The ICSI Story
To Infinity and Beyond

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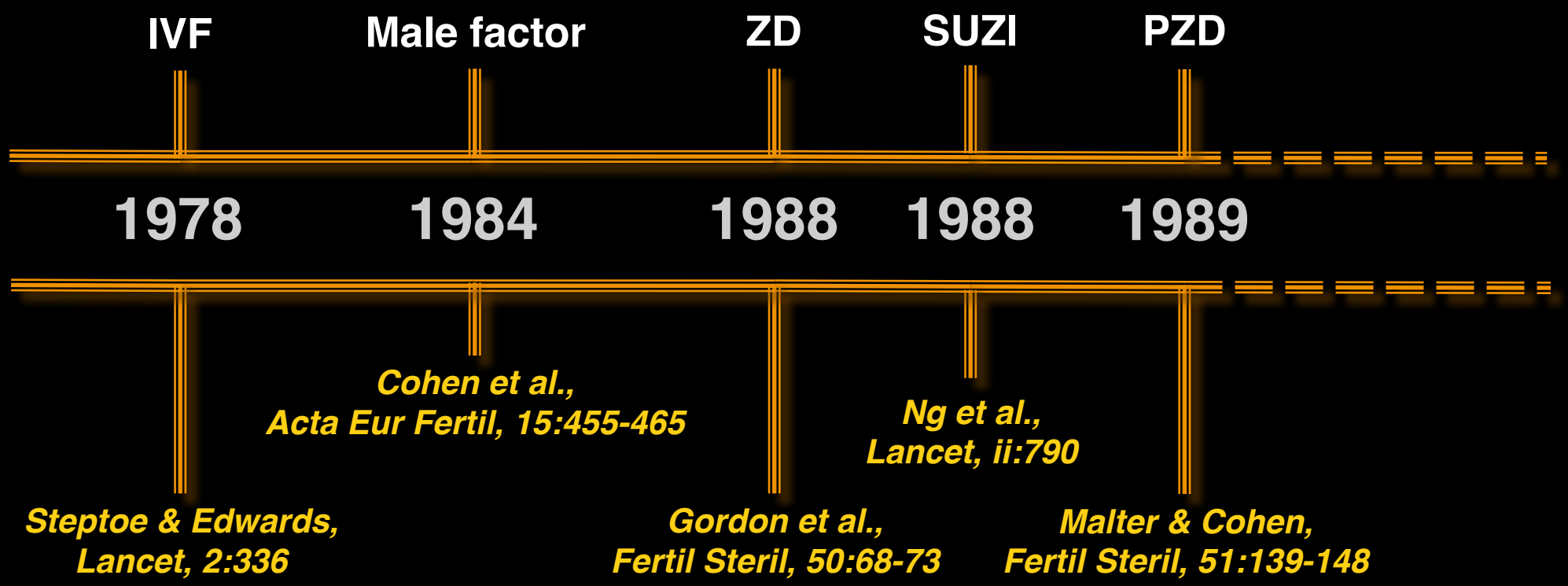
Assisted Reproduction



First ICSI Pregnancy

Age	38	
Oocyte harvested	14 (12 MII)	
	SUZI	ICSI
Injected	11	1
	No fert	1 (2PN)

24 hours later

1 (4 cell, 20% fragments)

