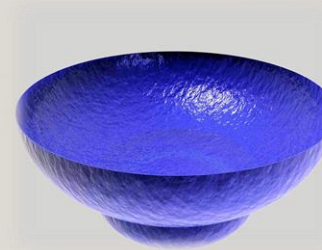
Later: Ice bag on the scrotum and bed rest for 24 hours.

If surgery has been made meticulously, **post-operative progress is actually painless**, and any scar will result invisible at ultrasonography three months later.

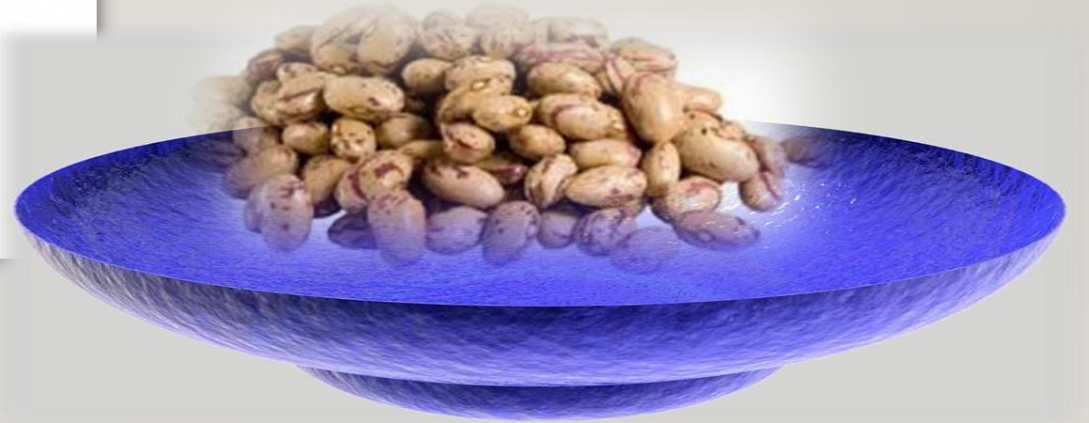
Colpi, Clinical Andrology, 2010



**Are there
beans in the
pot?**







Comparison of microdissection testicular sperm extraction, conventional testicular sperm extraction, and testicular sperm aspiration for nonobstructive azoospermia: a systematic review and meta-analysis

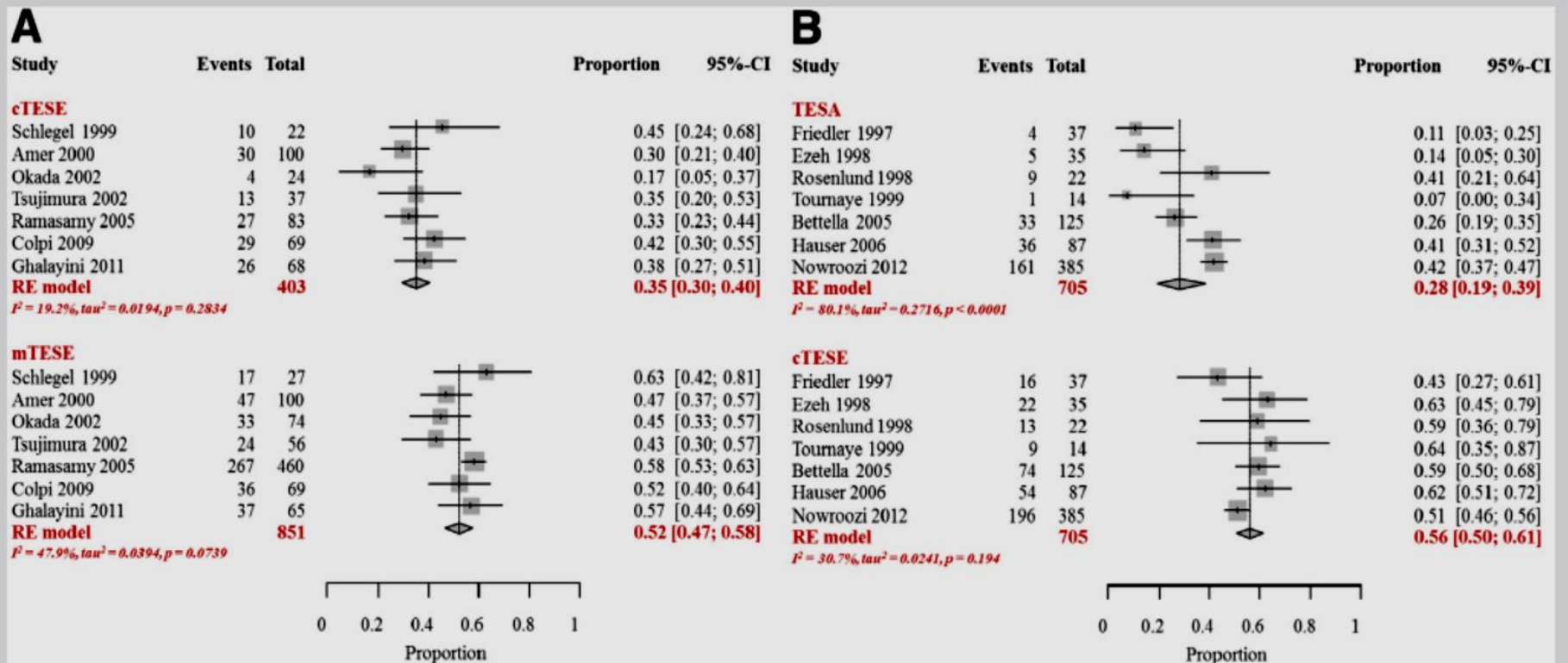
Aaron M. Bernie, M.D., M.P.H.,^a Douglas A. Mata, M.D., M.P.H.,^b Ranjith Ramasamy, M.D.,^c and Peter N. Schlegel, M.D., F.A.C.S.^a

TABLE 1

Selected characteristics of the 15 studies included in this systematic review.

First author, year (reference)	Location	Average age of patients (y)	Average FSH (mIU/mL)	Average T (ng/dL)	Average testicular volume (mL)
Friedler, 1997 (11)	Zerifin, Israel	32.7	—	—	—
Ezeh, 1998 (12)	Sheffield, UK	—	18.5	—	30.4
Rosenlund, 1998 (13)	Sweden	—	—	—	—
Schlegel, 1999 (7)	New York, NY	—	—	—	—
Tournaye, 1999 (14)	Brussels, Belgium	—	—	—	—
Amer, 2000 (15)	Giza, Egypt	33.5	15	—	—
Okada, 2002 (16)	Kobe, Japan	—	—	—	—
Tsujimura, 2002 (17)	Osaka, Japan	32.4 in cTESE 33.9 in mTESE	22.6 in cTESE 24.0 in mTESE	440 in cTESE 370 in mTESE	7.2 in cTESE 8.6 in mTESE
Bettella, 2005 (18)	Padova, Italy	37.6	—	—	—
Ramasamy, 2005 (19)	New York, NY	—	22	316 in cTESE, 303 in mTESE	—
Hauser, 2006 (20)	Tel Aviv, Israel	—	—	—	—
El-Haggar, 2008 (21)	Cairo, Egypt	30.4	18.7	—	9.88 in mTESE, 9.94 in TESA
Colpi, 2009 (22)	New York, NY	—	—	—	—
Ghalayini, 2011 (23)	Amman, Jordan	35.4 in cTESE, 34.8 in mTESE	—	390 in cTESE, 430 in mTESE	—
Nowroozi, 2012 (24)	Tehran, Iran	33	21.7	450	14.8

Bernie. Comparison of micro-TESE, cTESE, and TESA. *Fertil Steril* 2015.



