



XVII CONGRESSO
PAULISTA DE
UROLOGIA

3 A 6 DE SETEMBRO | 2022

Técnicas de obtenção de espermatozoides nas azoospermias obstrutivas

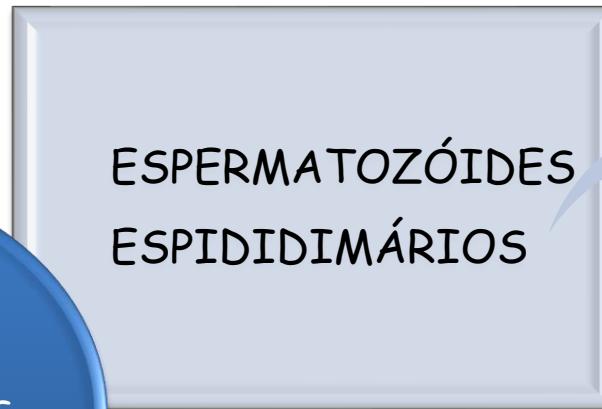
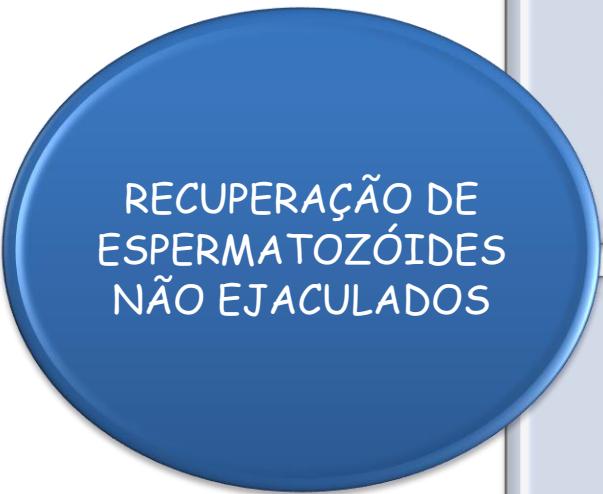
Edson Borges Jr.

Fertility Medical Group

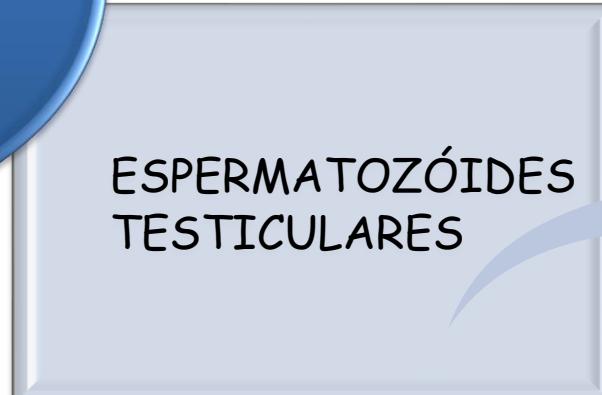
Instituto Sapientiae

Ausência de Conflito de Interesse

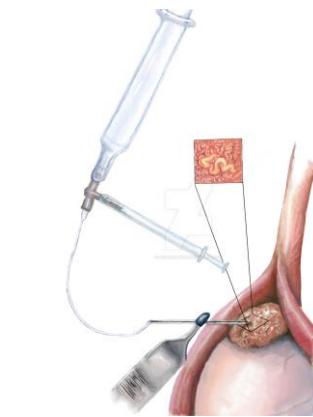
Resolução do Conselho Federal de Medicina nº
1.595/2.000



PERCUTANEOUS
EPIDYDIMAL
SPERM
ASPIRATION



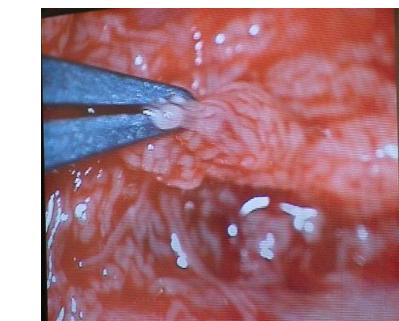
MICROSURGICAL
EPIDYDIMAL
SPERM
ASPIRATION



TESTICULAR
SPERM
ASPIRATION



Micro
TESTICULAR
SPERM
EXTRACTION



FERTILITY



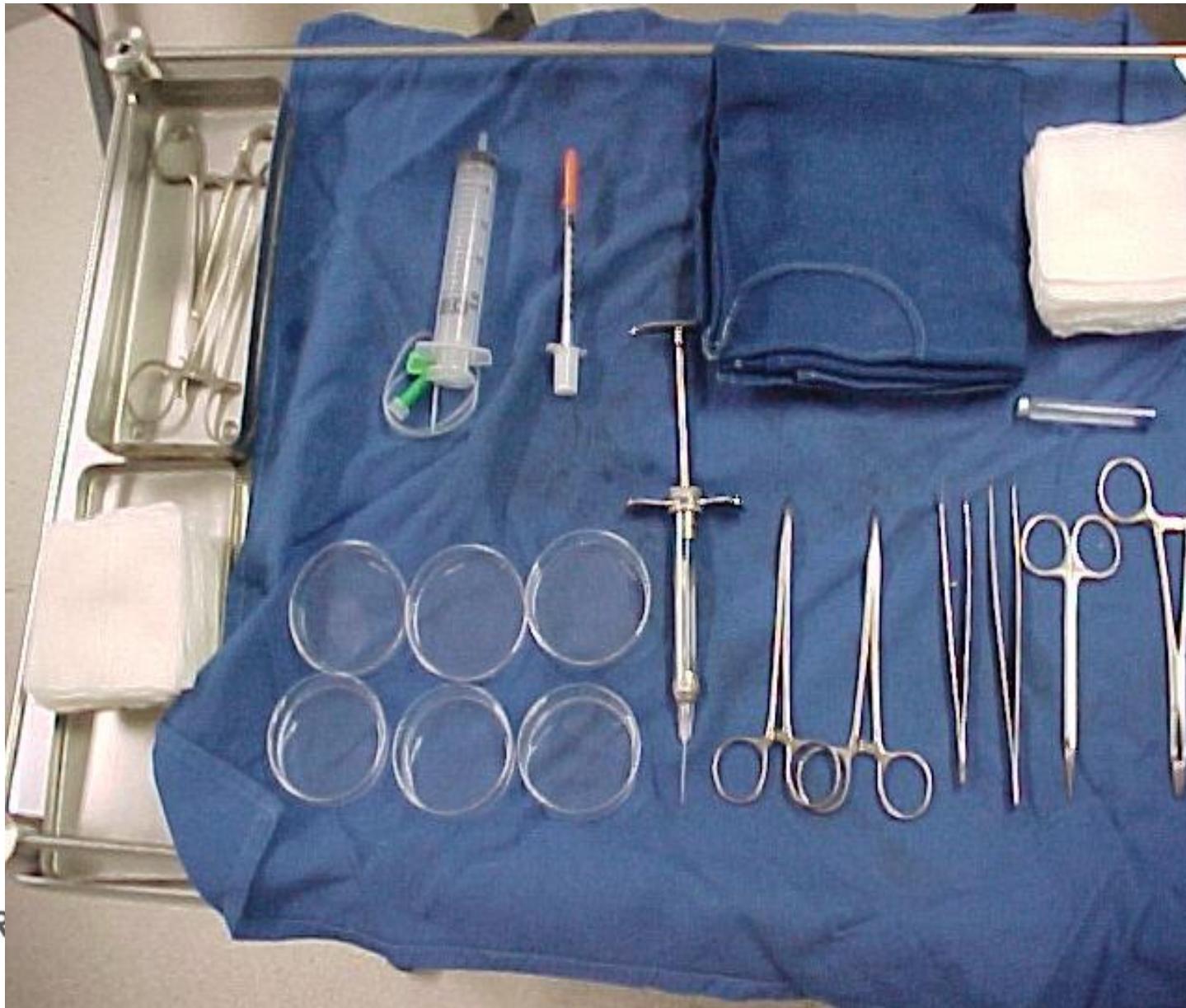
© 2014 Nucleus Medical Media. All Rights Reserved.