Replacement

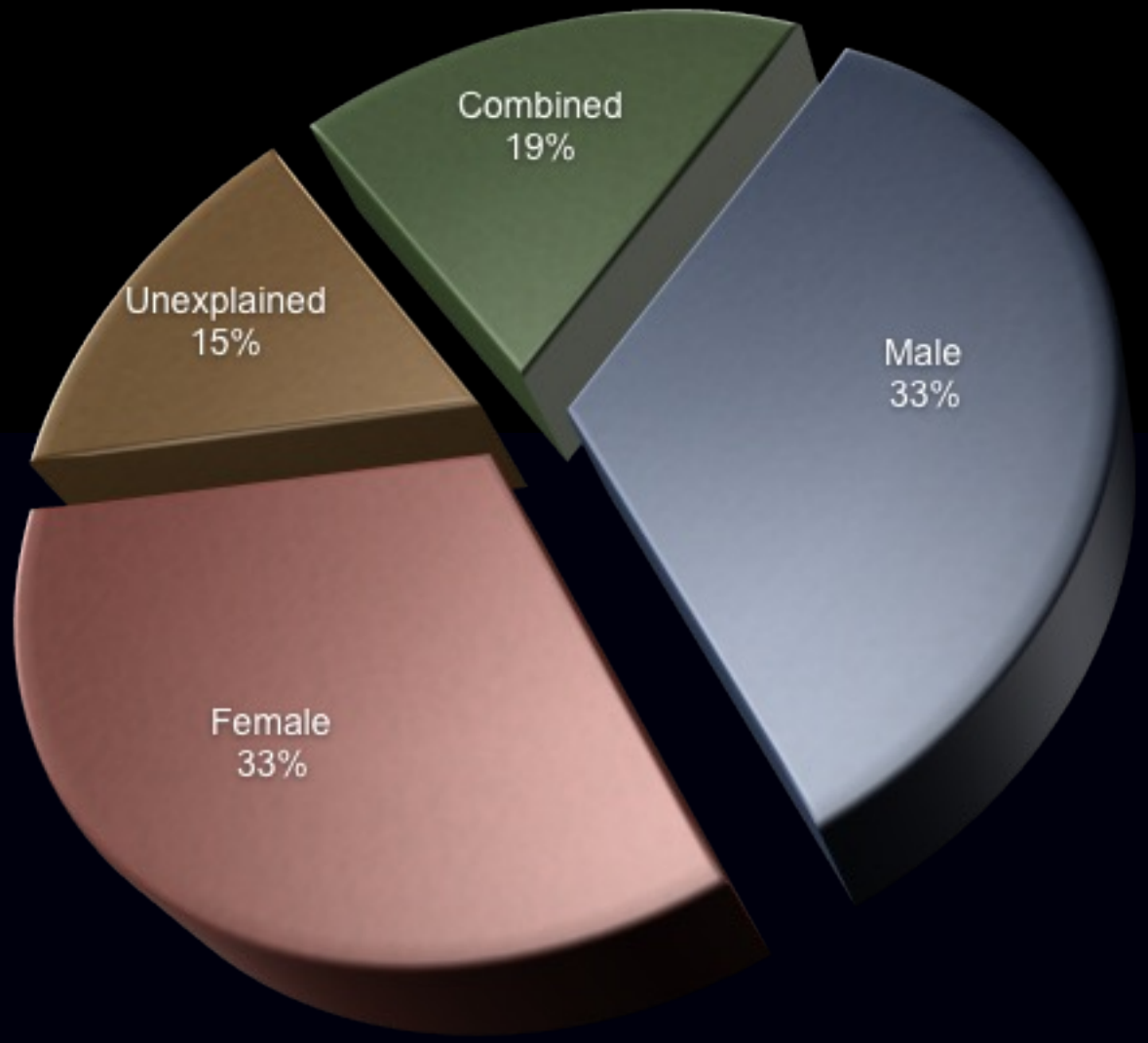
Pregnancy

Delivery 46,XY

January 14, 1992

Palermo et al., The Lancet, 1992

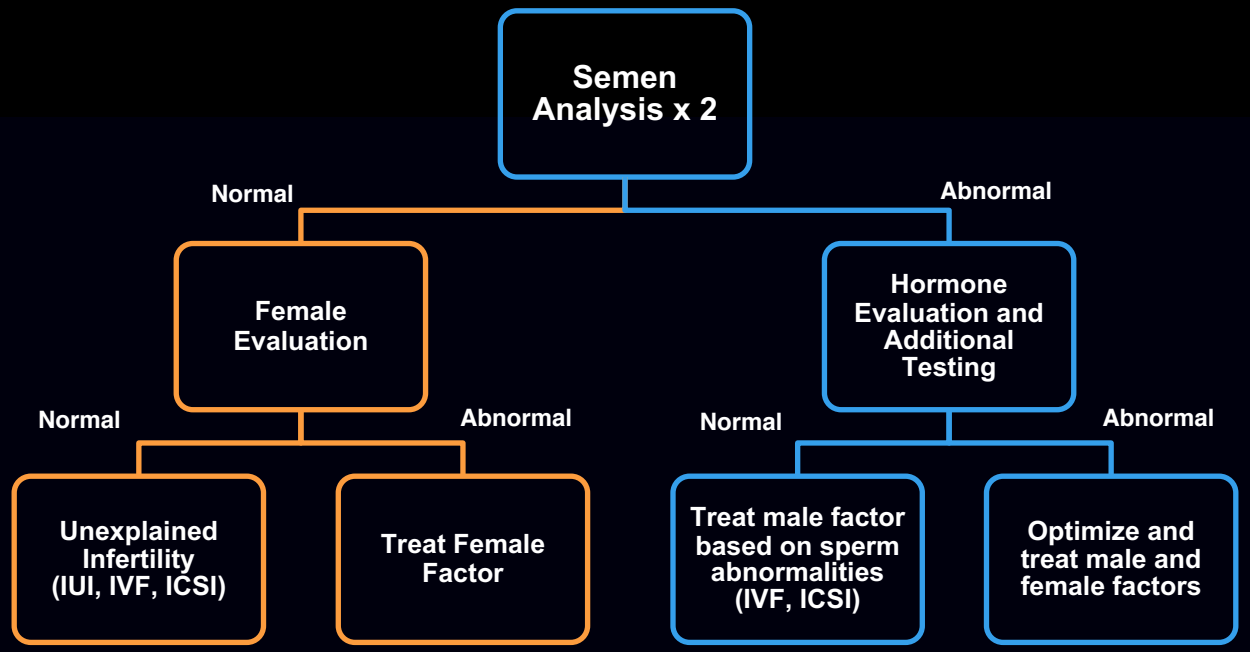
Infertility Indications



CDC – Reproductive Health, 2008

Evaluation of Male Partner

Developmental History | Medical and Medication History | Family History | Surgical History | Social and Sexual History | Physical, Genital and Rectal Examination



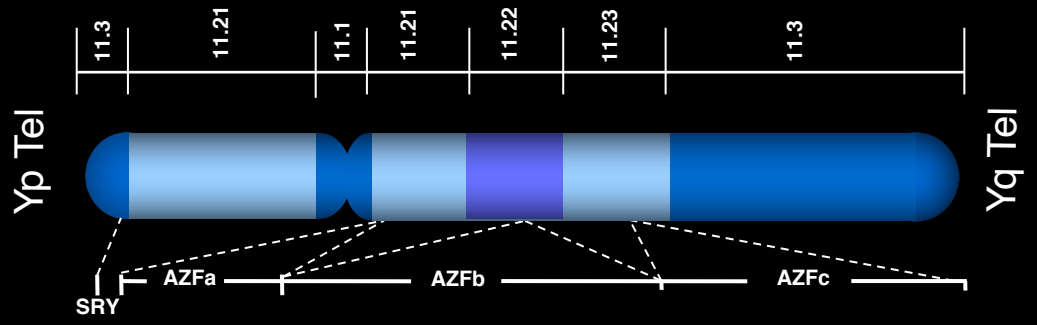
Azoospermic Men

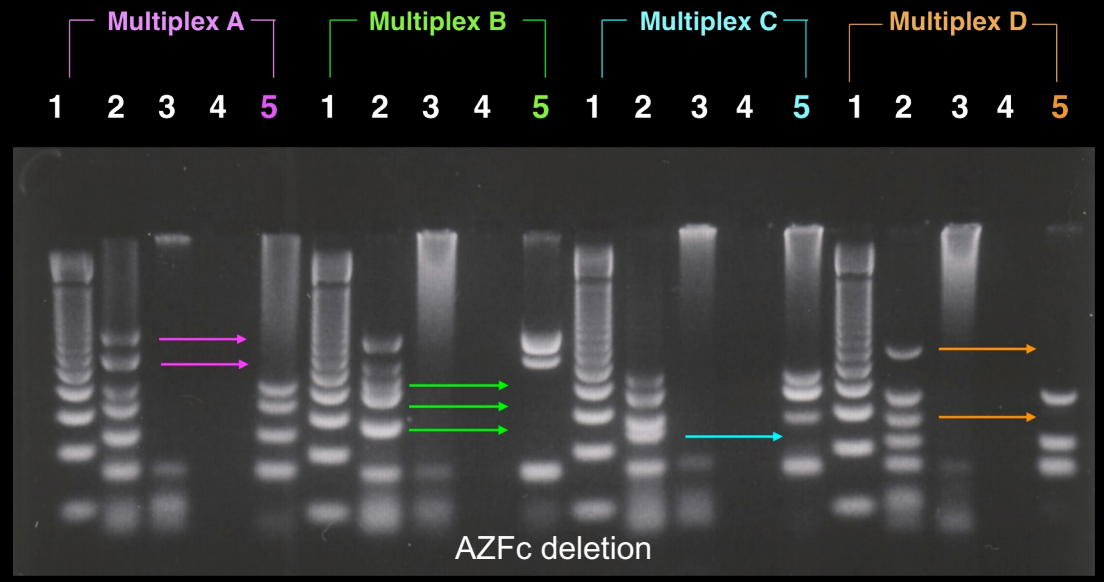
- **Autosomal: 126**
  - 6 - translocation
  - 21 - inversions
  - 3 - deletion
  - 54 - 46,XY __ qh(+)