(A) Meta-analysis of the association of cTESE vs. micro-TESE with SR outcome for men with nonobstructive azoospermia. (B) Meta-analysis of the association of TESA vs. cTESE with SR outcome for men with nonobstructive azoospermia.

Bernie. Comparison of micro-TESE, cTESE, and TESA. Fertil Steril 2015.

SRR del 35% con cTESE e 52% con MicroTESE.

Con MicroTESE si recupera 1.5 volte in più rispetto a TESA.

SSR del 56% con cTESE e 28% con TESA.

Con TESE si ottiene un recupero positivo 2 volte in più che con TESA.

PREDICTIVE FACTORS IN SUCCESSFUL TESTICULAR SPERM RECOVERY

According to an old paper, testicular histopathology seems to be the best predictor for a testicular SR in NOA, with:

Sensitivity= 58.8; Specificity= 88.5; PPV= 83.3; NPV= 68.7

Histopathology findings	sperm recovery
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Hypospermatogenesis	100%
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Incomplete SCOS	86%
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Complete SCOS	19%
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Incomplete Maturation Arrest	62%
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Complete Maturation Arrest	48%
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Tournaye, 1996

SRR stratified by histology:

100% in hypospermatogenesis, 46% in MA and 33% in SCO.

Hauser et al, 2006

Unfortunately, histological exam is usually available after SR, apart from few cases already submitted to testis biopsy.

MicroTESE

Richiede:

- strumentazione microchirurgica
- microscopio operatore (fino a 24X)



Richiede:

- Anestesia generale
- Tempi operatori maggiori della cTESE

Durata media:

- 1.8 h (range 0.5–6.6 h) in caso di recupero positivo
- 2.7 h (range 0.8–7.5 h) in caso di recupero negativo

Ramasamy, 2011

Our Operative Times (da MicroTESE No. 601 to No. 720)

Procedure	Mean operative time (minutes)
Monolateral MicroTESE with successful retrieval	86.9 (60-140)
Bilateral MicroTESE (successful or unsuccessful retrieval)	125.8 (85-180)
Monolateral MicroTESE plus Contralateral cTESE	106.6 (94-131)

Richiede:

- ESPERIENZA DEL CHIRURGO OPERATORE

Ishikawa (2010) ha dimostrato che la curva di apprendimento consente di ottenere tassi di recupero significativamente migliori dopo i primi 100 casi.

150 consecutive MicroTESE performed by the same surgeon	
	SSR
Group A (first 50)	32%
Group B (middle 50)	44%
Group C (last 50)	48%
(P < 0.05)	

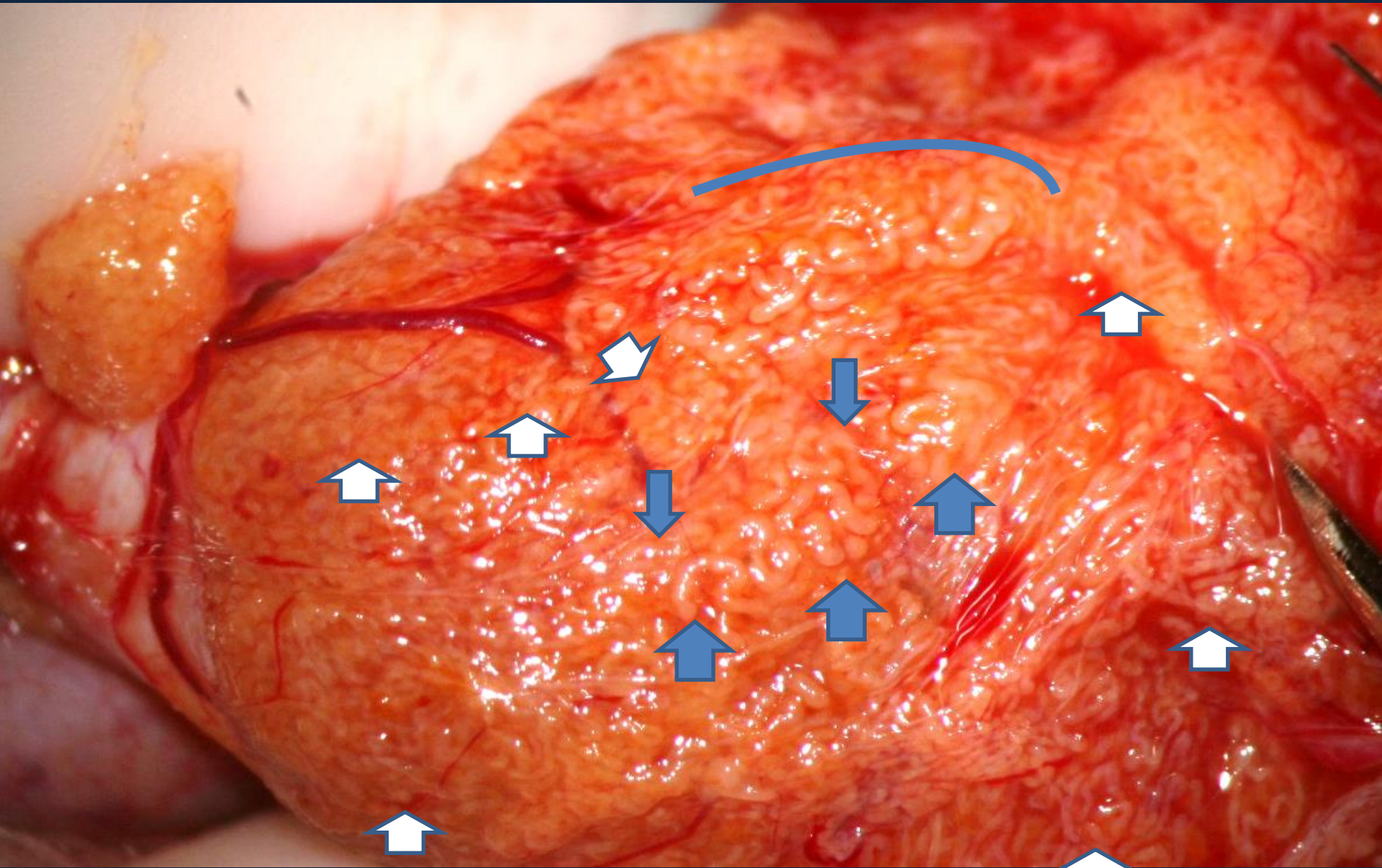
Schlegel sostiene che lo SRR continua lentamente a migliorare finchè l'esperienza microchirurgica supera i 500 casi.