nucleus
MEDICAL MEDIA



FERTILITY

PESA





FERTILITY



FERTILITY

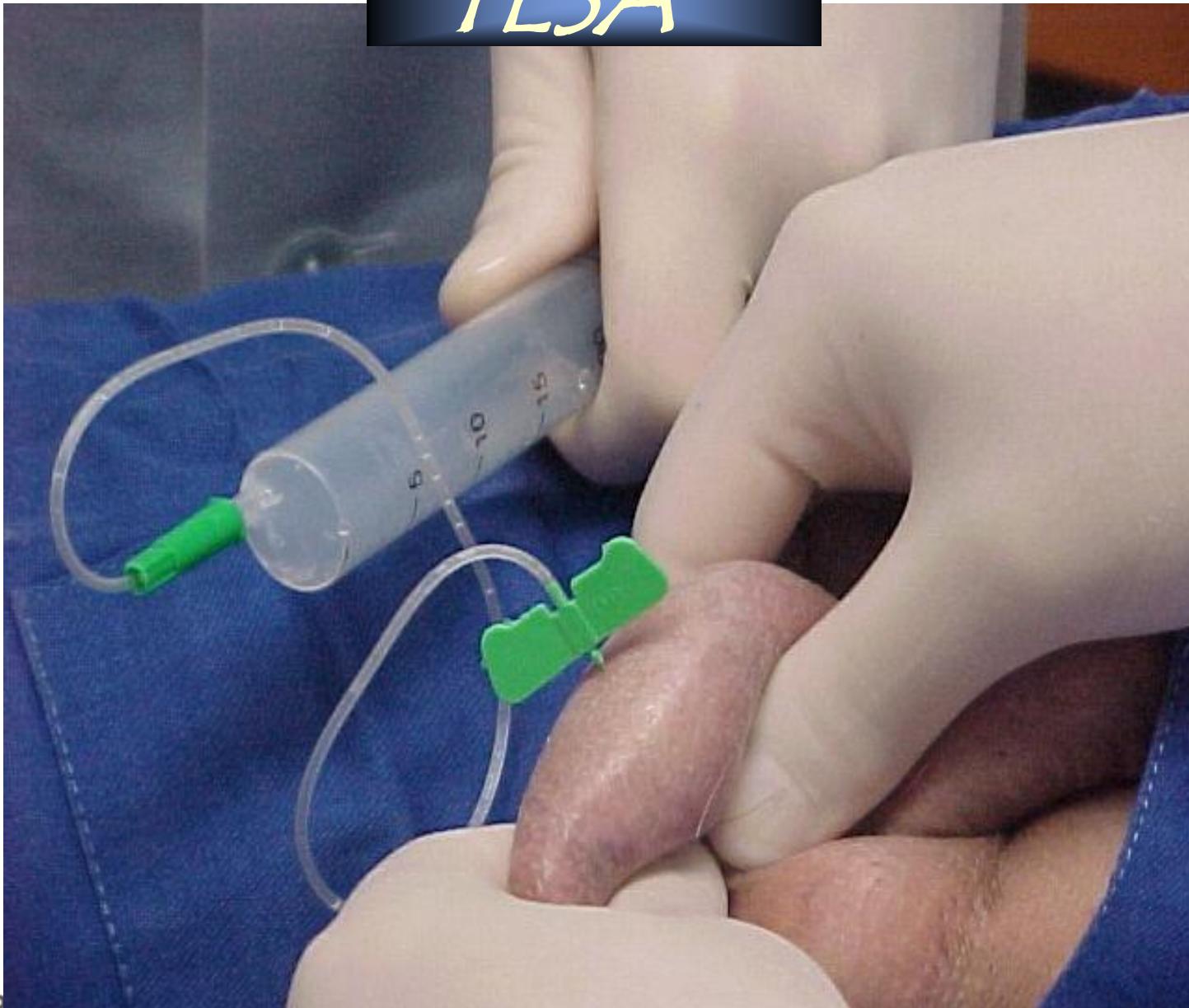


FERTILITY



FERTILITY

TESA



FERTILITY



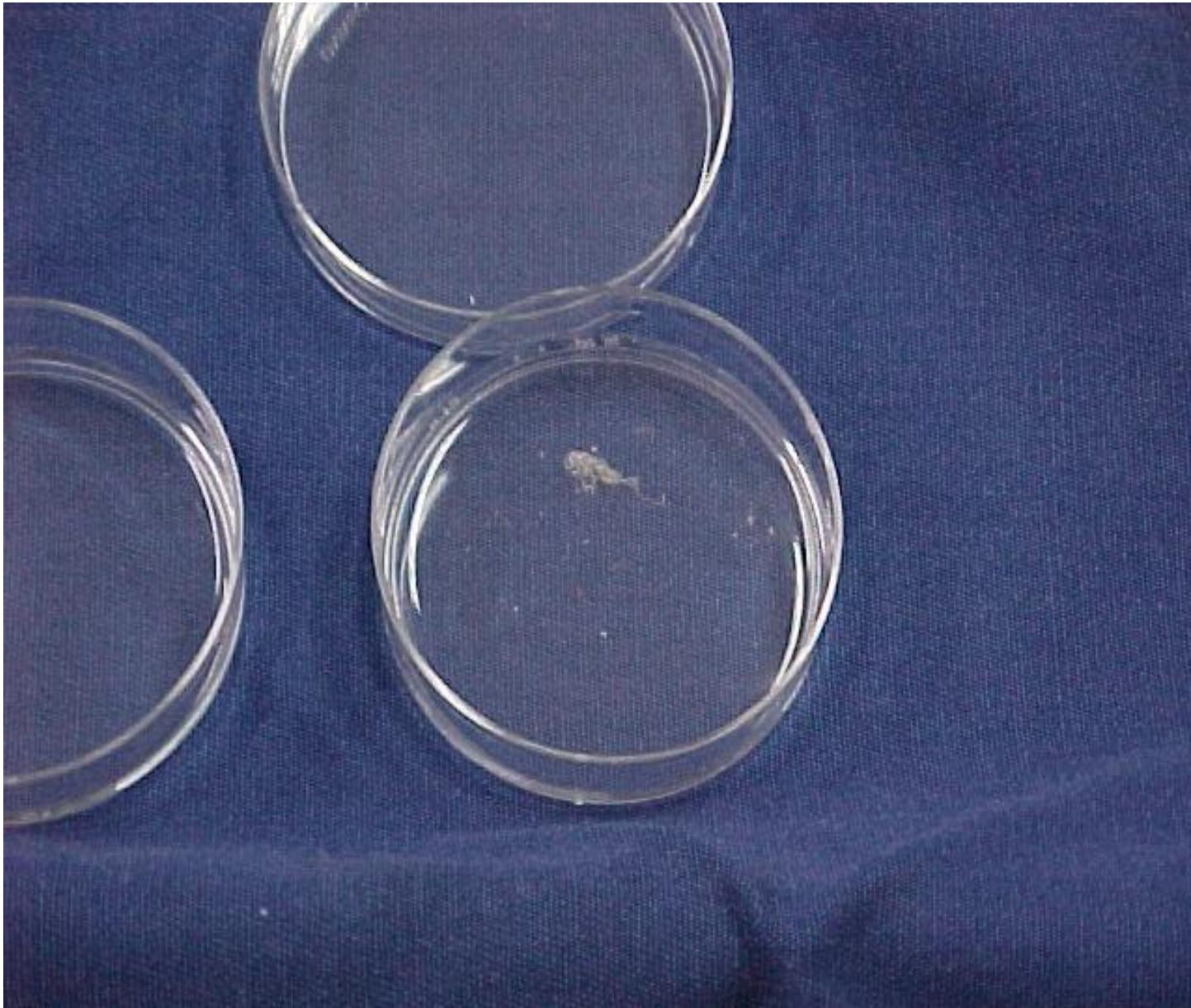
FERTILITY



FERTILITY



FERTILITY

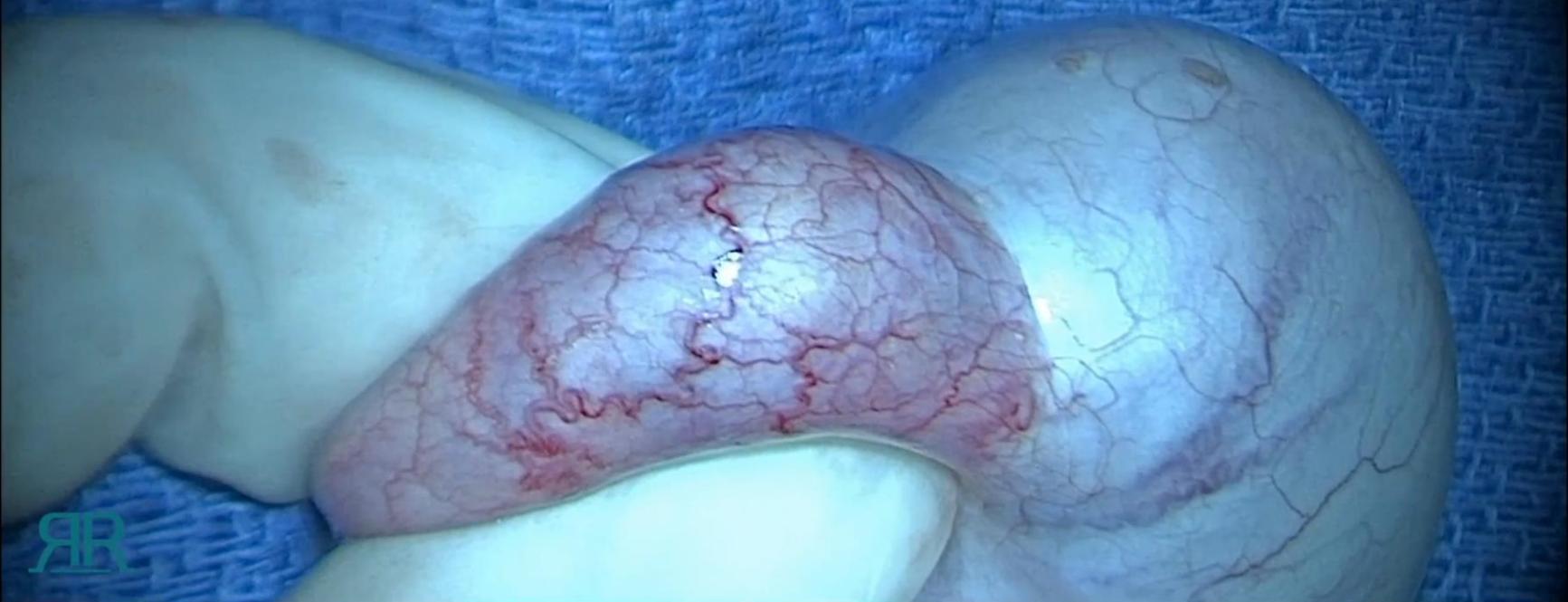


FERTILITY



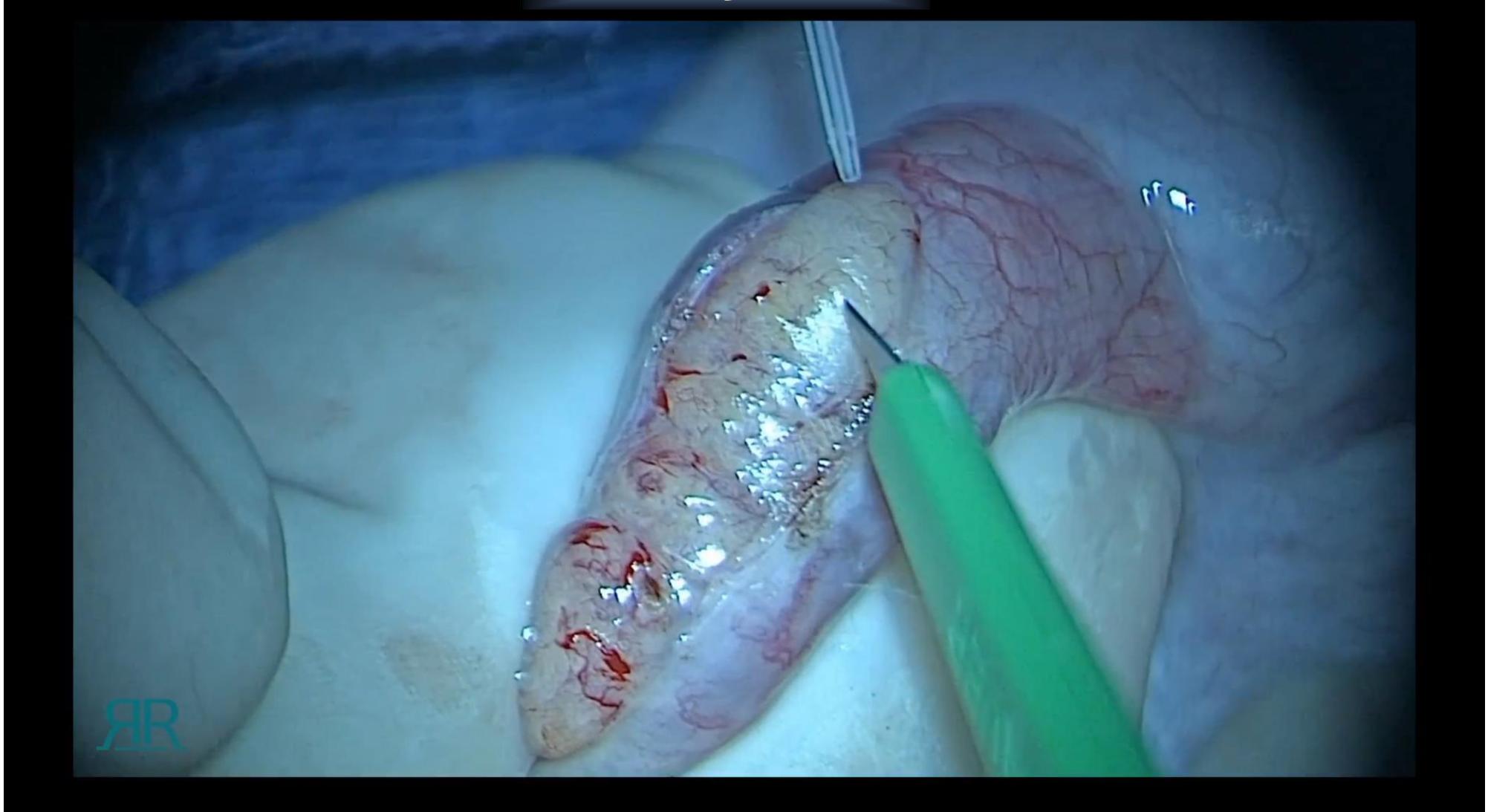
FERTILITY

MESA



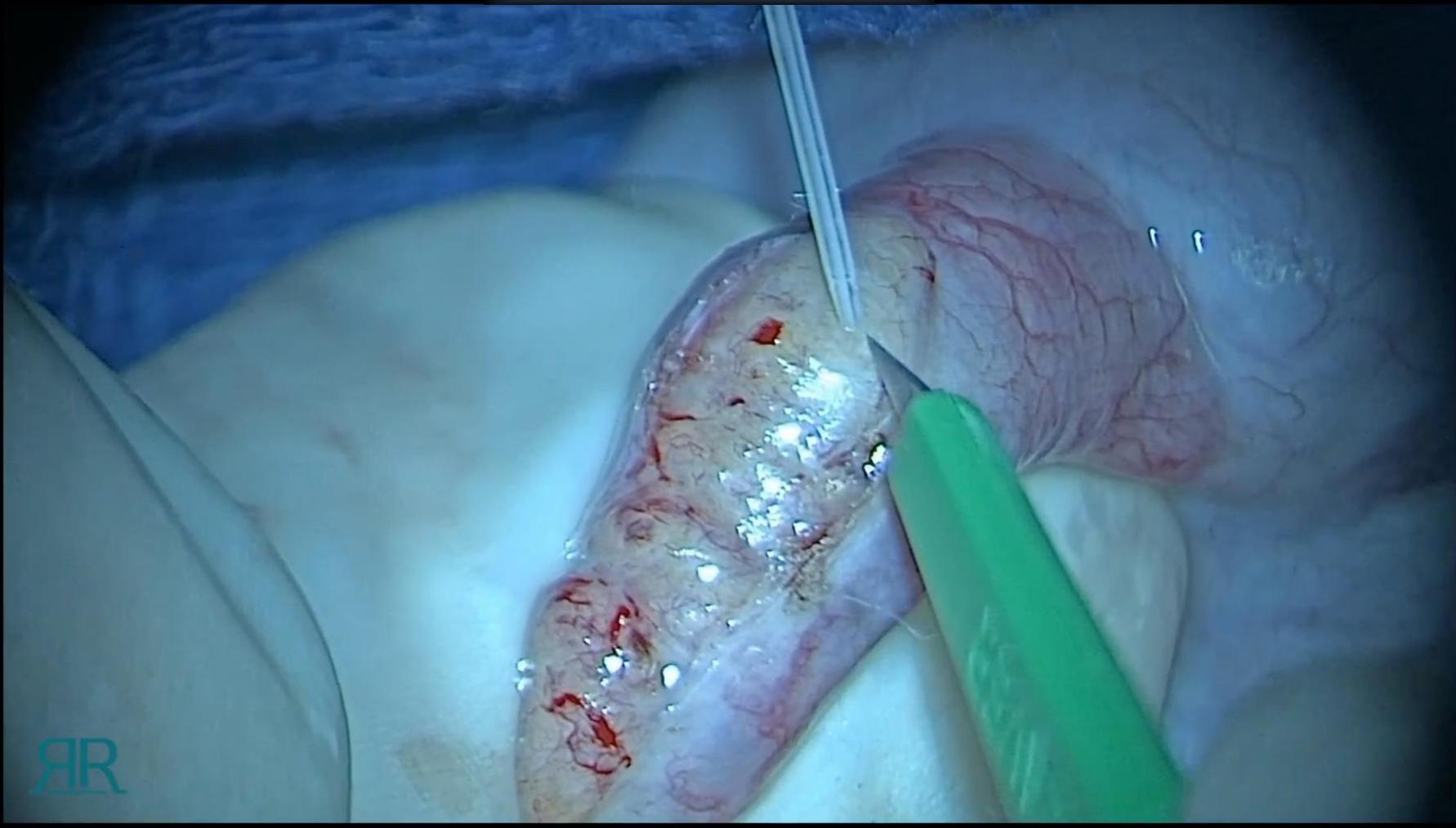
FERTILITY

MESA



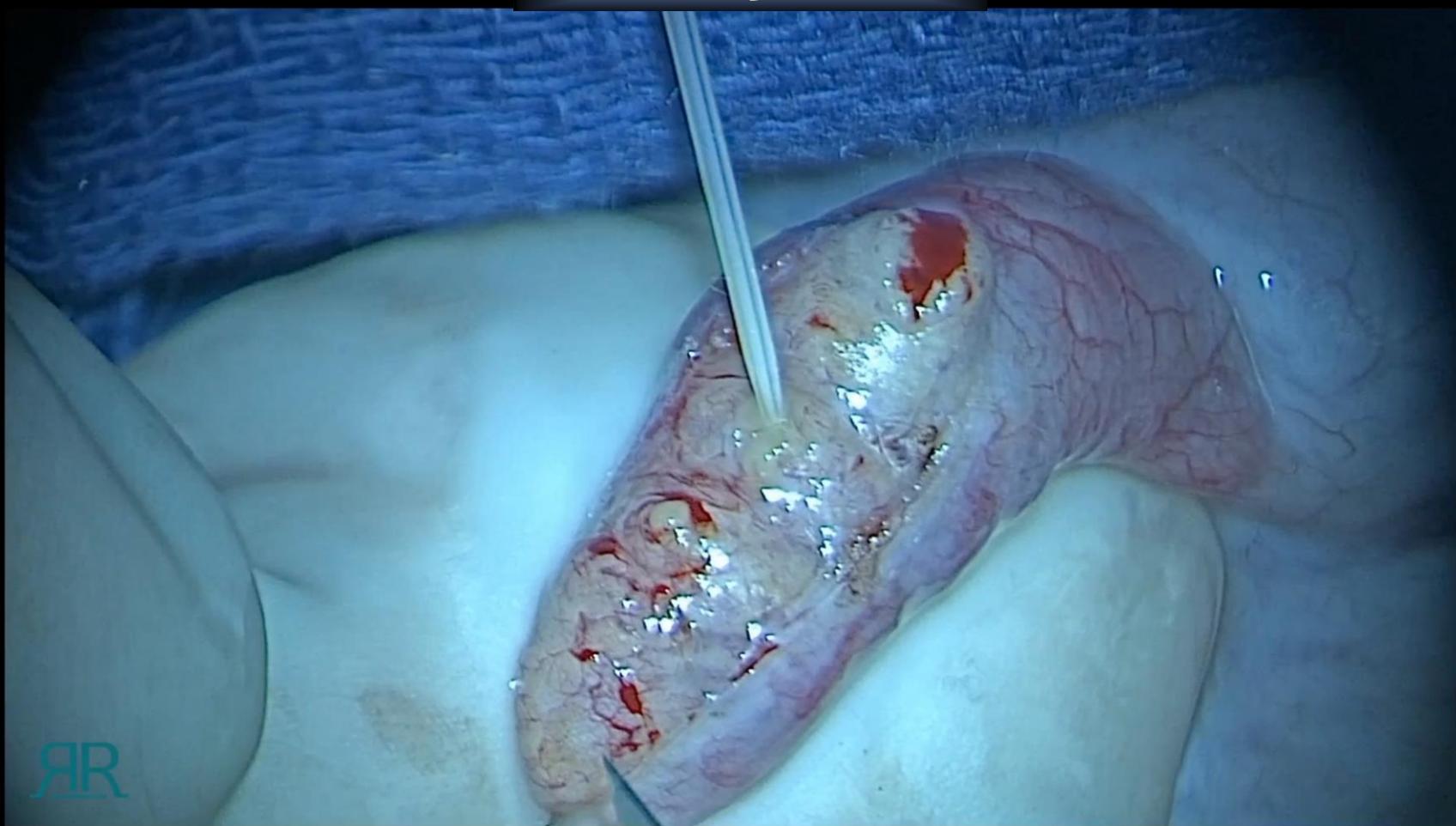
FERTILITY

MESA



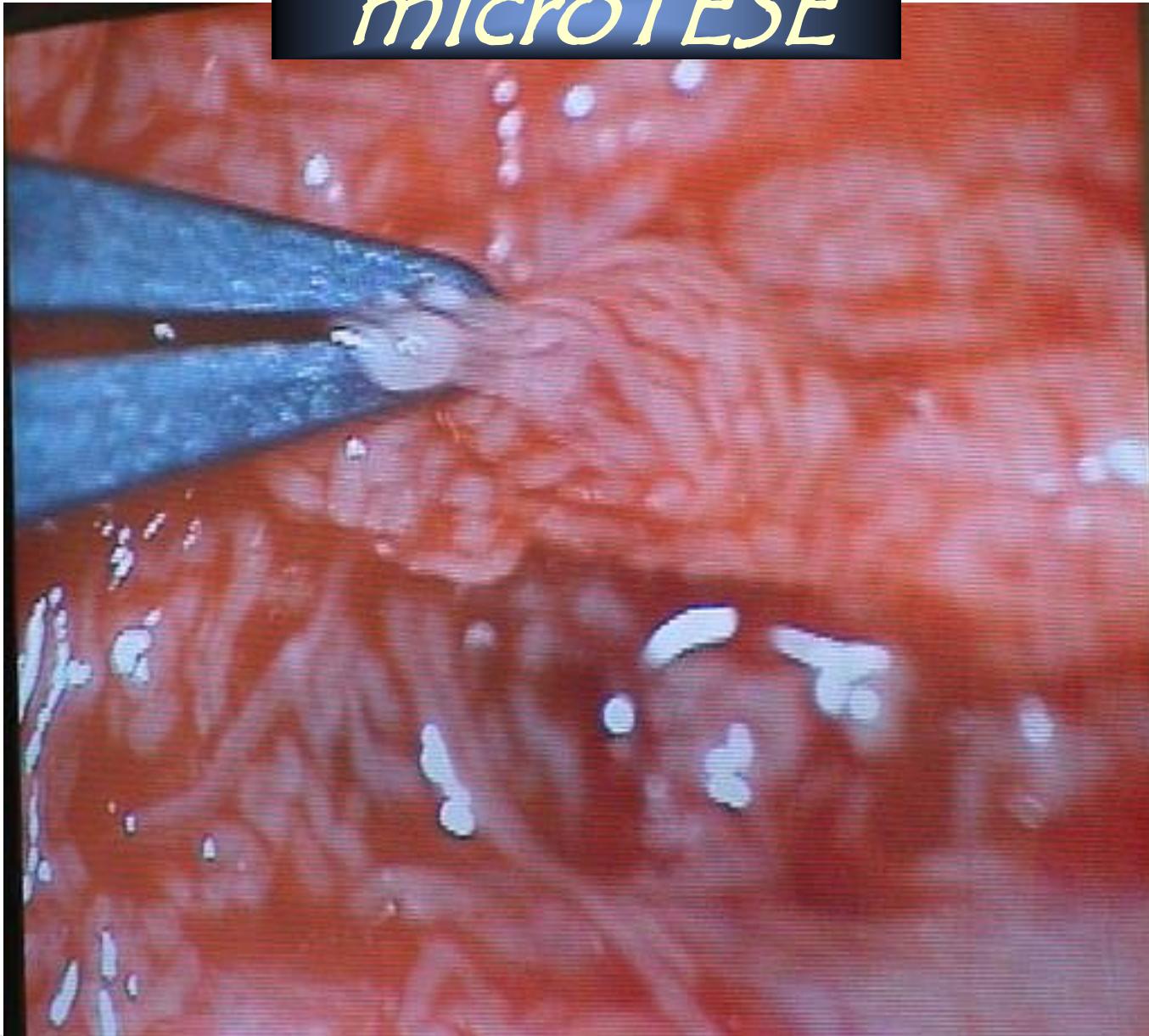
FERTILITY

MESA



FERTILITY

microTESE



FERTILITY

microTESE



FERTILITY



FERTILITY

¹Fertility – Assisted Fertilization Center, São Paulo, SP, Brazil, ²Sapientiae Institute – Educational and Research Center in Assisted Reproduction, São Paulo, SP, Brazil, and ³Institute of Biotechnology – Caxias do Sul University, Caxias do Sul, RS, Brazil

ORIGINAL ARTICLE

Assisted reproductive technology outcomes in azoospermic men: 10 years of experience with surgical sperm retrieval

Table II. ICSI outcomes from patients with obstructive azoospermia when the injected sperm were retrieved from the testicle (TESA) or epididymis (PESA).

Variable	Study group		
	OA-TESA (n=103)	OA-PESA (n=171)	p value
Normal fertilization rate (%)	57.9 \pm 9.5 (48.5–67.5)	65.2 \pm 4.1 (54.7–69.3)	0.0017
Abnormal fertilization rate (%)	13.2 \pm 6.3 (6.5–19.5)	12.7 \pm 5.3 (7.9–18.0)	0.9437
Fertilization failure rate (%)	28.9 \pm 8.9 (20.2–37.8)	22.1 \pm 6.0 (15.8–28.1)	0.1081
Non-cleaved rate (%)	9.87 \pm 5.9 (4.2–15.8)	7.46 \pm 3.9 (3.5–11.4)	0.4406
Pregnancy rate (%)	31.9 \pm 9.0 (23.0–41.0)	32.5 \pm 7.5 (25.9–40.0)	0.8803