    (chx 1,9,15,16)
  - 42 - Others

- **Gonosomal: 99**
  - 64 - Klinefelter's Syndrome
    - 49 - 47,XXY
    - 12 - 46,XY/47,XXY
    - 2 - 47,XXY/48,XXXY
    - 1 - 47,Xi(Xq)Y
  - 14 - 46,XY (delYq)
  - 3 - 45,X/46,XY
  - 2 - 45,X/46,XY,q-
  - 7 - 47,XYY
  - 9 - Others

Nakamura et al., Int J Urol 2001
Hamada et al., Clinics, 2013
Mazzilli et al., Asian J Androl 2022

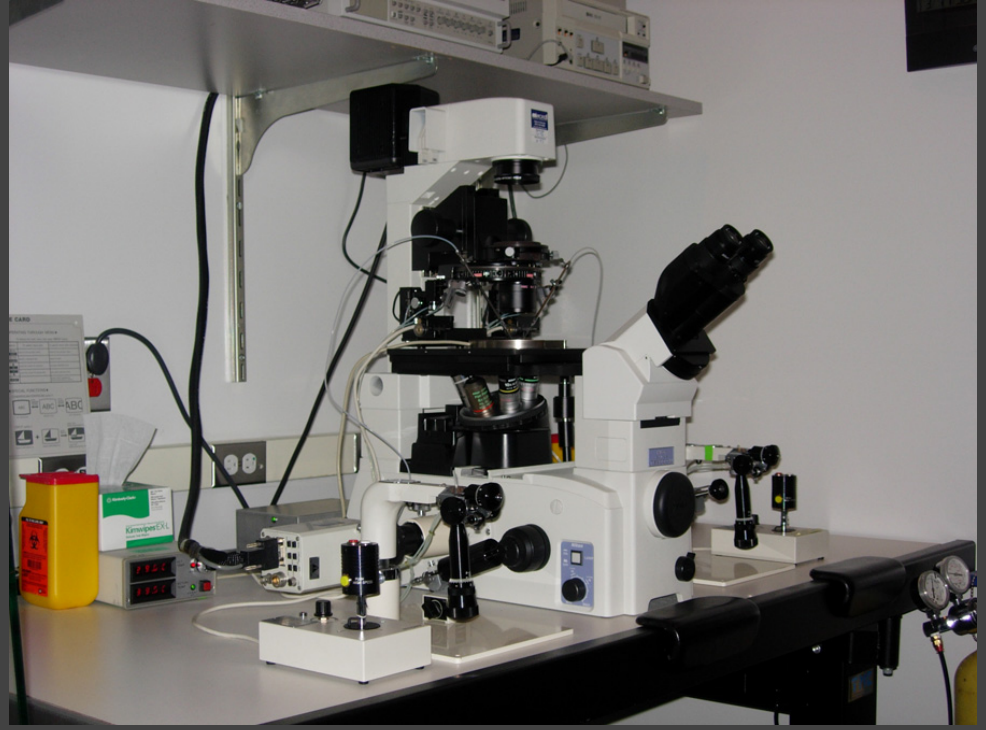




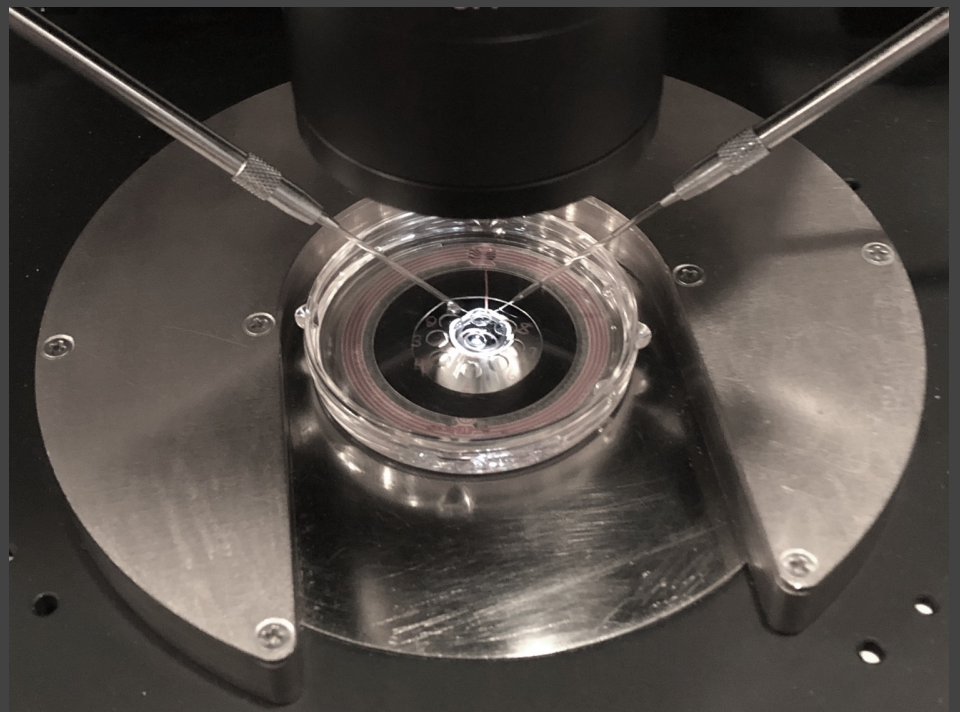
Concentration (x10⁶/ml)	Yq del (%)	
≥ 5	0/41	
< 5	1/42	(2.4)
< 1	6/103	(5.8)
0	69/834	(8.2)

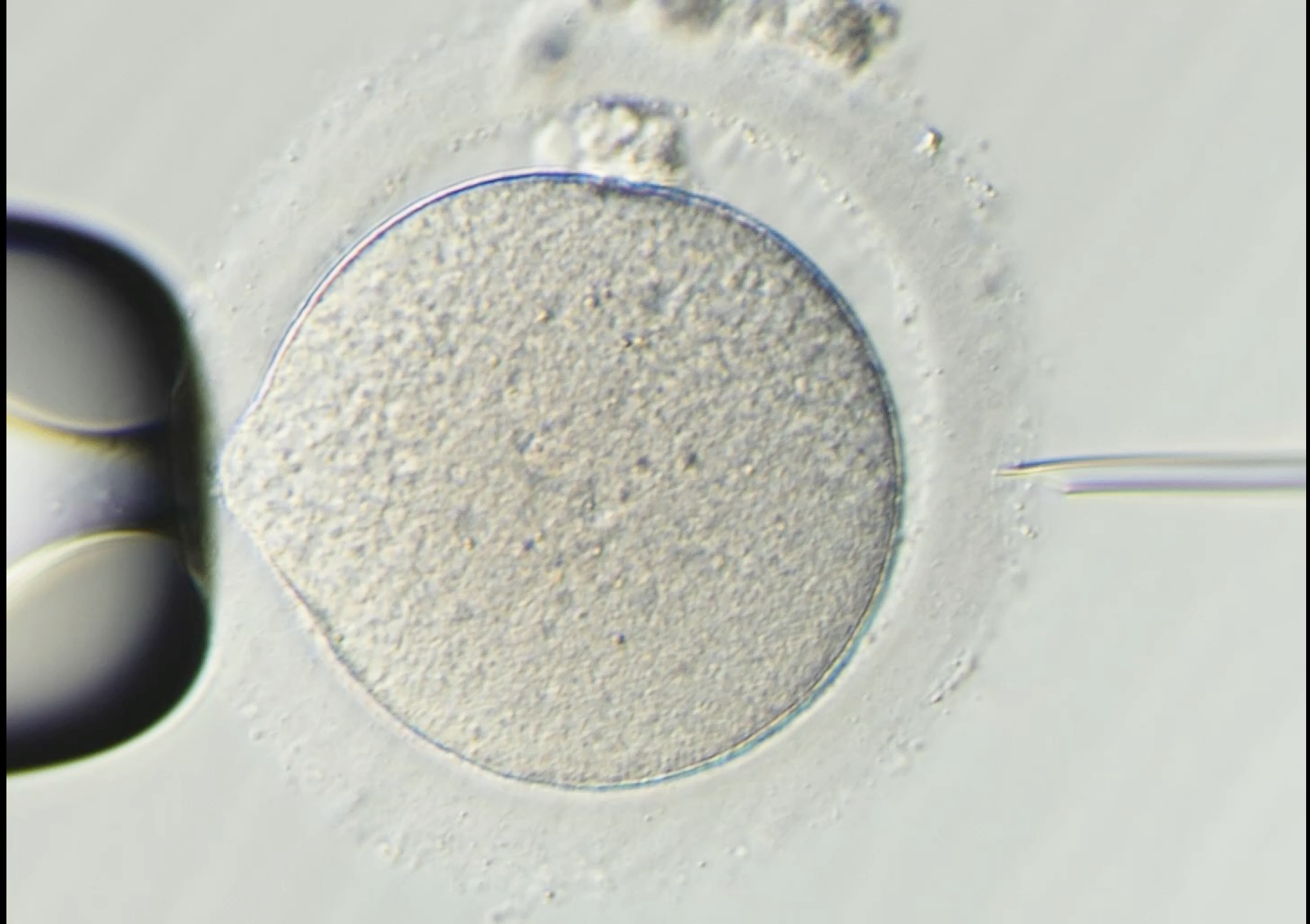
Hopps et al., Hum Reprod, 2003
Choi et al., Fertil Steril, 2004
Katagiri et al., RBMO, 2004
Zhou, et al., Asian J Androl., 2021