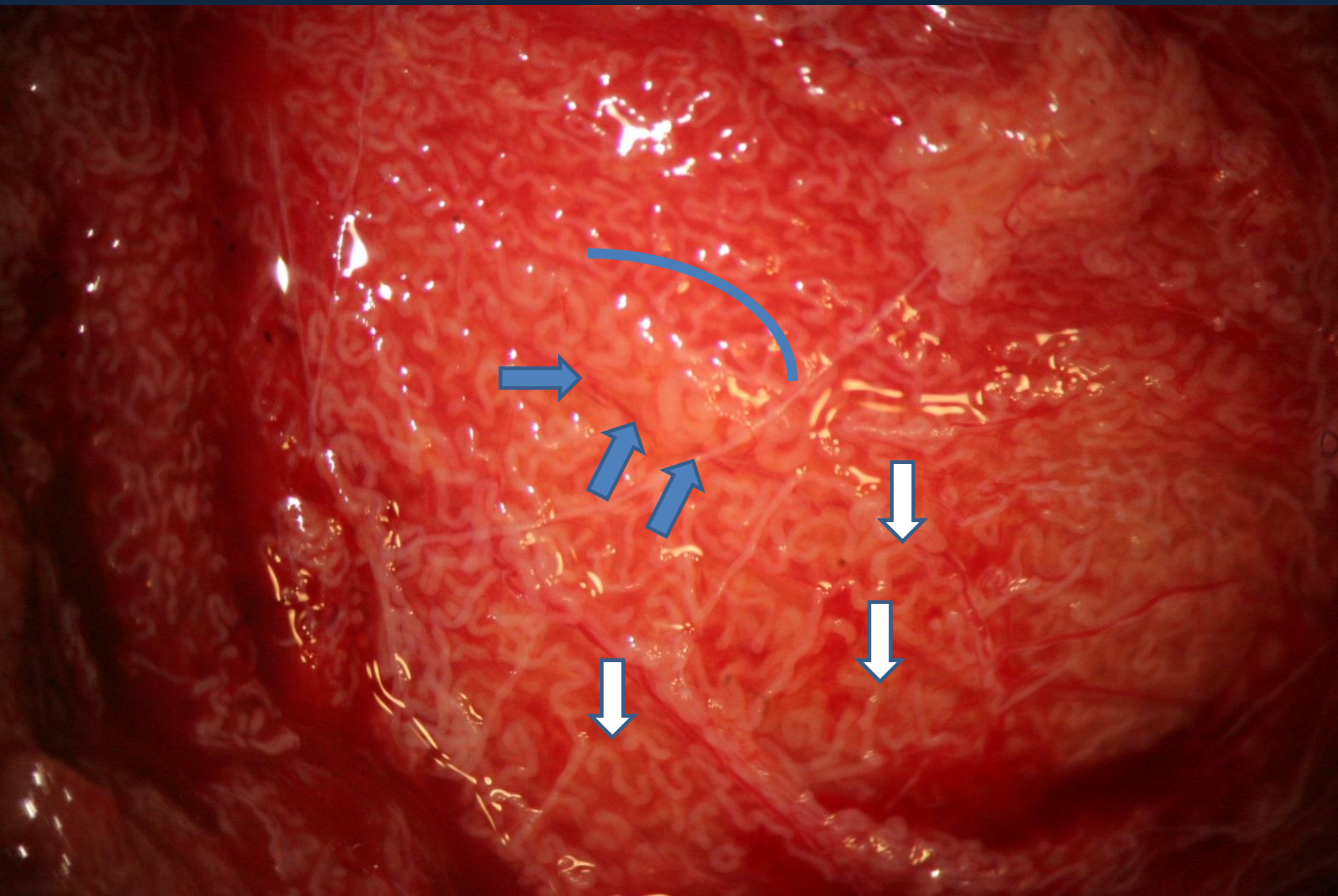
Superata tale soglia, cresce l'abilità nell'individuare anche minimali differenze nel calibro dei tubuli e si riducono i tempi operatori.