Abortion rate (%)	38.8 \pm 9.6 (29.6–48.4)	18.0 \pm 5.8 (12.2–23.8)	0.0387
Implantation rate (%)	9.4 \pm 5.6 (3.8–15.0)	10.5 \pm 4.0 (5.5–14.5)	0.6054

Values in percentage expressed as mean \pm SD (confidence interval of the frequencies).

¹Fertility – Assisted Fertilization Center, São Paulo, SP, Brazil, ²Sapientiae Institute – Educational and Research Center in Assisted Reproduction, São Paulo, SP, Brazil, and ³Institute of Biotechnology – Caxias do Sul University, Caxias do Sul, RS, Brazil

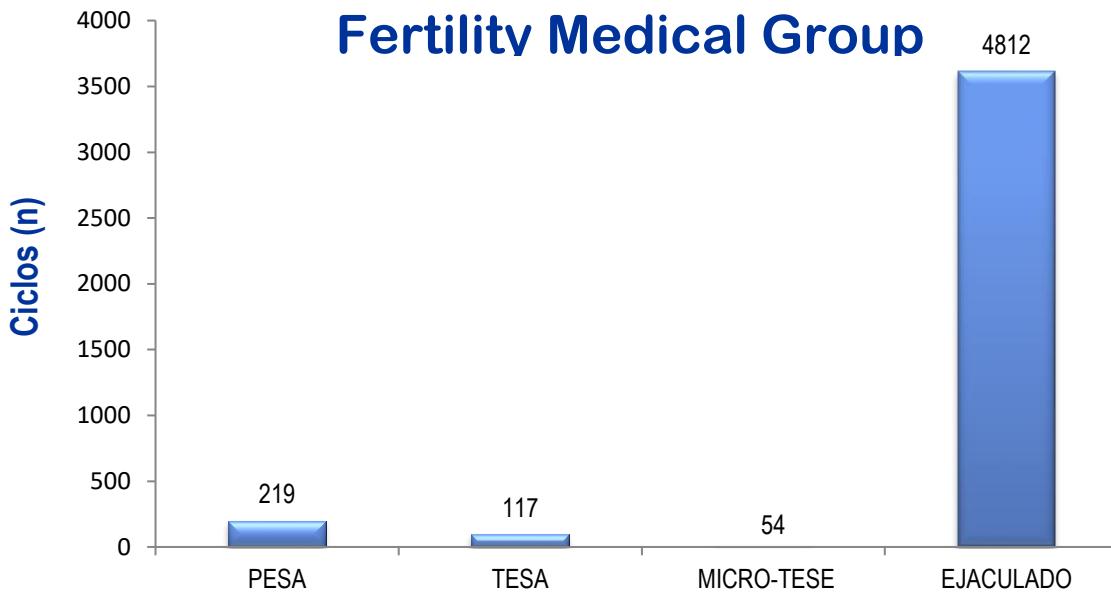
ORIGINAL ARTICLE

Assisted reproductive technology outcomes in azoospermic men: 10 years of experience with surgical sperm retrieval

Table II. ICSI outcomes from patients with obstructive azoospermia when the injected sperm were retrieved from the testicle (TESA) or epididymis (PESA).

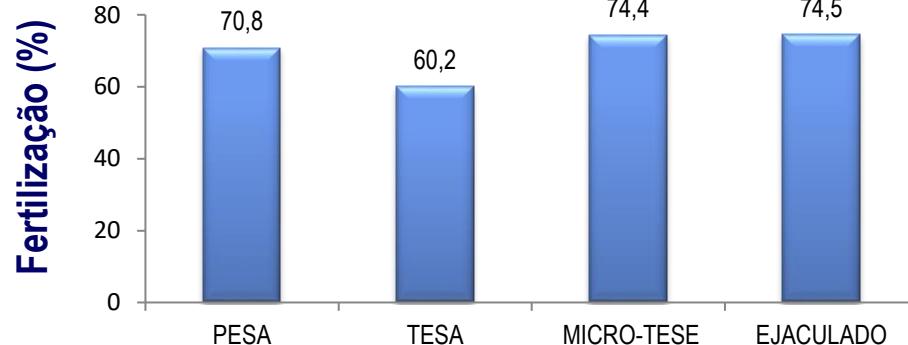
Variable	Study group		
	OA-TESA (n=103)	OA-PESA (n=171)	p value
Normal fertilization rate (%)	57.9 \pm 9.5 (48.5–67.5)	65.2 \pm 4.1 (54.7–69.3)	0.0017
Abnormal fertilization rate (%)	13.2 \pm 6.3 (6.5–19.5)	12.7 \pm 5.3 (7.9–18.0)	0.9437
Fertilization failure rate (%)	28.9 \pm 8.9 (20.2–37.8)	22.1 \pm 6.0 (15.8–28.1)	0.1081
Non-cleaved rate (%)	9.87 \pm 5.9 (4.2–15.8)	7.46 \pm 3.9 (3.5–11.4)	0.4406
Pregnancy rate (%)	31.9 \pm 9.0 (23.0–41.0)	32.5 \pm 7.5 (25.9–40.0)	0.8803
Abortion rate (%)	38.8 \pm 9.6 (29.6–48.4)	18.0 \pm 5.8 (12.2–23.8)	0.0387
Implantation rate (%)	9.4 \pm 5.6 (3.8–15.0)	10.5 \pm 4.0 (5.5–14.5)	0.6054

Values in percentage expressed as mean \pm SD (confidence interval of the frequencies).