No. of	Ejaculated	Testicular
Cycles	14	11
Patients	6	7
Density (x 10⁶/ml)	2.5	0.0003
Oocytes inseminated	149	102
Oocytes fertilized (%)	80 (53.7)	42 (41.2)
Clinical pregnancies	5 (35.7)	4 (36.4)

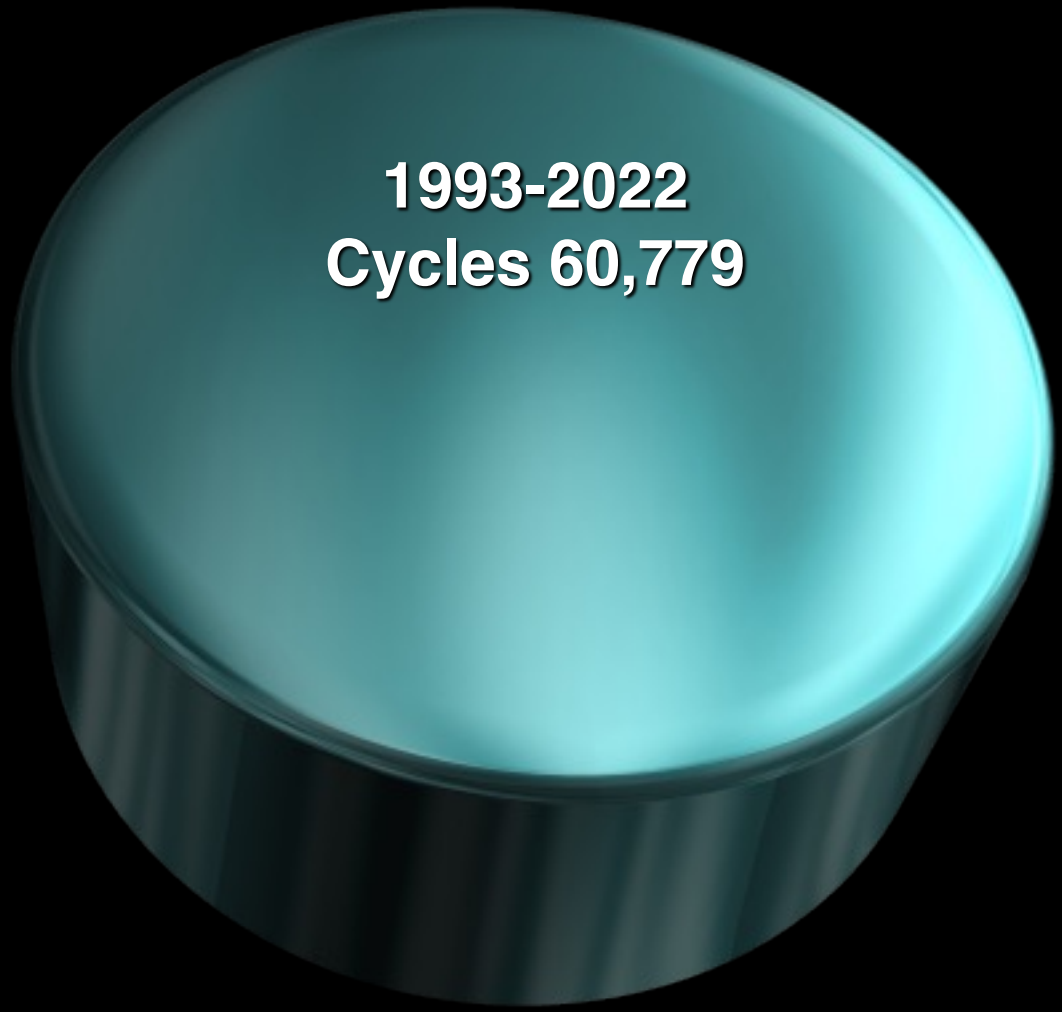

Kimwipes EX-L


morrell
KIMTECH



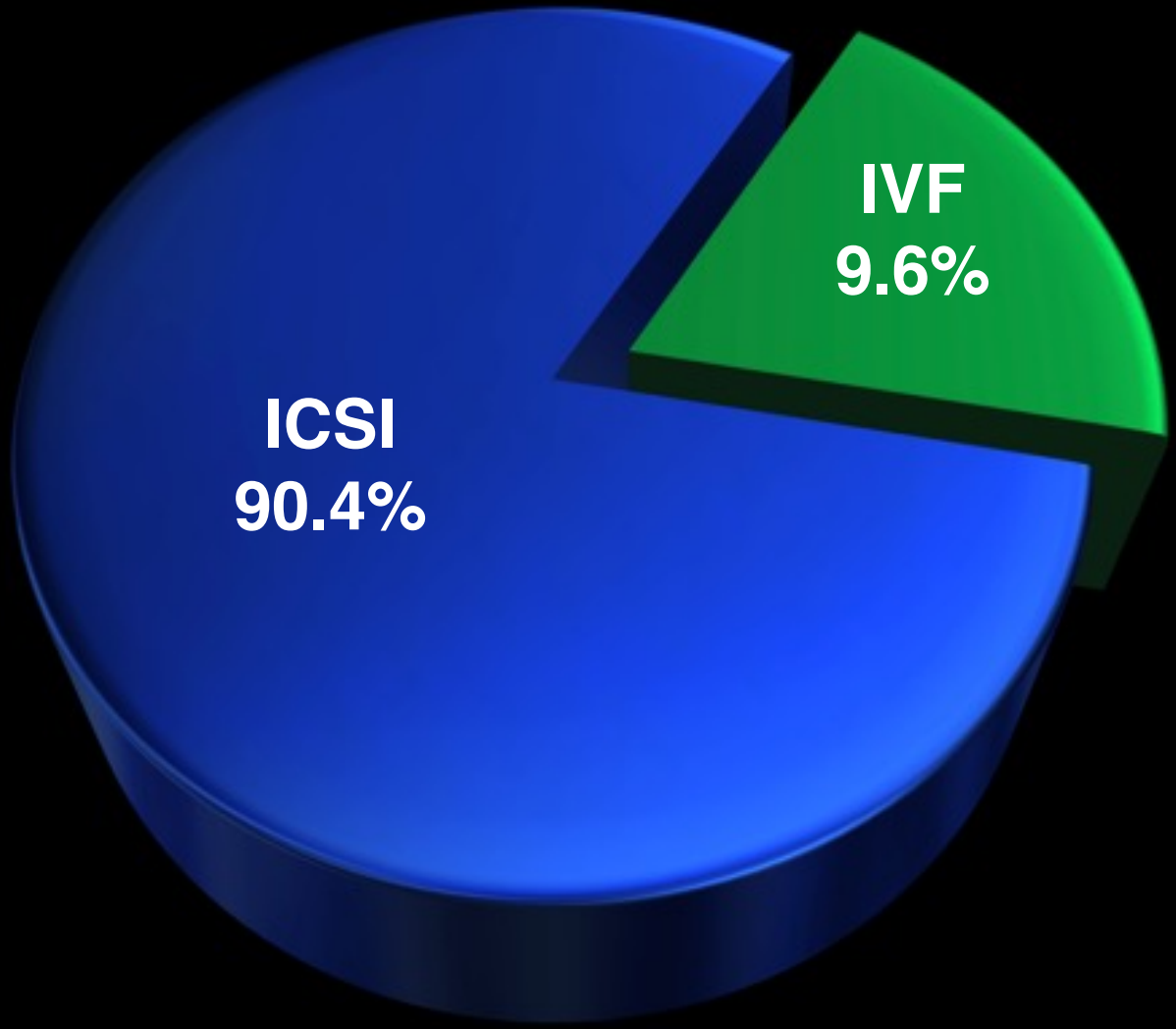