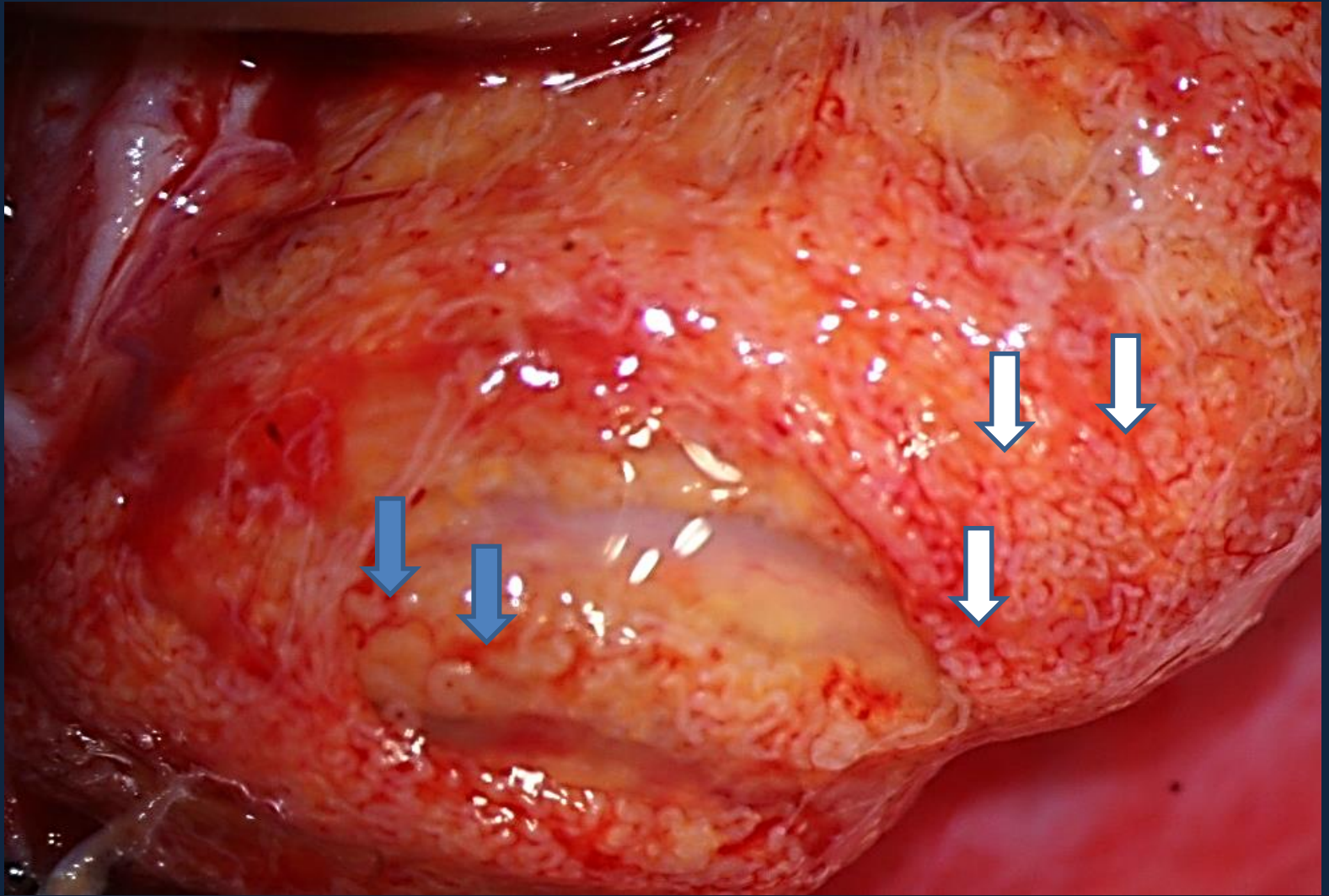
MICROTESE

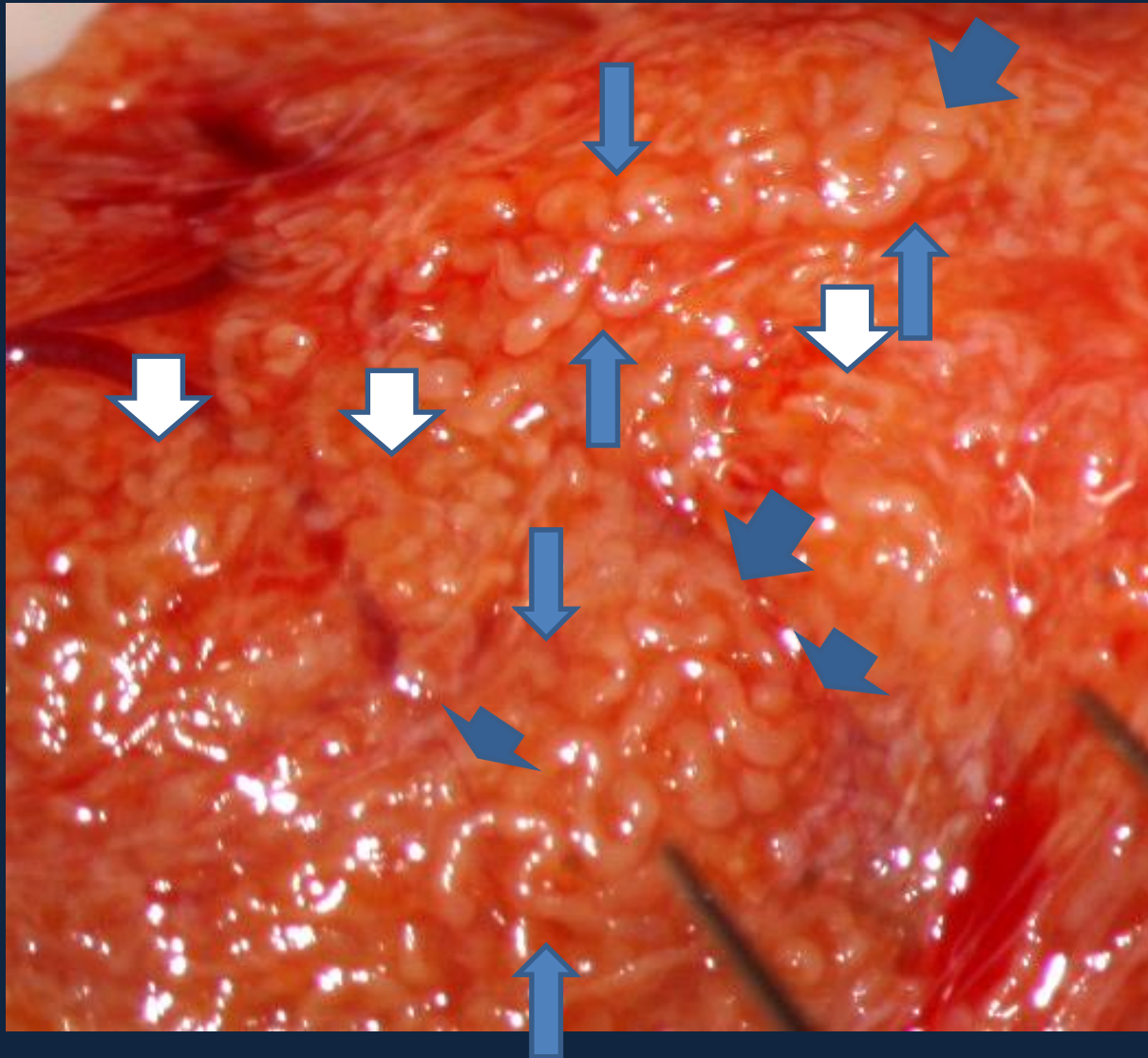
Dilated Tubules *:
more probable residual spermatogenesis

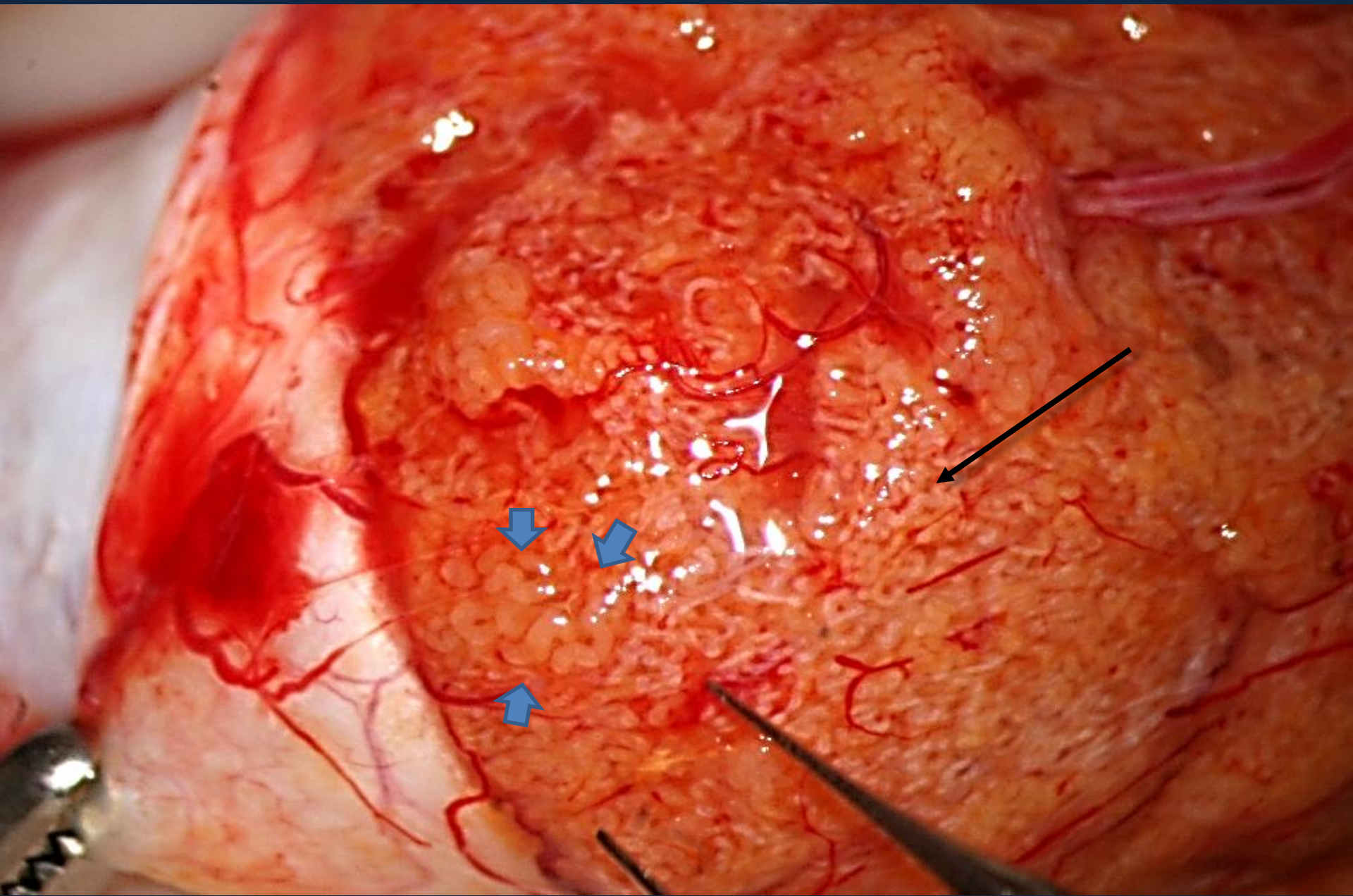
* *actually, their caliber is similar (or slightly less)
to those found in a normal spermatogenesis testis*