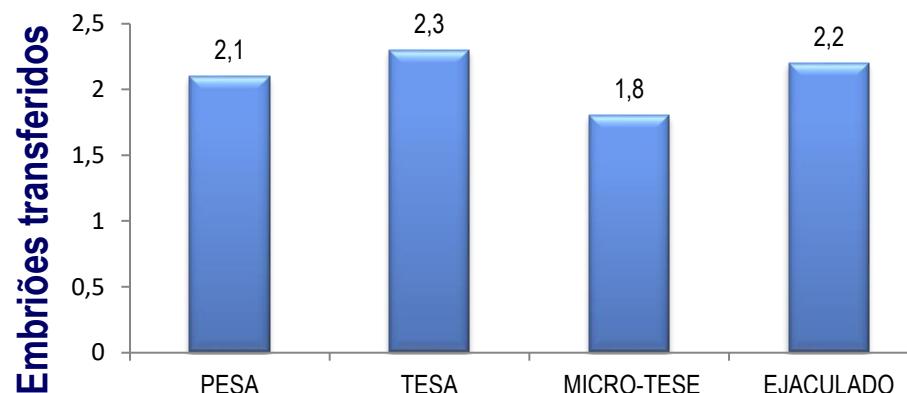
	PESA	TESA	MICRO-TESE	EJACULADO
Ciclos (n)	219	117	54	4812
Idade ± DP	34.9 ± 4.6	34.8 ± 5.4	32.2 ± 2.7	35.8 ± 4.7
Folículos ± DP	20.4 ± 15.4	18.1 ± 11.3	15.9 ± 14.4	15.8 ± 12.4
Oocitos recuperados ± DP	14.2 ± 10.8	13.3 ± 9.3	11.0 ± 11.4	11.0 ± 9.0
Oocitos micromanipulados ± DP	9.8 ± 6.4	8.9 ± 5.1	8.0 ± 6.9	7.8 ± 5.8

Fertility Medical Group



	P
PESA VS TESA	< 0.001
PESA VS MICRO-TESE	> 0.05
PESA VS EJACULADO	> 0.05
TESA VS MICRO-TESE	> 0.05
TESA VS EJACULADO	< 0.001
MICRO-TESE VS EJACULADO	> 0.05

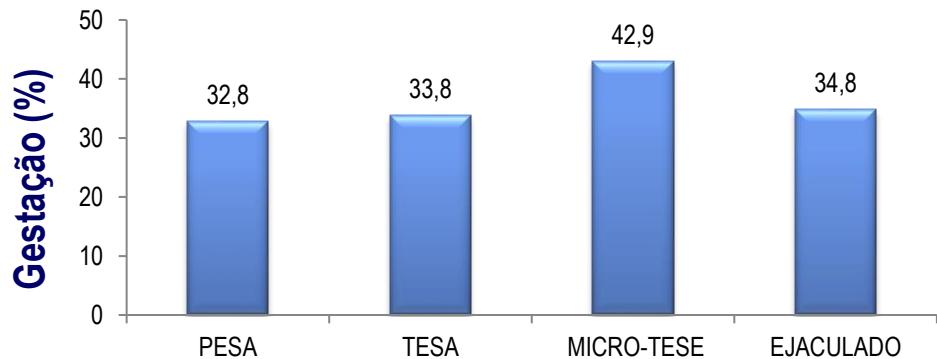
ANOVA



	P
PESA VS TESA	> 0.05
PESA VS MICRO-TESE	> 0.05
PESA VS EJACULADO	> 0.05
TESA VS MICRO-TESE	> 0.05
TESA VS EJACULADO	> 0.05
MICRO-TESE VS EJACULADO	> 0.05

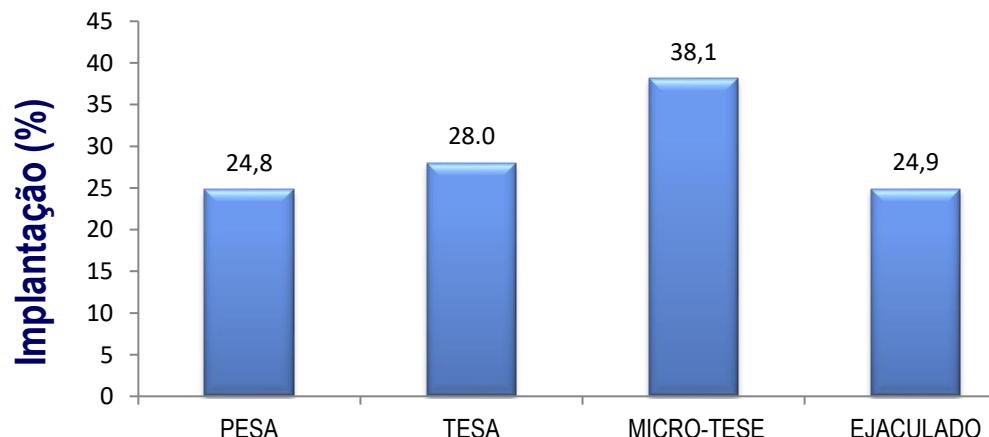
ANOVA

Fertility Medical Group



	P
PESA VS TESA	> 0.05
PESA VS MICRO-TESE	> 0.05
PESA VS EJACULADO	> 0.05
TESA VS MICRO-TESE	> 0.05
TESA VS EJACULADO	> 0.05
MICRO-TESE VS EJACULADO	> 0.05

QUI-QUADADRO



	P
PESA VS TESA	> 0.05
PESA VS MICRO-TESE	> 0.05
PESA VS EJACULADO	> 0.05
TESA VS MICRO-TESE	> 0.05
TESA VS EJACULADO	> 0.05
MICRO-TESE VS EJACULADO	> 0.05

ANOVA



FERTILITY

Predictive Factors of Repeat Sperm Aspiration Success

**Edson Borges, Jr., Daniela Paes de Almeida Ferreira Braga,
Tatiana Carvalho de Sousa Bonetti, Fabio Firmback Pasqualotto, and
Assumpto Iaconelli Jr.**



- ☞ 189 pacientes AO=80 - ANO=109; 290 TESA
 - ❖ TESA 1X: 143
 - ❖ TESA 2X: 46
 - ❖ TESA 3X: 42
 - ❖ TESA 4X: 19



Predictive Factors of Repeat Sperm Aspiration Success

**Edson Borges, Jr., Daniela Paes de Almeida Ferreira Braga,
Tatiana Carvalho de Sousa Bonetti, Fabio Firmback Pasqualotto, and
Assumpto Iaconelli Jr.**



<i>TESA attempt</i>	<i>Positive sperm retrieval in a previous attempt</i>	<i>Negative sperm retrieval in a previous attempt</i>
Second	26/28 (92.8%)	3/18 (16.6%)
Third	23/28 (82.1%)	8/14 (57.1%)
Fourth	10/14 (71.4%)	2/5 (40.0%)

Cumulative sperm retrieval rate following a previous fail 35.1%



FERTILITY

MALE FACTOR

Fertility and Sterility® Vol. 83, No. 3, March 2005
Copyright ©2005 American Society for Reproductive Medicine.

Etiology-specific outcomes of intracytoplasmic sperm injection in azoospermic patients

Fábio F. Pasqualotto, M.D., Ph.D.^a Lia Mara Rossi, B.Sc., M.Sc.^{a,b}

Patrícia Guilherme, B.Sc., M.Sc.^a Valdemar Ortiz, M.D., Ph.D.^c Assumpto Iaconelli, Jr., M.D.,^a and Edson Borges, Jr., M.D.^a

^aFertility – Center for Assisted Reproduction, São Paulo; ^bJundiaí Medical School, São Paulo; and ^cDepartment of Urology, Federal University of São Paulo, São Paulo, Brazil

	PESA	TESA
2PN (%) ^a	61.4	59.4
1 PN + 3 PN (%)	24.1	12.8
Gestação / ciclo (%)	31.76	22.66
Gestação / paciente (%)	42.60*	26.15
Aborto (%)	11.11	44.11*

MALE FACTOR

Fertility and Sterility® Vol. 83, No. 3, March 2005
Copyright ©2005 American Society for Reproductive Medicine.

Etiology-specific outcomes of intracytoplasmic sperm injection in azoospermic patients

Fábio F. Pasqualotto, M.D., Ph.D.,^a Lia Mara Rossi, B.Sc., M.Sc.,^{a,b}

Patrícia Guilherme, B.Sc., M.Sc.,^a Valdemar Ortiz, M.D., Ph.D.,^c Assumpto Iaconelli, Jr., M.D.,^a and Edson Borges, Jr., M.D.^a

^a Fertility – Center for Assisted Reproduction, São Paulo; ^b Jundiaí Medical School, São Paulo; and ^c Department of Urology, Federal University of São Paulo, São Paulo, Brazil

	NOA	Vasectomia	Congenita	Infecção	P
Ciclos / pacientes	102 / 84	99 / 84	25 / 20	31 / 24	
Gestações	22	31	7	7	
2PN (%)	47.3	64.1 ^a	67.7 ^a	58.9	0.041
NF (%)	32.2 ^b	19.2	21.0	30.7 ^b	0.005
Gestação / ciclo (%)	21.56	31.31	28	22.58	0.812
Gestação / paciente (%)	26.2	36.1	35	29.2	0.812
Implantação (%)	12.5	13.4	28.5 ^c	12.9	0.032
Aborto (%)	45.6 ^d	25.8	28.57	28.57	0.614

Does acquired obstructive azoospermia have less impact than congenital azoospermia on ICSI results? Systematic review and meta-analysis

Leonardo Seligra Lopes¹  | Willy Baccaglini¹ | Bruno von Muhlen¹ |
Felipe Placco Araujo Glina¹ | Sergio Albertini Daiuto¹ | Cristiano Linck Pazeto¹ |
Sidney Glina^{1,2}

Comparing specifically post-vasectomy azoospermia and congenital groups, both presented:

- a similar fertilization rate (62.4% vs. 53.4%, respectively; OR 1.10; 95% CI, 0.79, 1.54; $p = .56$; $I_2 = 89\%$).
- a similar pregnancy rate per cycle (39.4% vs. 35.6%, respectively; OR 1.26; 95% CI, 0.96, 1.66; $p = .09$; $I_2 = 0\%$).
- Higher live birth rate was identified in the congenital group compared to vasectomy group (28.4% × 19.5%; OR 1.54; 95% CI, 1.11, 2.15; $p = .01$; $I_2 = 0\%$).

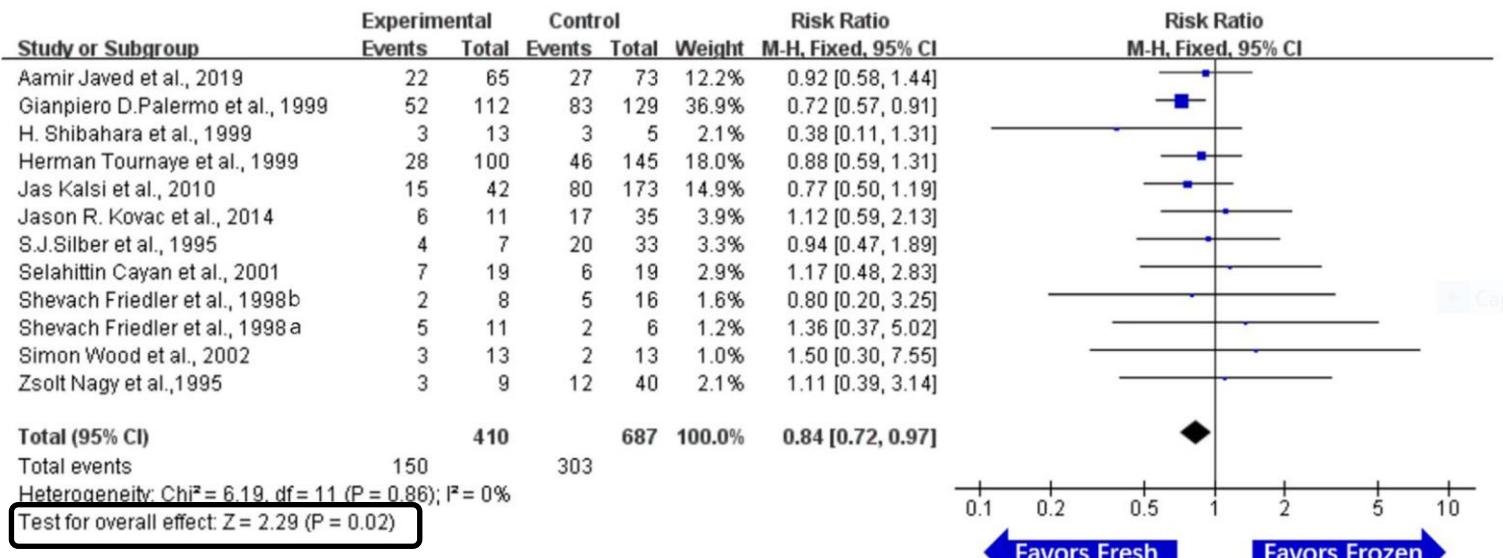


Check for
updates

Impact on using cryopreservation of testicular or epididymal sperm upon intracytoplasmic sperm injection outcome in men with obstructive azoospermia: a systematic review and meta-analysis

Hanchao Liu¹ · Yun Xie¹ · Linzhi Gao² · Xiangzhou Sun¹ · Xiaoyan Liang² · Chunhua Deng¹ · Yong Gao³ · Guihua Liu²

b



Epididymal
Clinical pregnancy rate



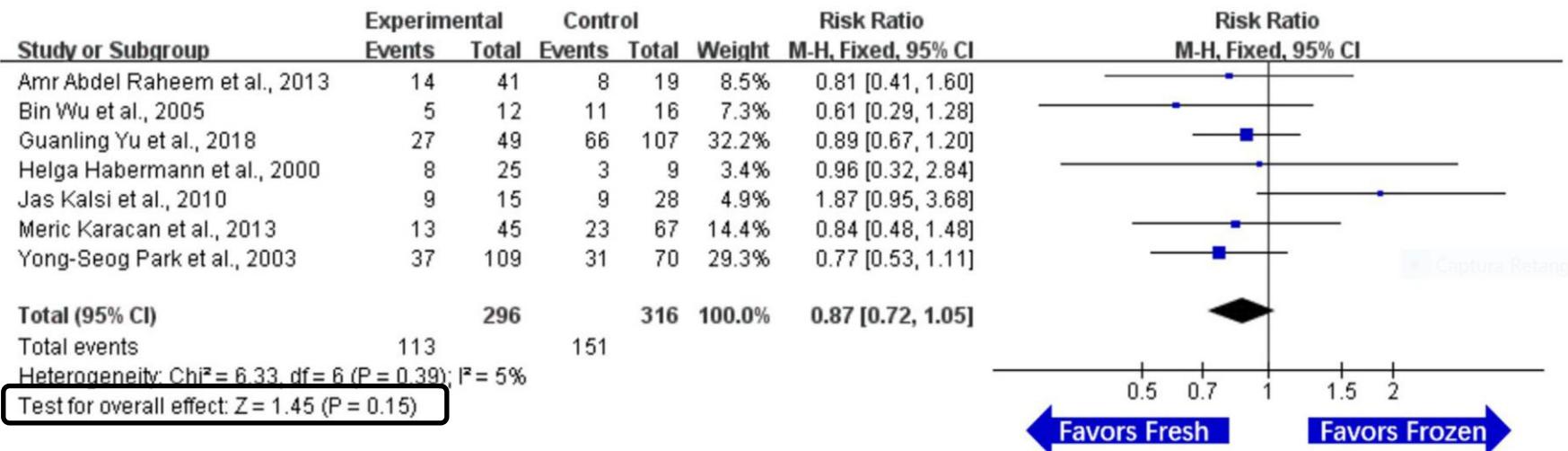
FERTILITY



Impact on using cryopreservation of testicular or epididymal sperm upon intracytoplasmic sperm injection outcome in men with obstructive azoospermia: a systematic review and meta-analysis

Hanchao Liu¹ · Yun Xie¹ · Linzhi Gao² · Xiangzhou Sun¹ · Xiaoyan Liang² · Chunhua Deng¹ · Yong Gao³ · Guihua Liu²

b



**Tesicular
Clinical pregnancy rate**

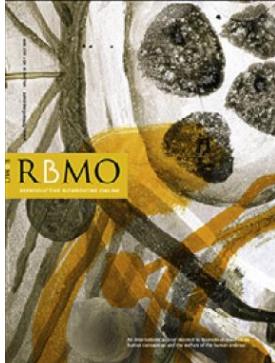
The obstructive interval predicts pregnancy rates in post-vasectomy patients undergoing ICSI with surgical sperm retrieval



BIOGRAPHY

Edson Borges Jr obtained his MD degree in 1984 (University of Campinas), PhDs in urology in 2005 (Federal University of São Paulo) and in gynaecology in 2007 (São Paulo State University). He is founder and managing partner of Fertility Medical Group and director at Sapientiae Institute, São Paulo, Brazil.

Edson Borges Jr^{1,2,*}, Daniela Paes de Almeida Ferreira Braga^{1,2},
Assumpto Iaconelli Jr^{1,2}, Amanda Souza Setti^{1,2}



RBMO VOLUME 39 ISSUE 1 2019

TABLE 2 INFLUENCE OF THE OBSTRUCTIVE INTERVAL ON SSR OUTCOMES

SSR parameter ^a	95% CI				
	Estimate (β)	SE	P-value	Lower bound	Upper bound
Presence of spermatozoa during PESA	-0.032	0.012	0.009	-0.056	-0.009
Presence of motile spermatozoa during PESA	-0.031	0.012	0.010	-0.054	-0.008
Need to convert to TESA	0.012	0.004	0.003	0.004	0.019

CI = confidence interval; PESA = percutaneous epididymal sperm aspiration; SE = standard error; SSR = surgical sperm retrieval; TESA = testicular sperm aspiration.

^a Adjusted for paternal age, smoking habit, previous vasectomy reversal attempt, hormonal profile and abnormalities found in the male partner physical examination.



TABLE 4 INFLUENCE OF THE OBSTRUCTIVE INTERVAL ON THE OUTCOMES OF ICSI WITH SSR

ICSI outcome	95% CI				
	Estimate (β)	SE	P-value	Lower bound	Upper bound
Fertilization rate ^a	-0.098	0.302	NS	-0.696	0.500
Day 2 high-quality embryos rate ^a	-0.001	0.003	NS	-0.007	0.005
Day 3 high-quality embryos rate ^a	0.001	0.003	NS	-0.003	0.007
Blastocyst development rate ^a	-0.011	0.004	0.014	-0.019	-0.002
Clinical pregnancy rate ^b	-0.016	0.007	0.031	-0.031	-0.001
Implantation rate ^b	-1.107	0.530	0.039	-2.157	-0.056
Miscarriage rate ^b	0.006	0.009	NS	-0.012	0.025

^a Adjusted for maternal and paternal ages, paternal smoking habit, previous vasectomy reversal attempt, paternal hormonal profile and abnormalities found in the male partner physical examination, and number of retrieved oocytes.

^b Adjusted for the same variables cited above plus number of transferred embryos. CI = confidence interval;

ICSI = intracytoplasmic sperm injection; NS = not significant; SE = standard error; SSR = surgical sperm retrieval.

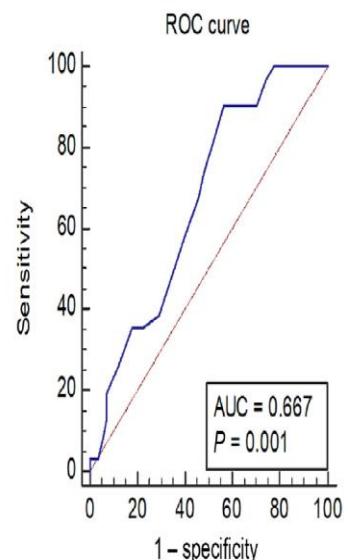


FIGURE 1 Receiver operating characteristic (ROC) curve for predicting clinical pregnancy using obstruction interval as test variable.

Successful twin pregnancy with intracytoplasmic sperm injection using surgical sperm retrieval after 25 years of vasectomy: a case report

Edson Borges Jr.^{1,2}, Amanda Souza Setti^{1,2}, Daniela Paes de Almeida Ferreira Braga^{1,2}, Assumpto Iaconelli Jr.^{1,2}

¹Fertility Medical Group, São Paulo, SP - Brazil.

²Instituto Sapientiae - Centro de Estudos e Pesquisa em Reprodução Humana Assistida, São Paulo, SP - Brazil.

The couple from this clinical case consisted of a 55 years old male with an obstructive interval of 25 years post vasectomy, and a 38 years old female partner.

A clinical pregnancy was confirmed by the detection of two gestational sacs with foetal heartbeats.





FERTILITY
MEDICAL GROUP

HOME GRUPO FERTILITY ▾ INFERTILIDADE ▾ TRATAMENTOS ▾ DIAGNÓSTICOS EDUCAÇÃO ▾ NEWS ▾ CONTATO

ENSINO

INSTITUTO SAPIENTIAE

PESQUISA

AULAS MINISTRADAS

ATIVIDADES

VÍDEOS TÉCNICOS

EVENTOS

E-BOOKS

ENQUETES

LINKS ÚTEIS

AULAS MINISTRADAS

2022 2021 2020 2019 2018 2017 2016 2015

<https://fertility.com.br/aulas-ministradas/>



FERTILITY

Obrigado!

Dr. Edson Borges Jr.

www.fertility.com.br

E-mail: edson@fertility.com.br



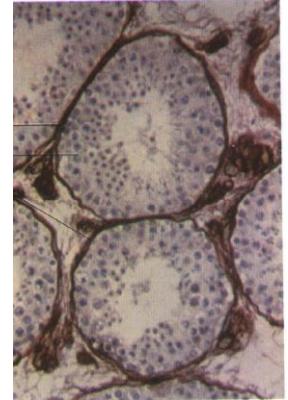
FERTILITY



FERTILITY



FERTILITY



Biópsia Testicular

- Possibilidade de congelar espermatozoides / tecido testicular
- Diagnóstico de CA *in situ* (CIS): 1 - 3% pacientes com inferilidade masculina
- Realização com técnica microcirúrgica no momento do ICSI



FERTILITY

**ESPERMATOZOIDE
EJACULADO**

Espermatozoide
do epidídimo

Espermatozoide
do testículo

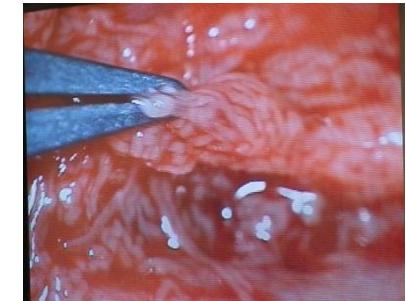
PERCUTANEOUS
EPIDYDIMAL
SPERM
ASPIRATION