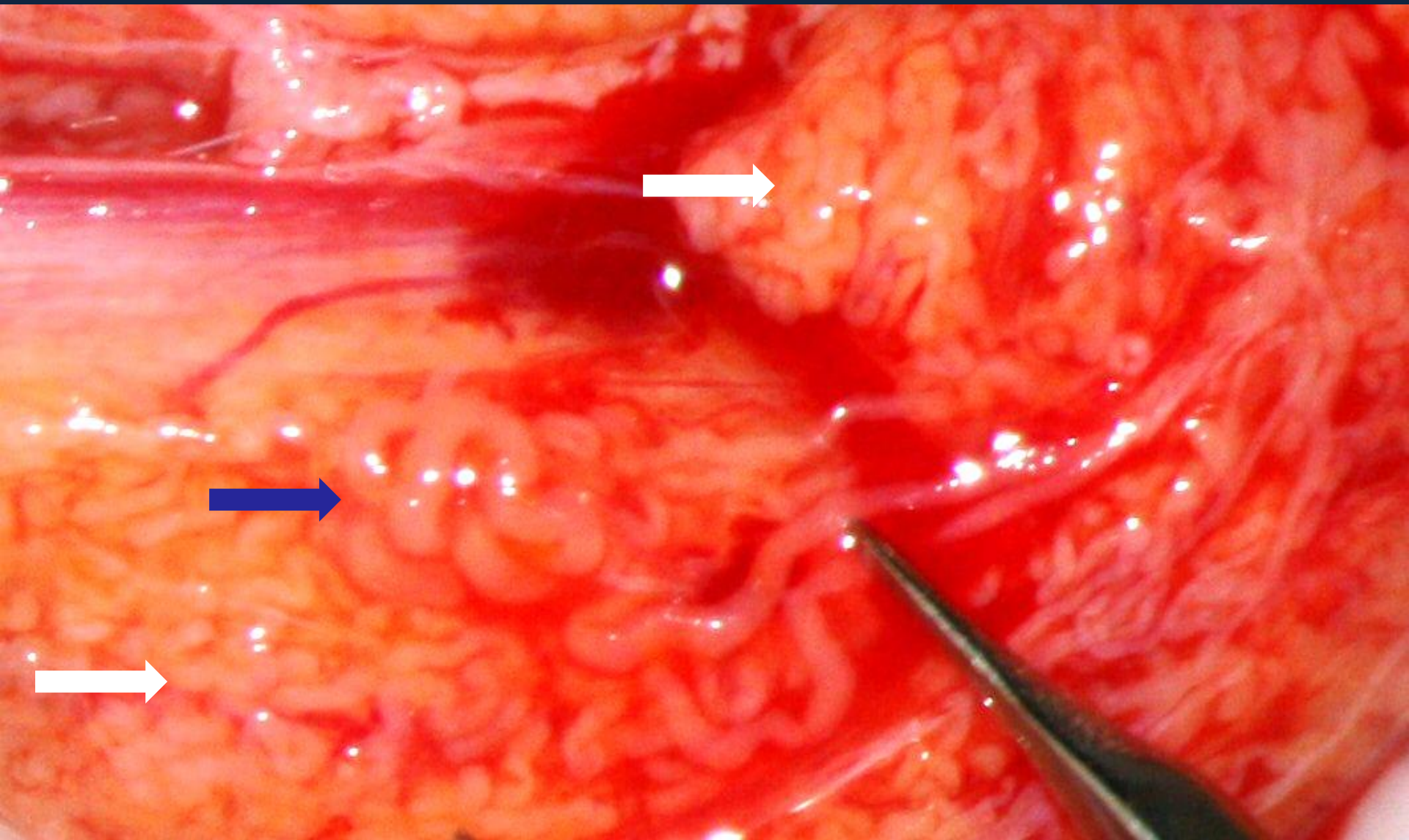


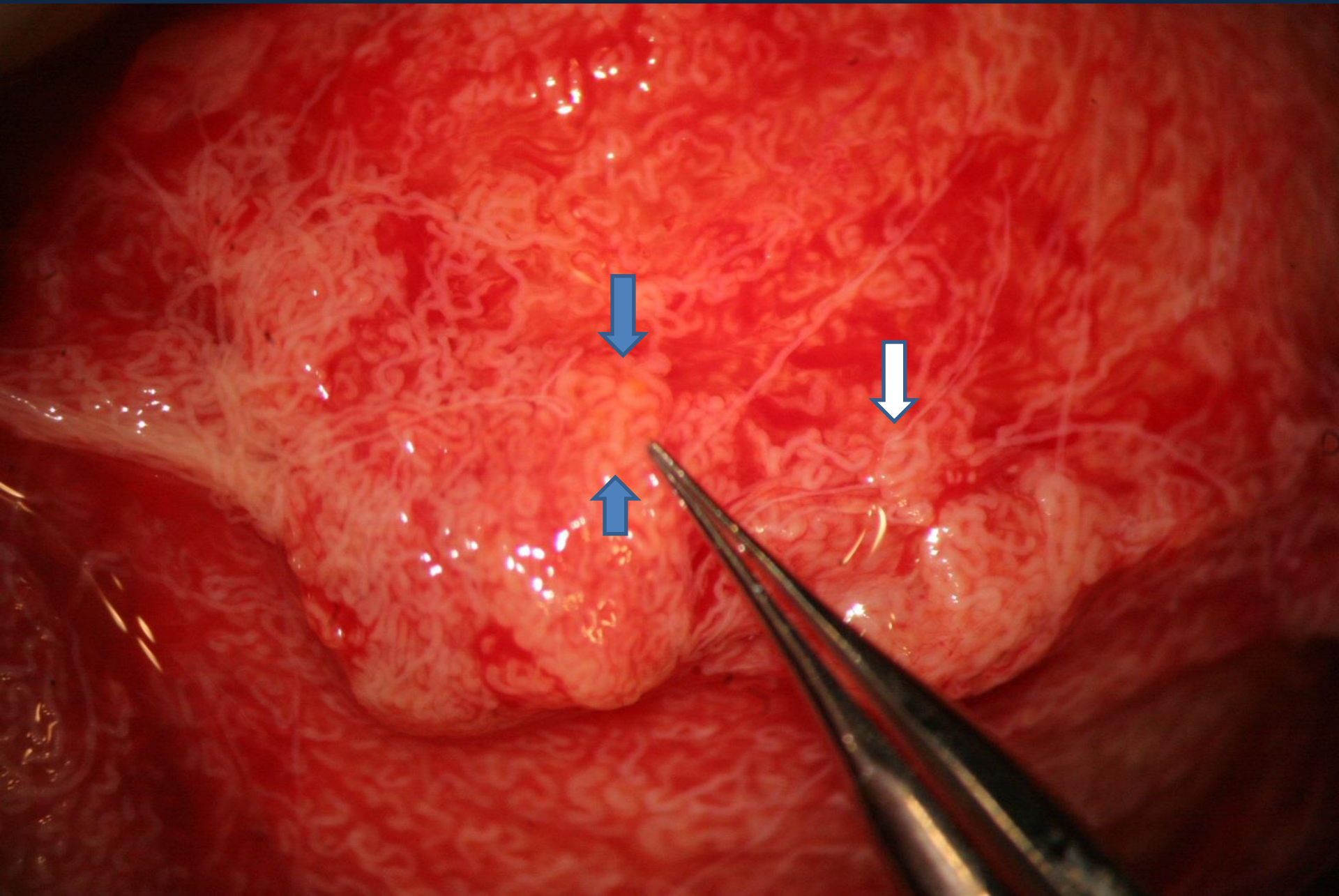


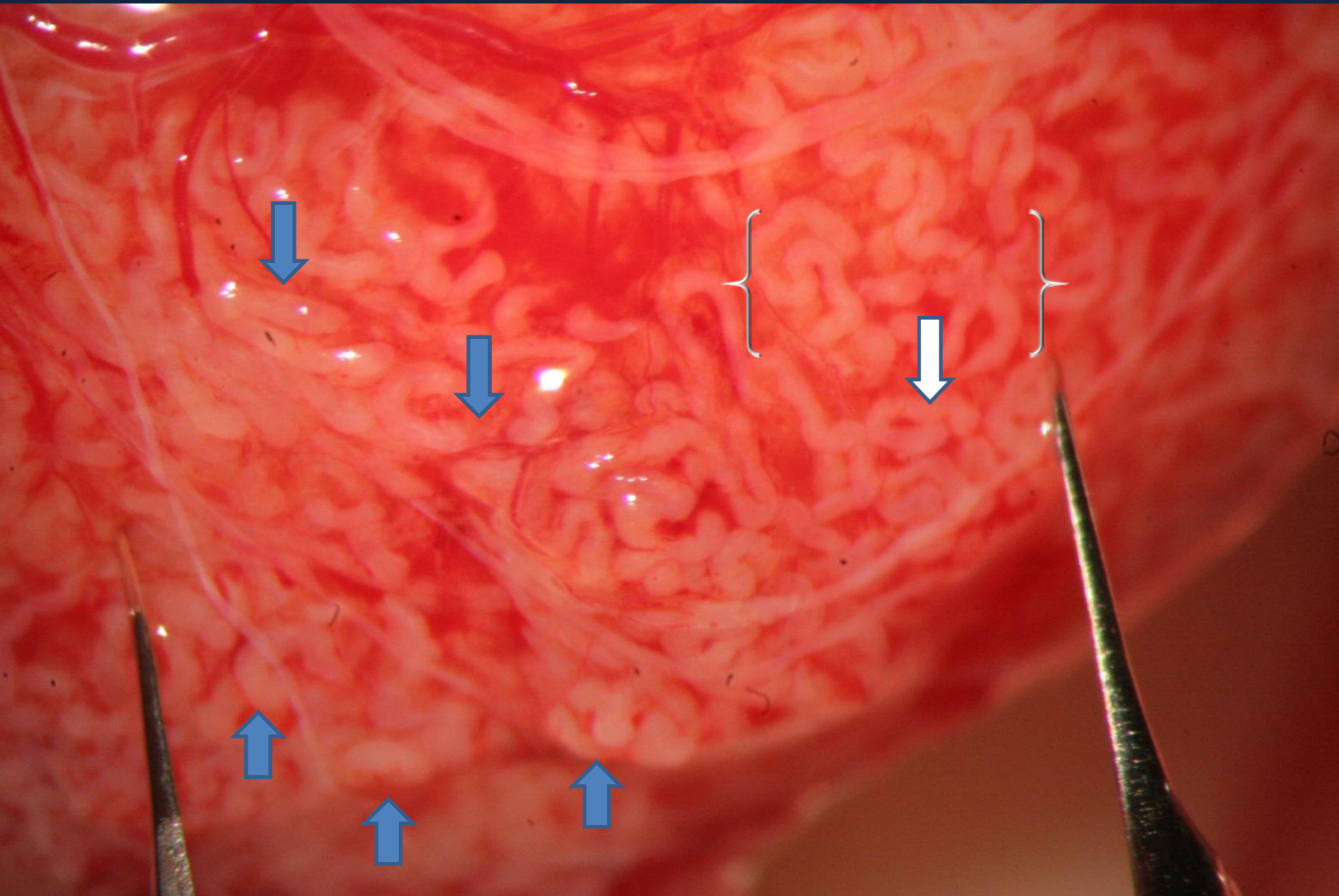












RELEVANCE OF TUBULAR CALIBER LOOK DURING MICROTese

«The seminiferous tubule caliber pattern as evaluated at high magnification during microdissection testicular sperm extraction predicts sperm retrieval in patients with non-obstructive azoospermia».

E. Caroppo, E.M. Colpi, G. Gazzano, L. Vaccalluzzo, E. Piatti, G. D'Amato, G.M. Colpi

Andrology, 2018

We retrospective reviewed the clinical data of 143 male patients: 64 underwent unilateral and 79 bilateral microTESE, for a total amount of 222 testis. (from January 2015 to July 2017).

During all procedures we carefully recorded the caliber pattern of all tubules retrieved: dilated tubules (DT), not dilated tubules (NDT) and slightly dilated tubules (SDT).

The embryologists were blinded to the pattern of tubules retrieved during MicroTESE.

A fragment of one or more of the tubules of the same diameter (DT, SDT or NDT) found was sent for histological analysis (conducted by examining at least 100 different tubule sections).

All surgical procedures were performed by the same urologist.
All testis specimen processing were made by the same two embryologists.
All histological analysis were made by a unique pathologist.