TESTICULAR
SPERM
. ASPIRATION
. EXTRACTION



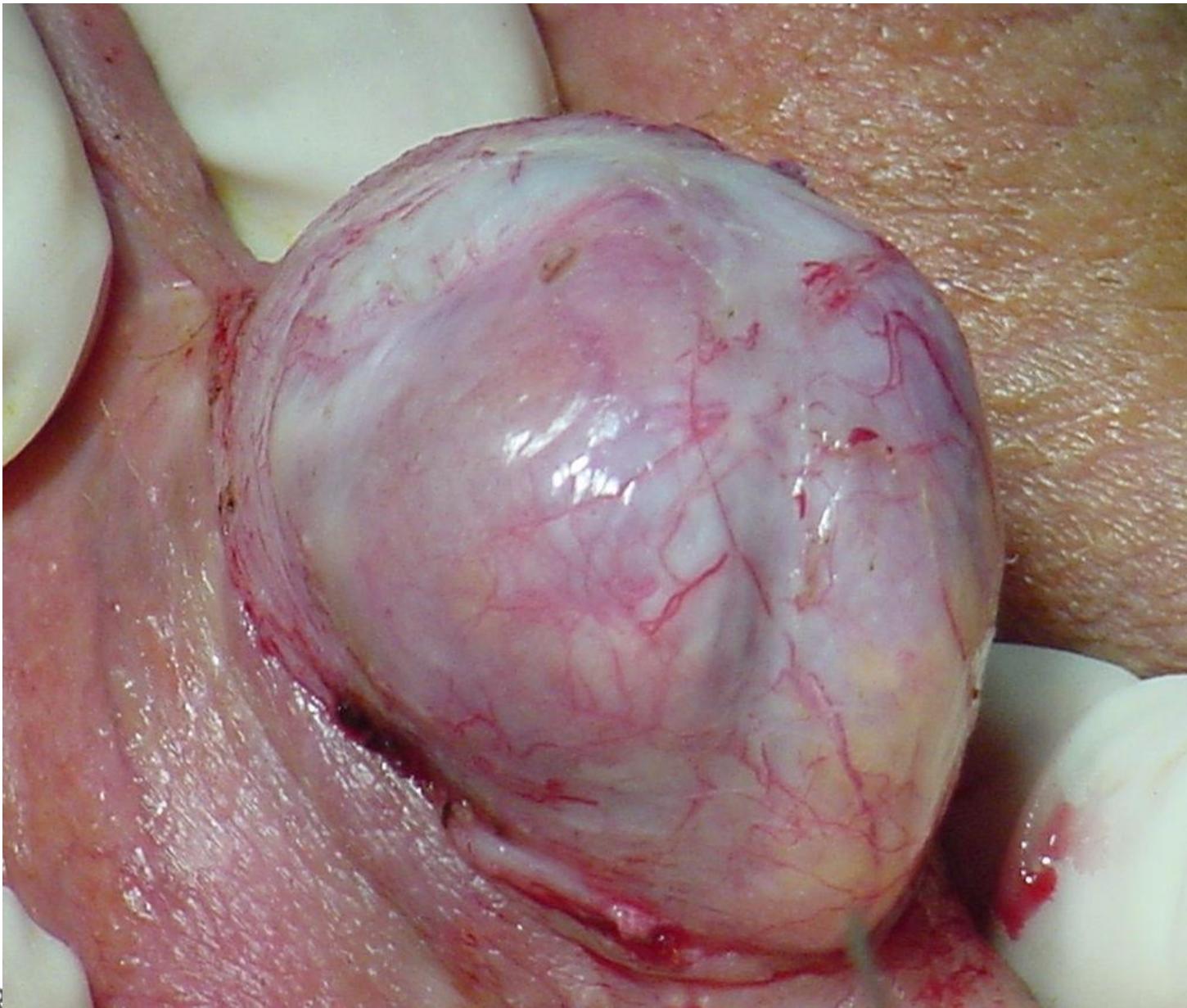
MICRO
TESTICULAR
SPERM
EXTRACTION



FERTILITY



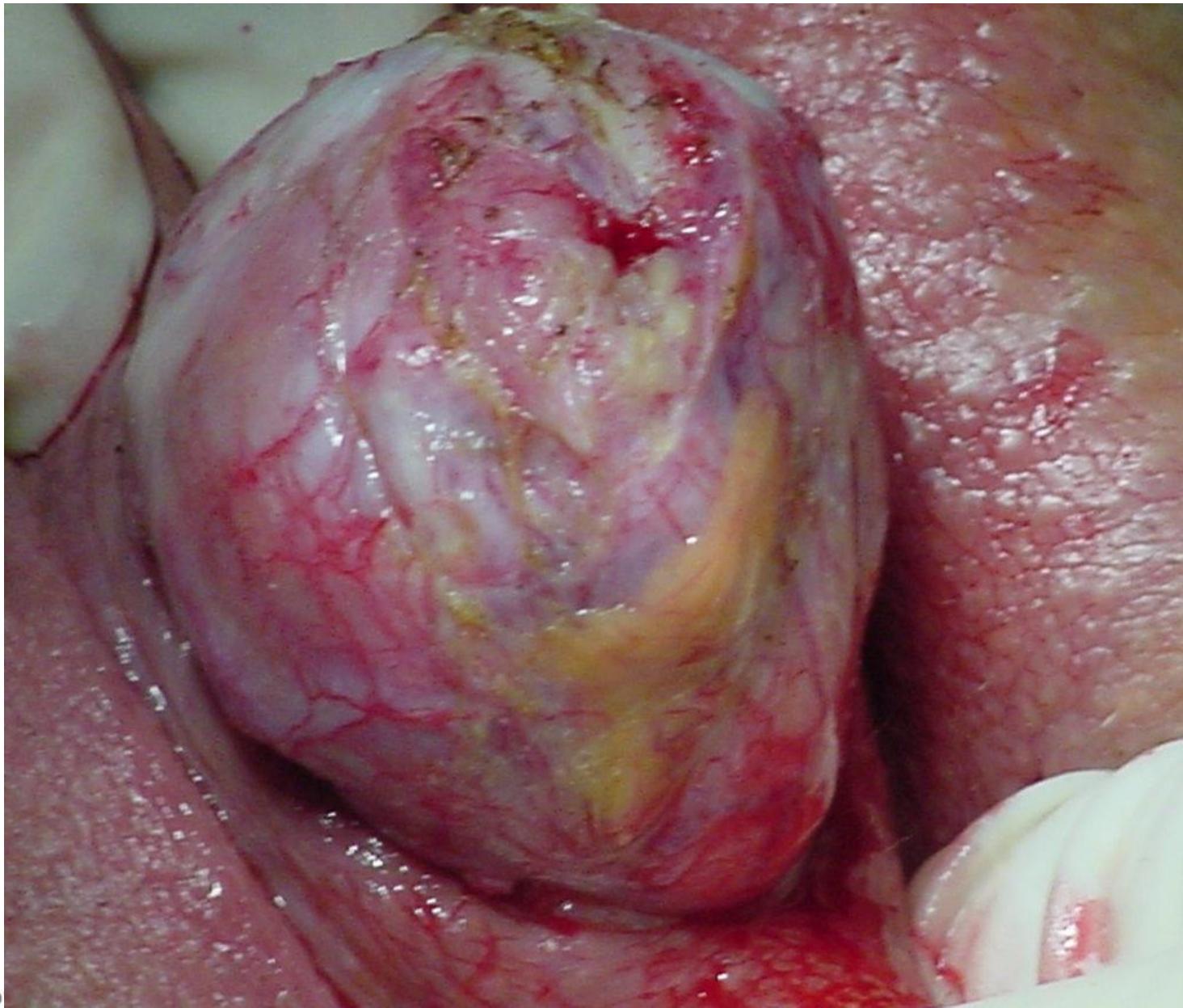
FERTILITY



FERMENT



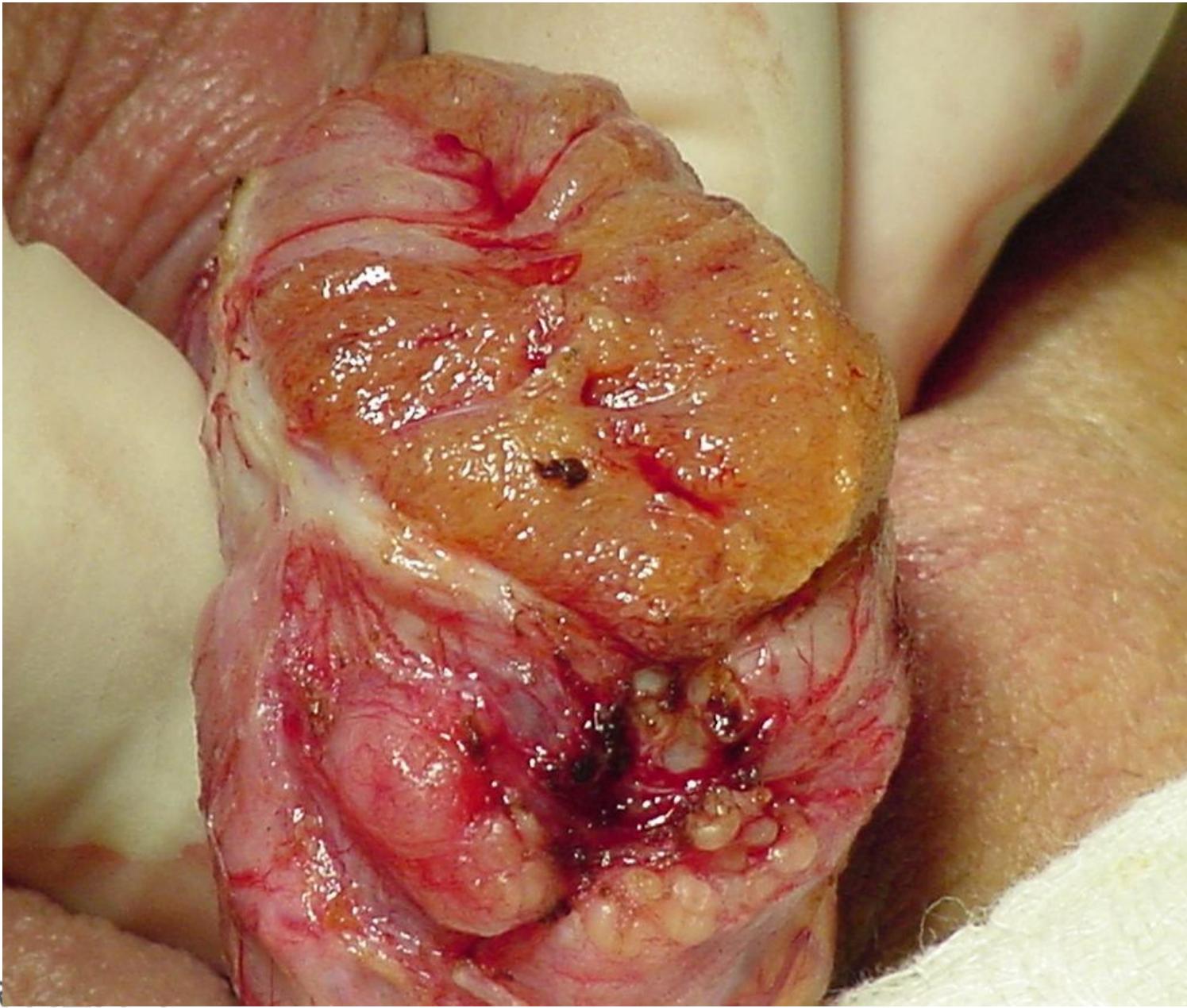
FERTILITY



FER HEMI



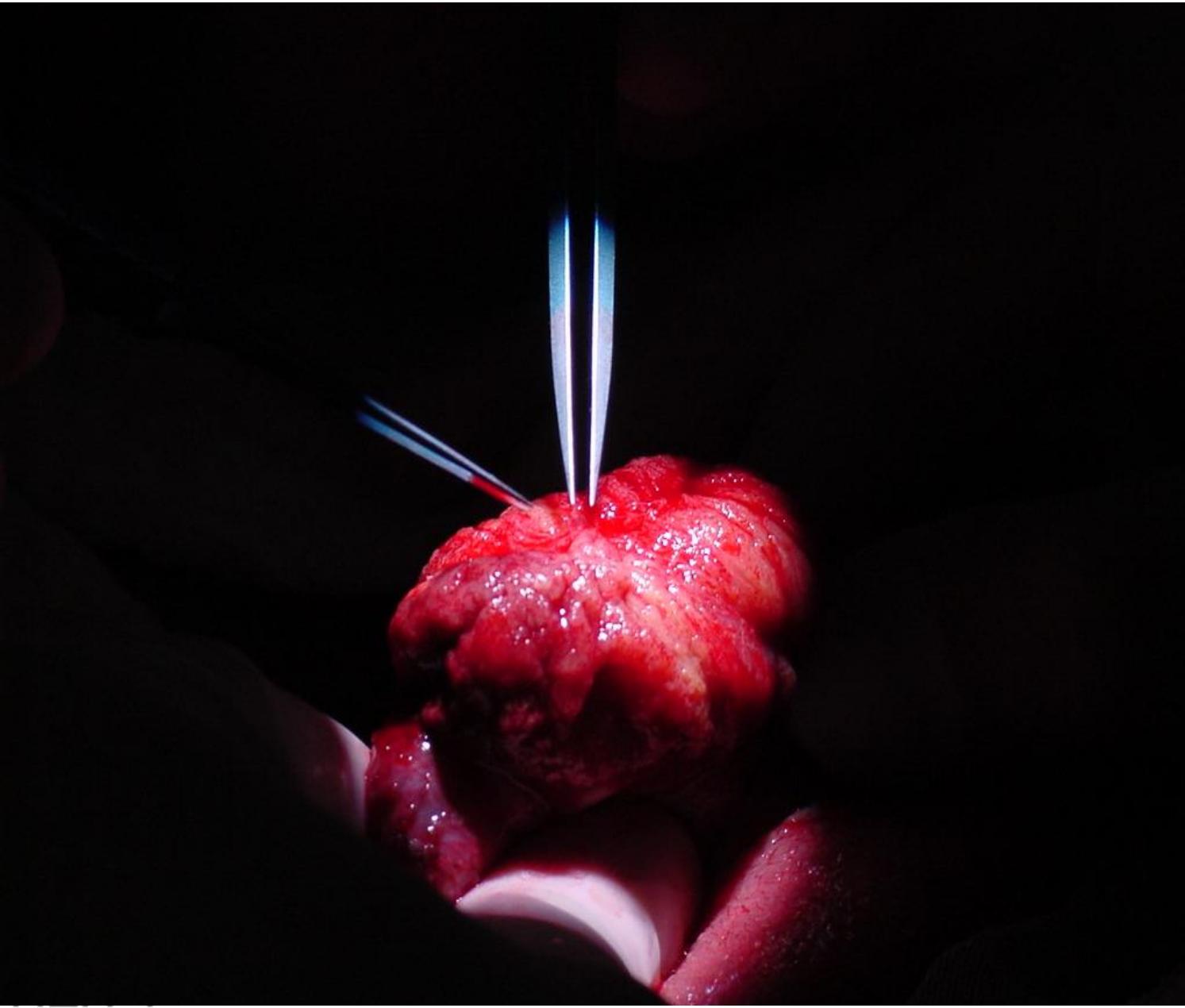
FER



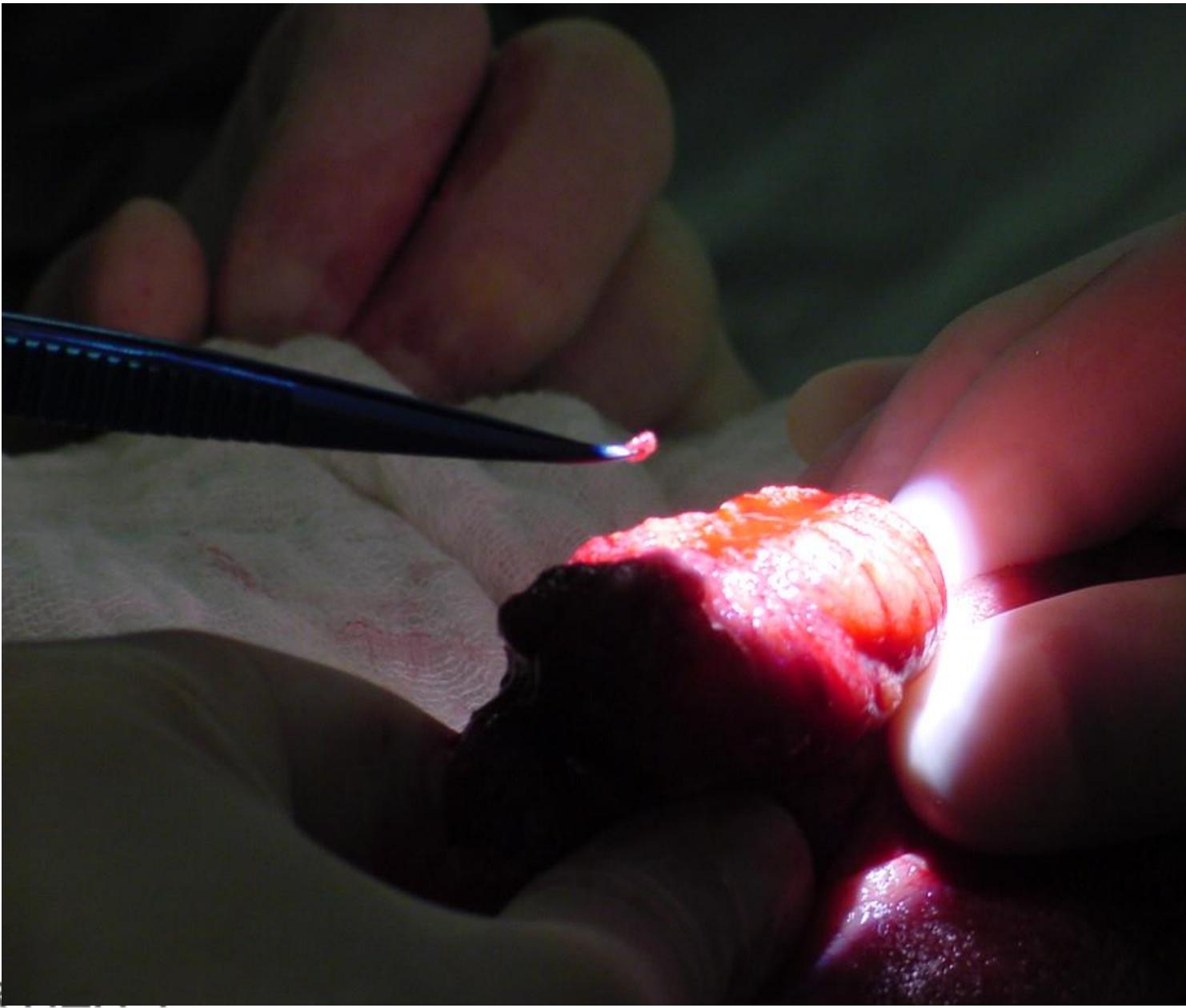
FER



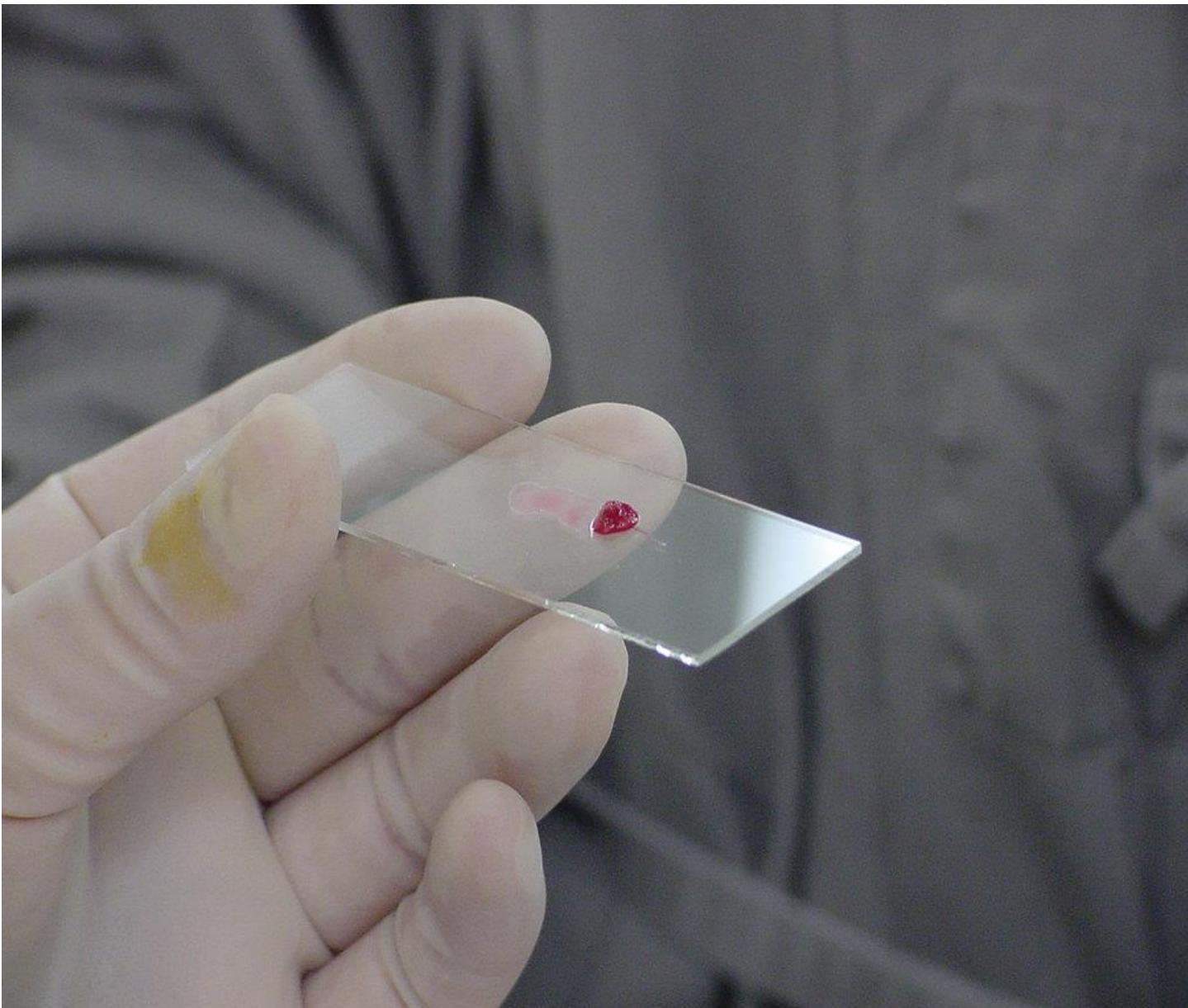
FERTILITY



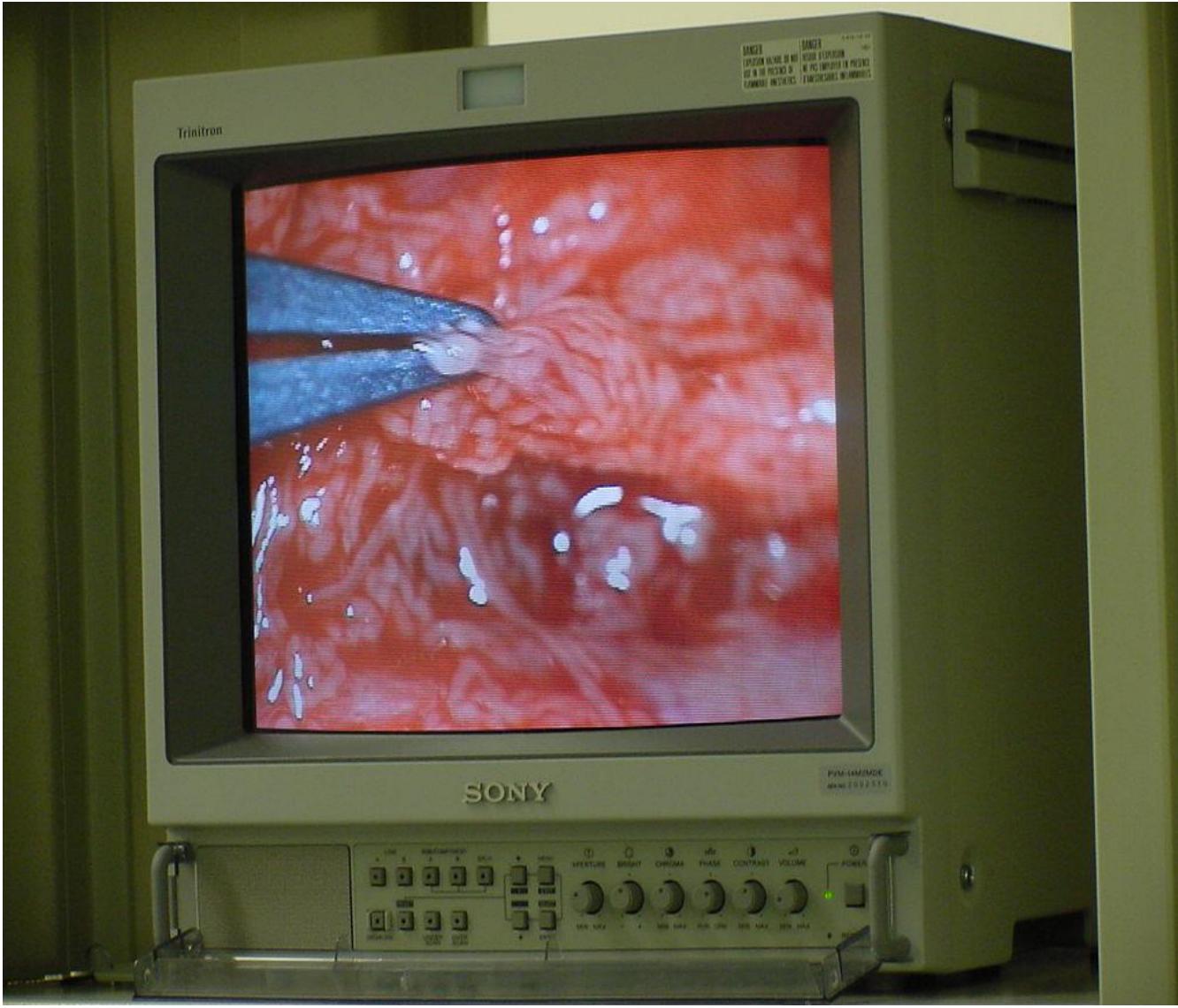
FER



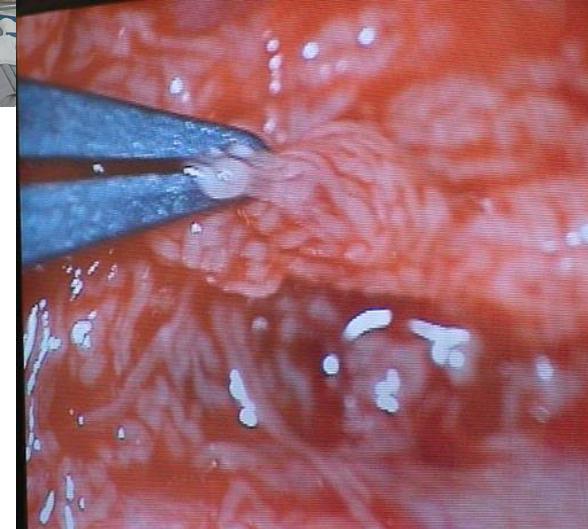
FER



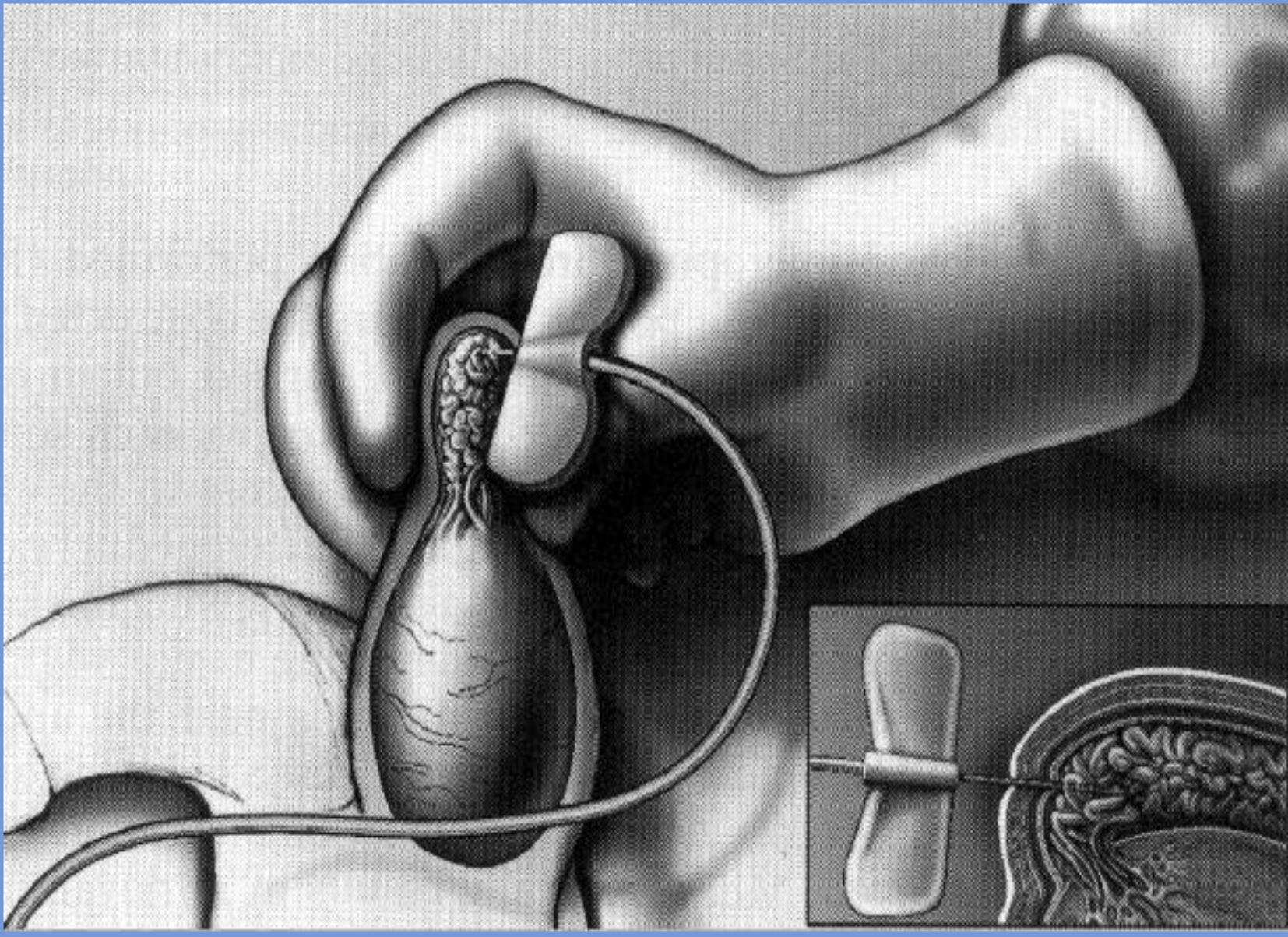
FERTILITY



PESA / TESA / microTESE



FERTILITY



FERTILITY



FERTILITY



FERTILITY



FERTILITY

Pregnancy after vasectomy: surgical reversal or assisted reproduction?

U. Valerie¹, S. De Brucker², M. De Brucker^{1,3,*}, V. Vloeberghs¹,
P. Drakopoulos¹, S. Santos-Ribeiro¹, and H. Tournaye¹

¹Department of Gynaecology and Fertility, Universitair Ziekenhuis Brussel, Laarbeeklaan 101, B1090 Brussels, Belgium ²Department of Urology, Universitair Ziekenhuis Brussel, Laarbeeklaan 101, B1090 Brussels, Belgium ³Department of Obstetrics and Gynaecology, CHU Tivoli, Avenue Max Buset, B7100 La Louvière, Belgium

→ Recanalisation of the vas seems to be a reasonable alternative for patients who do not wish to undergo immediate IVF/ICSI.

→ In those who opt for ART immediately, the cumulative pregnancy rates seem comparable but the pregnancies occurred earlier.