«The seminiferous tubule caliber pattern as evaluated at high magnification during microdissection testicular sperm extraction predicts sperm retrieval in patients with non-obstructive azoospermia».

E. Caroppo, E.M. Colpi, G. Gazzano, L. Vaccaluzzo, E. Piatti, G. D'Amato, G.M. Colpi

Andrology, 2018

	Successful sperm retrieval	Sperm retrieval failure	P
Patients age (years)	36 (33-40) [26-62]	35 (33-38) [26-46]	0.23
Testis volume (ml)	7,5 (6-9) [1.5-17]	7.1 (5.5-8.5) [1.6 – 17]	0.15
FSH mIU/ml	21 (16-28) [1.8-47.6]	20.8 (14.7-29.5) [1.47-68.7]	0.96
LH mIU/ml	6.9 (4,4-10) [0.2-27.4]	6.2 (4.4-10.9) [0.5-48]	0.944
Total testosterone (ng/dl)	422 (350-550) [220-890]	380 (277-497) [134-860]	0.007
Testis histology			<0.0001 [#]
SCO (%)	45 (31.5%)	98 (68,5%)	
Focal SCO (%)	= 68,9%	= 35,3%	
MA (%)	11 (38%)	18 (62%)	
Hypo (%)	27 (96,4%)	1 (3.6%)	
Hyalinosis (%)	2 (22,2%)	7 (77,8%)	
Carcinoma In Situ (%)	1 (50%)	1 (50%)	
Tubules caliber pattern			<0.0001 [§]
Dilated (%)	63 (90%)	7 (10%)	
Slightly dilated (%) (at 24x)	25 (47%)	28 (53%)	
Not dilated (%)	7 (7%)	92 (93%)	
Sperm count per tubule caliber pattern			<0.0001 [#]
Dilated	50000 (9000-300000) [500-5,2x10 ⁶]	/	
Slightly dilated	1000 (500-1000) [500-450000]	/	
Not dilated tubules	500 (500-500) [up to 500]	/	

Spermatozoi recuperati in 95 dei 222 testicoli (42,8%), cioè in 83 dei 143 pazienti **(58,0%)**.

Recuperi positivi:

nel 90% dei Tubuli Dilatati,

nel **47% dei Tubuli Apparentemente Dilatati (a 24X)**, e

nel 7% dei Tubuli Non Dilatati

(p<0.0001).