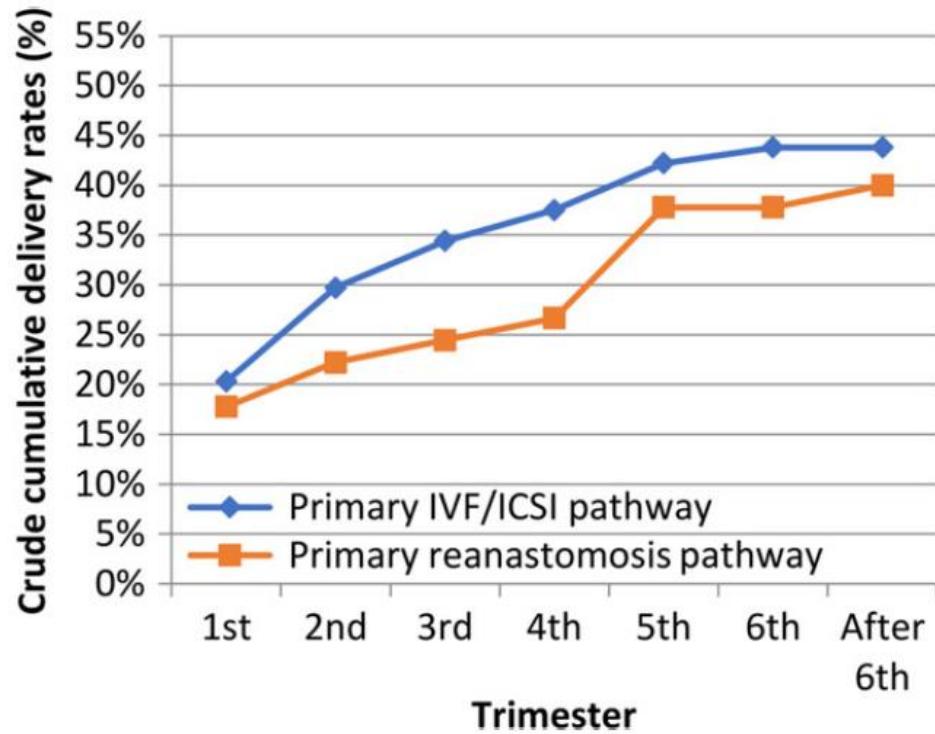
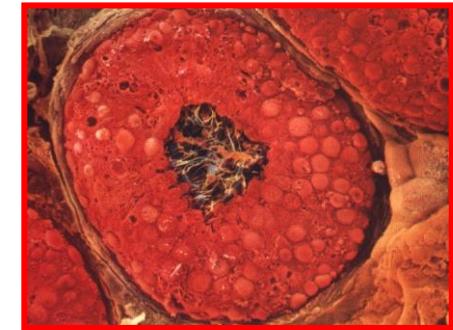


Figure 3 Crude cumulative delivery rates in the primary IVF/ICSI pathway ($n = 64$) and the primary reanastomosis pathway ($n = 45$).

AZOOSPERMIA OBSTRUTIVA (excretora)

- **CONGÊNITA**

- Agenesia congênita bilateral dos ductos deferentes



- **ADQUIRIDA**

- Processos inflamatórios-infecciosos dos epidídimos / traumas
- Vasectomia
- Neurológica
- Obstrução ductos ejaculatórios



FERTILITY

TESE MICROCIRURGICA

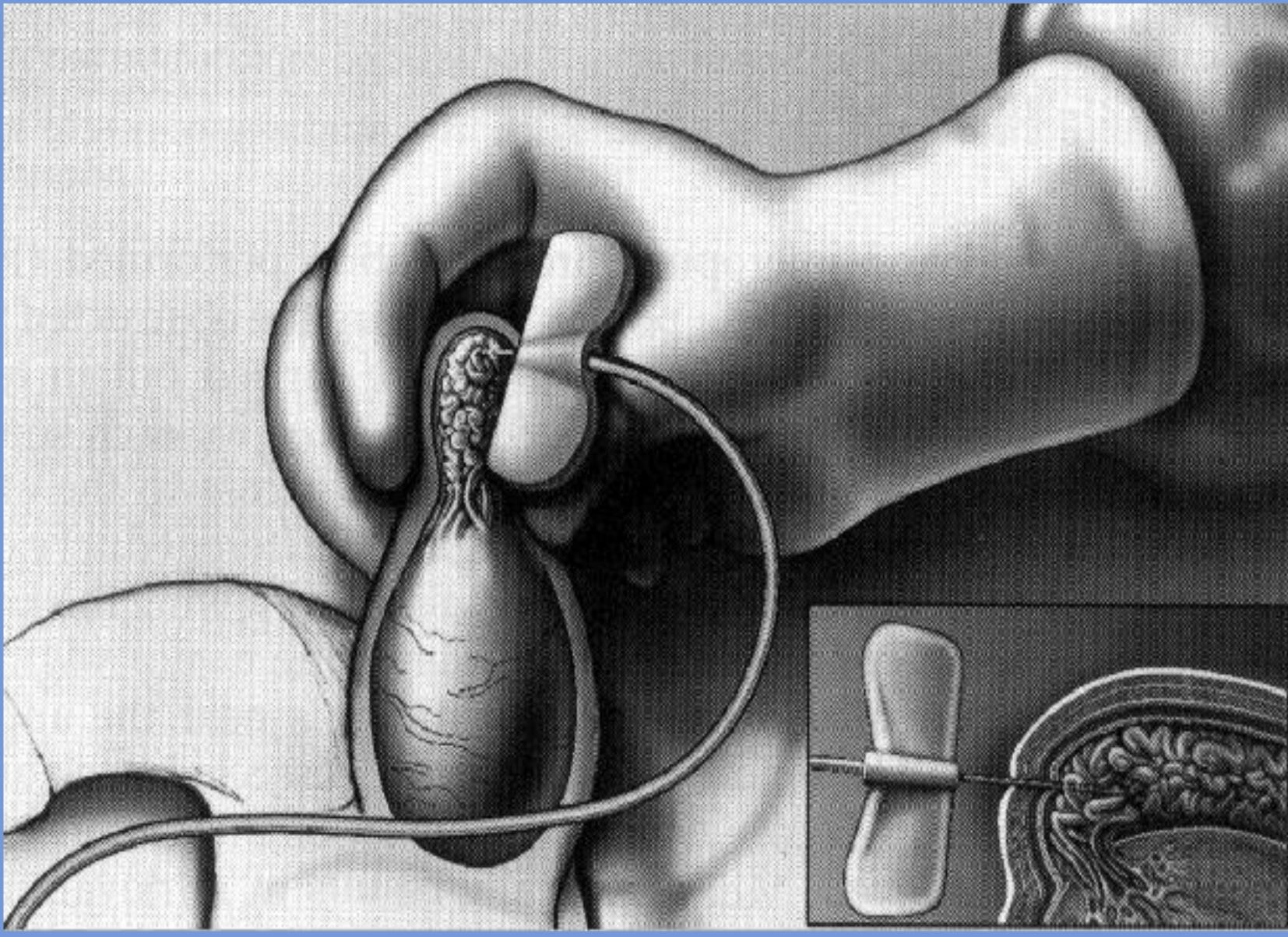
MicroTESE

PETER SCHLEGEL. *HUMAN REPROD*, 14: 131-135, 1999

SHERMAN J. SILBER. *HUMAN REPROD*, 15: 2278-2284, 2000



FERTILITY



FERTILITY

REVERSÃO DA VASECTOMIA

TABLE 2 Operative outcomes of the vasovasostomy and the vasoepididymostomy

First Author	Year	Patient number	Mean patient's age (years)	Mean obstructive interval (years)	Surgical procedure	Anastomosis method	Operative time (min)	Patency rate (%)	Pregnancy rate (%)
Bolduc ³⁸	2007	747	37	6.8	MVV	1 layer	—	86	33
Pate ³⁹	2008	106	40	8.2	MVV	1 layer/2 layer	—	98	—
Jee ⁴⁰	2010	25	39	7.1	MVV	1 layer	106	96	40
		25	39	6.9	LAVV	1 layer	78	72	28
Peng ^{41,a}	2011	73	31	—	MVE	LIVE	—	72	33
Parekattil ⁴²	2012	28	—	—	MVV	2 layer	97	80	—
Schwarzer ⁴³	2012	1303	41	8.2	MVV/MVE	3 layer	110	89	59
Li ⁴⁴	2013	34	39	9.2	MVV/MVE	2 layer	120	94	68
Mui ²⁸	2014	1229	41	10.0	MVV/MVE	1 layer/2 layer	—	84	—
Chen ^{6,a}	2014	62	31	—	MVV/MVE	2 layer	—	57	29
Kavoussi ⁴⁵	2015	27	—	—	MVV/MVE	1 layer/2 layer	141	89	22
Nyame ³⁴	2016	86	40	8.0	MVV	2 layer	165	89	—
		20	43	9.5	MVV	1 layer	120	93	—
Wang ^{46,a}	2017	56	38	24.1	MVV	2 layer	—	88	43

LAVV, loupe-assisted vasovasostomy; LI, longitudinal intussusception vasoepididymostomy; MVE, microscopic vasoepididymostomy; MVV, microsco

^aReport for only the patients with obstructive azoospermia following childhood herniorrhaphy.

patency rate=87%
pregnancy rate=49%

Vasovasostomy and vasoepididymostomy: Review of the procedures, outcomes, and predictors of patency and pregnancy over the last decade

Takeshi Namekawa | Takashi Imamoto | Mayuko Kato | Akira Kmiya | Tomohiko Ichikawa

Reprod Med Biol. 2018;1-13.

MALE FACTOR

Fertility and Sterility® Vol. 83, No. 3, March 2005
Copyright ©2005 American Society for Reproductive Medicine.

Etiology-specific outcomes of intracytoplasmic sperm injection in azoospermic patients

Fábio F. Pasqualotto, M.D., Ph.D.,^a Lia Mara Rossi, B.Sc., M.Sc.,^{a,b}

Patrícia Guilherme, B.Sc., M.Sc.,^a Valdemar Ortiz, M.D., Ph.D.,^c Assumpto Iaconelli, Jr., M.D.,^a and Edson Borges, Jr., M.D.^a

^a Fertility – Center for Assisted Reproduction, São Paulo; ^b Jundiaí Medical School, São Paulo; and ^c Department of Urology, Federal University of São Paulo, São Paulo, Brazil

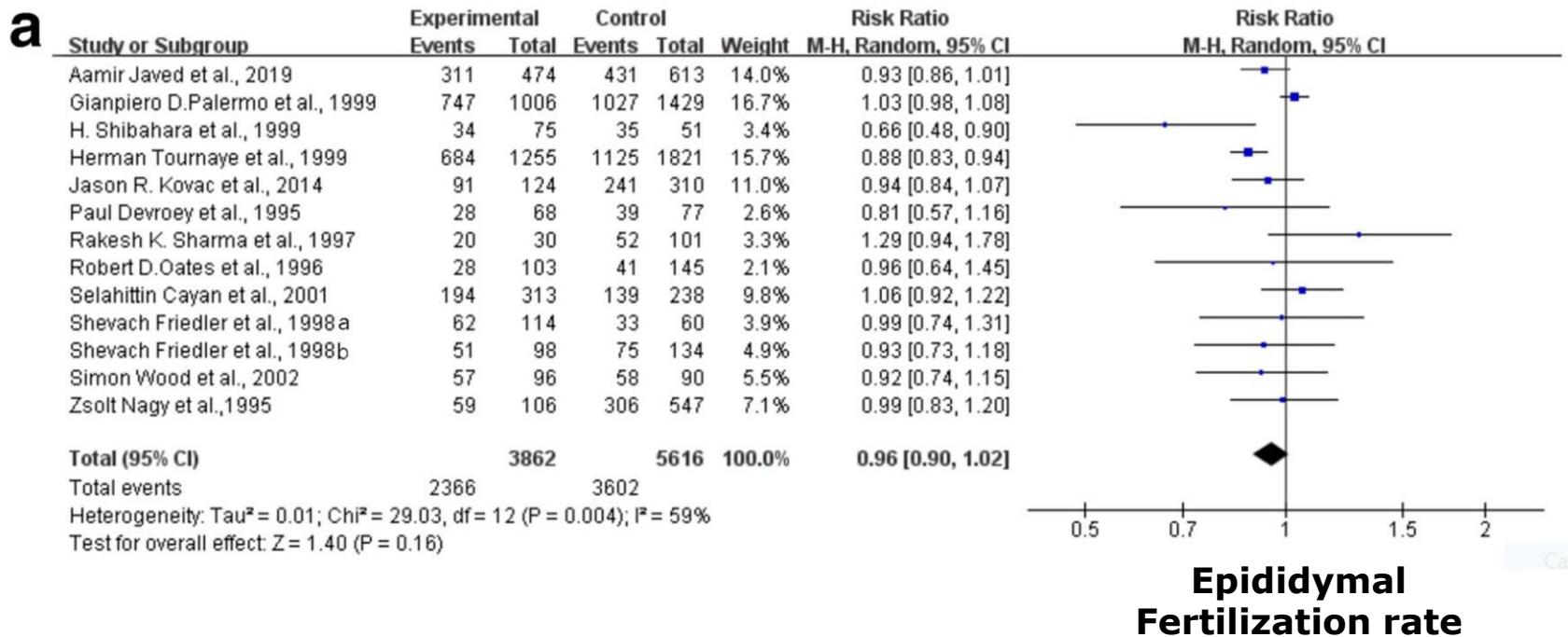
	NOA	Vasectomia	Congenita	Infecção	P
Ciclos / pacientes	102 / 84	99 / 84	25 / 20	31 / 24	
Gestações	22	31	7	7	
2PN (%)	47.3	64.1 ^a	67.7 ^a	58.9	0.041
NF (%)	32.2 ^b	19.2	21.0	30.7 ^b	0.005
Gestação / ciclo (%)	21.56	31.31	28	22.58	0.812
Gestação / paciente (%)	26.2	36.1	35	29.2	0.812
Implantação (%)	12.5	13.4	28.5 ^c	12.9	0.032
Aborto (%)	45.6 ^d	25.8	28.57	28.57	0.614



Check for
updates

Impact on using cryopreservation of testicular or epididymal sperm upon intracytoplasmic sperm injection outcome in men with obstructive azoospermia: a systematic review and meta-analysis

Hanchao Liu¹ · Yun Xie¹ · Linzhi Gao² · Xiangzhou Sun¹ · Xiaoyan Liang² · Chunhua Deng¹ · Yong Gao³ · Guihua Liu²





Check for
updates

Impact on using cryopreservation of testicular or epididymal sperm upon intracytoplasmic sperm injection outcome in men with obstructive azoospermia: a systematic review and meta-analysis

Hanchao Liu¹ · Yun Xie¹ · Linzhi Gao² · Xiangzhou Sun¹ · Xiaoyan Liang² · Chunhua Deng¹ · Yong Gao³ · Guihua Liu²

a