Model	Chi-Square	P	Cox& Snell R Square	Nagelkerke R Square	% cases correctly predicted	Intercept odds ratio	AUC
Prediction of sperm retrieval success							
Histology	63.329	<0.0001	0.25	0.336	72.7	38.9	0.7
Tubules pattern	133.731	<0.0001	0.45	0.608	82.4	0.84	0.89
Tub. pattern plus Histology	156.749	<0.0001	0.51	0.68	86.8	28.4	0.93
Prediction of tubules caliber pattern*							
Histology	33.843	<0.0001	0.143	0.2	75.9	0.85	0.67

Stepwise binary logistic regression revealed that only the seminiferous tubules pattern and testis histology were significantly predictive of SSR: the combination of both variates correctly classified 86,8% of testes, with an excellent diagnostic accuracy as demonstrated by ROC AUC estimate computed on the predictive probability (0.93).

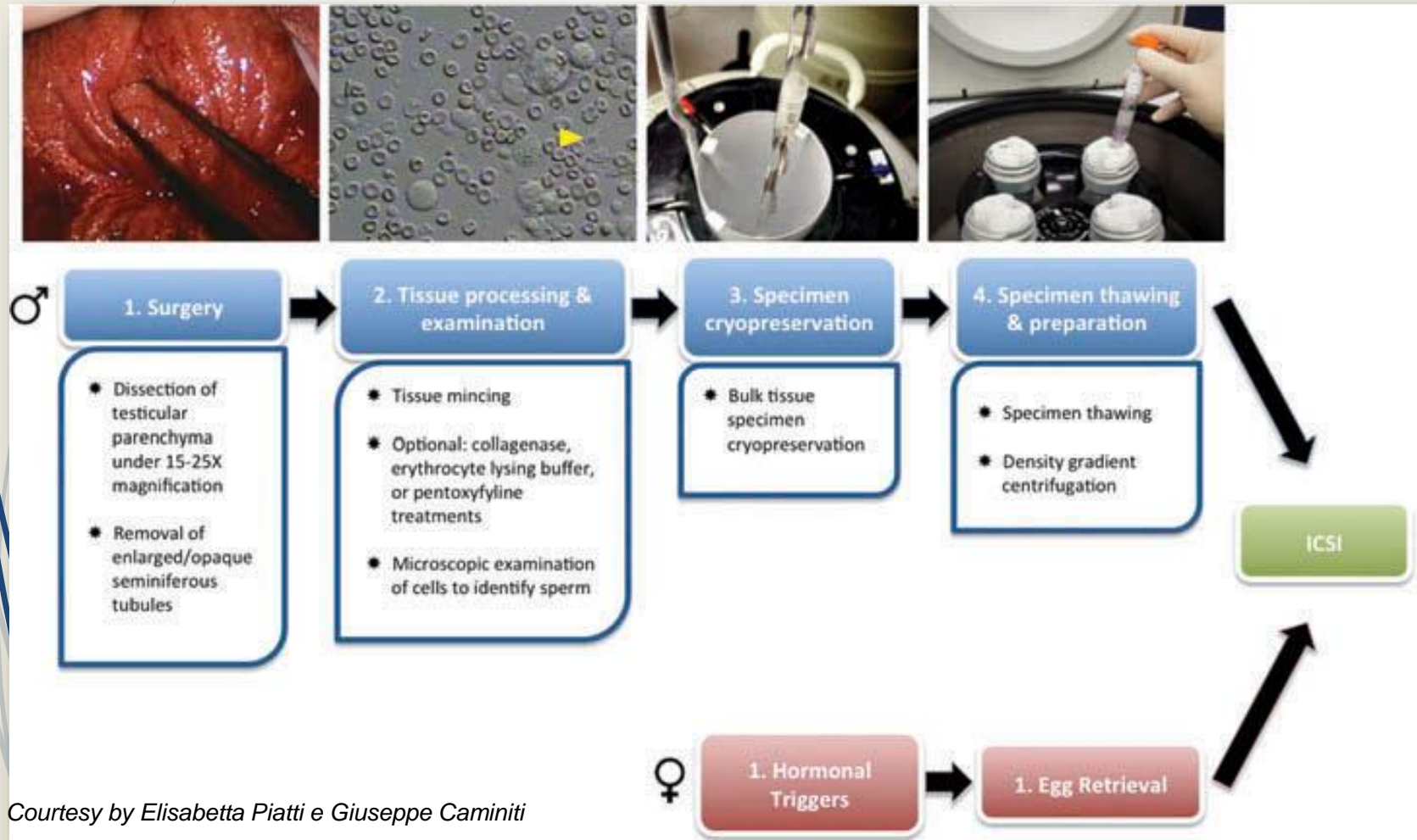


**IL PROCESSAMENTO
DEL TESSUTO TESTICOLARE:
CRITICITA' e OTTIMIZZAZIONE**

Preparazione del campione da MicroTESE

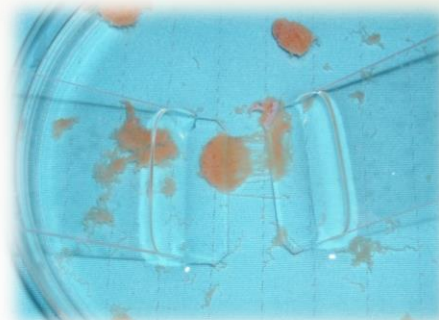
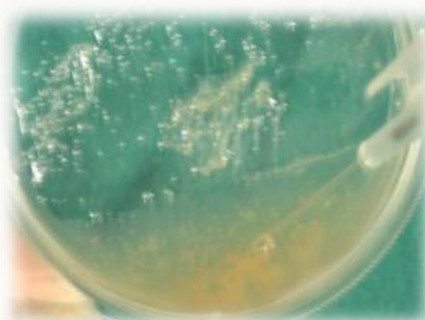
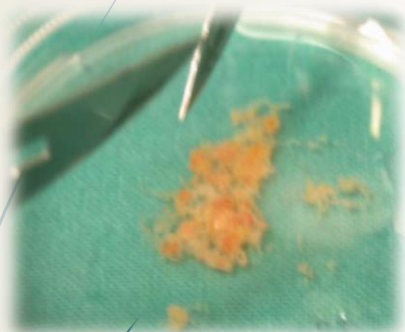
In sala operatoria deve essere presente un microscopio ottico per i Biologi

La presenza di **due biologi** consente di dissociare i tubuli di più prelievi rapidamente e dare in pochi minuti una risposta sulla presenza o assenza di spermatozoi nelle microaliquote di tessuto in esame.

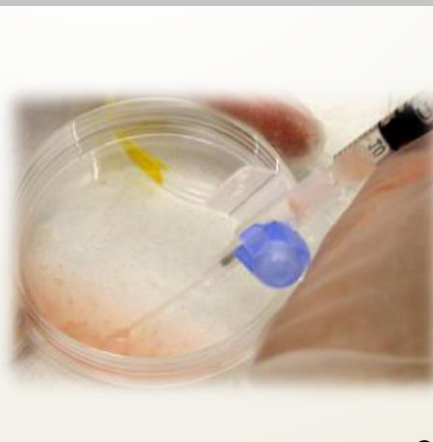
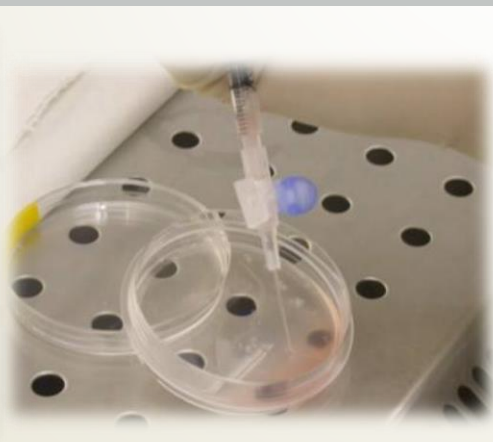


Dissociazione meccanica dei tubuli

La dissociazione avviene secondo il metodo di Schlegel (con vetrini sterili o lame di bisturi utilizzate come i vetrini) per far fuoriuscire il contenuto dei tubuli seminiferi. Si depositano più prelievi in differenti piastre Petri da 60mm.



Si passa più volte la sospensione ottenuta in angiocatetere (sec. Schlegel) e una goccia da ciascuna piastra viene esaminata al microscopio a 40x a luce diretta per dare indicazioni se continuare con i prelievi.



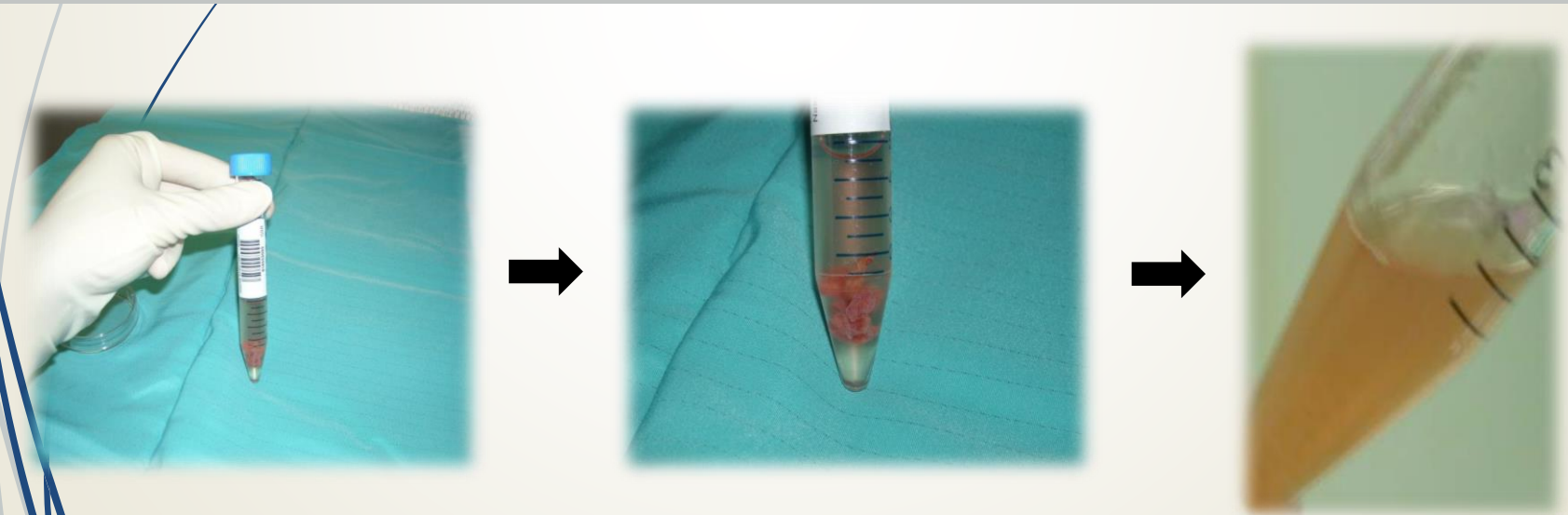
Il vetrino potrà essere poi fissato e colorato.



Preparazione all'uso a fresco o alla crioconservazione

Al termine della procedura di prelievo il materiale deve risultare sminuzzato completamente e reso il più possibile omogeneo. Il contenuto delle diverse piastre può essere o meno unificato.

Si prepara il campione lavando con terreno HTF contenente albumina per centrifugazione a 600g e si concentra lasciando un volume finale in funzione del pellet ottenuto (in genere tra 0,3 e 1,5 ml).

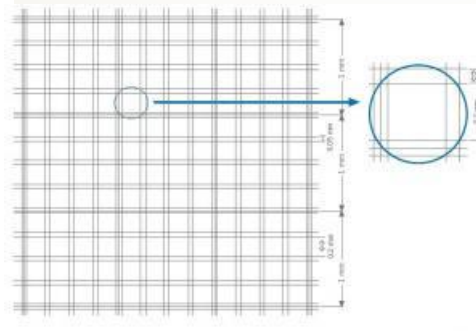
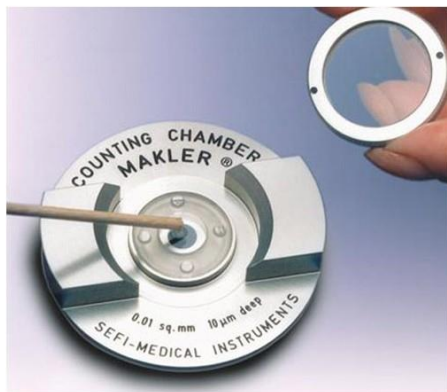


Courtesy by Elisabetta Piatti e Giuseppe Caminiti

Stima della concentrazione di spermatici maturi recuperati

Per dare una corretta quantificazione degli spermatozoi presenti si utilizza una camera di conta (Makler o Burker).

In caso di numero scarso di spermatozoi si fornisce una stima semi-quantitativa andando a valutare il numero medio (cioè di più letture) di spermatozoi presenti in un volume di $10 \mu\text{l}$ sottostante ad un vetrino copri-oggetto da $22 \times 22 \text{ mm}$ a ingrandimento $40 \times$, analizzando in modo sistematico l'intera superficie.



Crioconservazione

In funzione del numero di spermatozoi ottenuto si crioconserva il campione con congelamento rapido, in modo da rendere possibile il **recupero di un numero adeguato di spermatozoi** per un ciclo di ICSI, **evitando tuttavia di dover sprecare materiale in eccesso.**

Proposte di Ottimizzazione

Il recupero di una buona percentuale di spermatozoi vitali dopo crioconservazione potrebbe essere stimato **valutando la vitalità pre-congelamento** quando possibile.

È noto che esistono fattori intrinseci (apoptosi abortiva, difetti maturativi, patologie a carico dell' epididimo) che rendono più vulnerabili gli spermatozoi alla frammentazione e questo influisce inevitabilmente sulla qualità degli spermatozoi recuperati.

Vari tipi di trattamento del materiale prelevato e il congelamento per vitrificazione potrebbero essere modificazioni utili all'ottenimento di un maggior numero di spermatozoi utilizzabili per ICSI.

MICROTESE in PREVIOUS TESE FAILURES

“Salvage micro-dissection testicular sperm extraction: outcome in men with non-obstructive azoospermia with previous failed sperm retrievals”. (Kalsi et al, 2014)

Positive SRR in 27 out of 58 cases (46.5%), without any correlation with FSH levels.

This finding confirms data in previous papers, reporting positive SRRs from 45% (Tsujiura, 2006) to 60% (Ramasamy, 2007).

GENERAL WARNING in NOA PATIENTS


About half of NOA patients are hypogonadic, and require endocrine post-surgical follow-up, and testosterone replacement when needed.

Bobjer, 2012

TRYING TO INCREASE Successful Testicular Sperm Retrievals

PREOPERATIVE MEASURES

- > Limiting patient exposure to certain harmful physical such as heat (Jung & Schuppe 2007), and chemical agents
- > Supplementation therapy (Nutraceuticals ?)
- > Treating NOA due to Hypogonadism
- > Pre-treating NOA with low testosterone (Raman 2002, Schlegel 2009, Cavallini 2011)



“The testicular sperm extraction procedure should be offered to all men with NOA, but should *only* be undertaken in a Centre with expertise in MicroTESE and where an ICSI laboratory with expertise in handling these samples is available”

(Canadian Guidelines, Jarvi et al. 2015)