Cornell ART



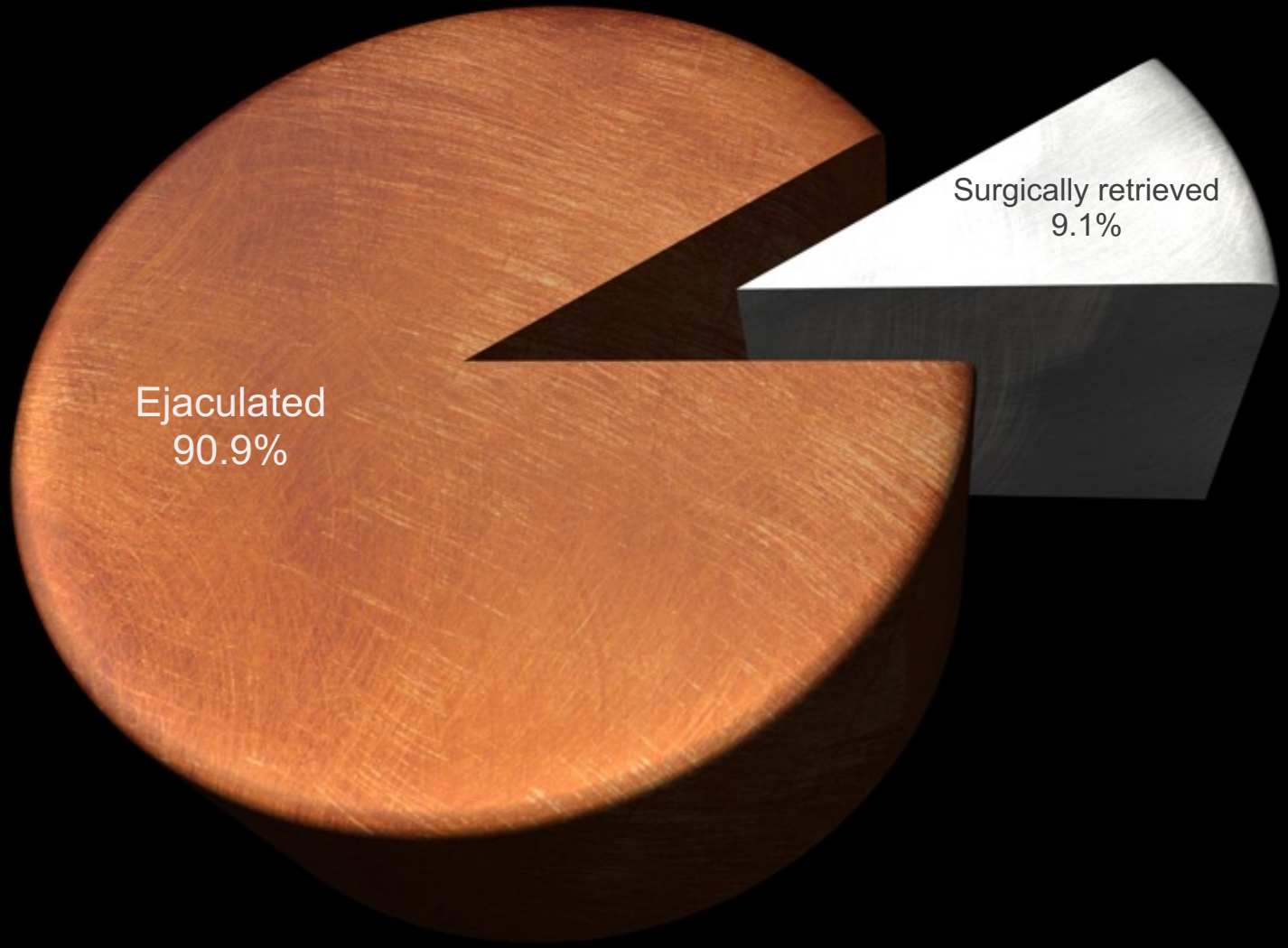
ICSI Prevalence

2012 to 2022
n = 26,874



44,585 ICSI Cycles

September 1993 – August 2022



ICSI

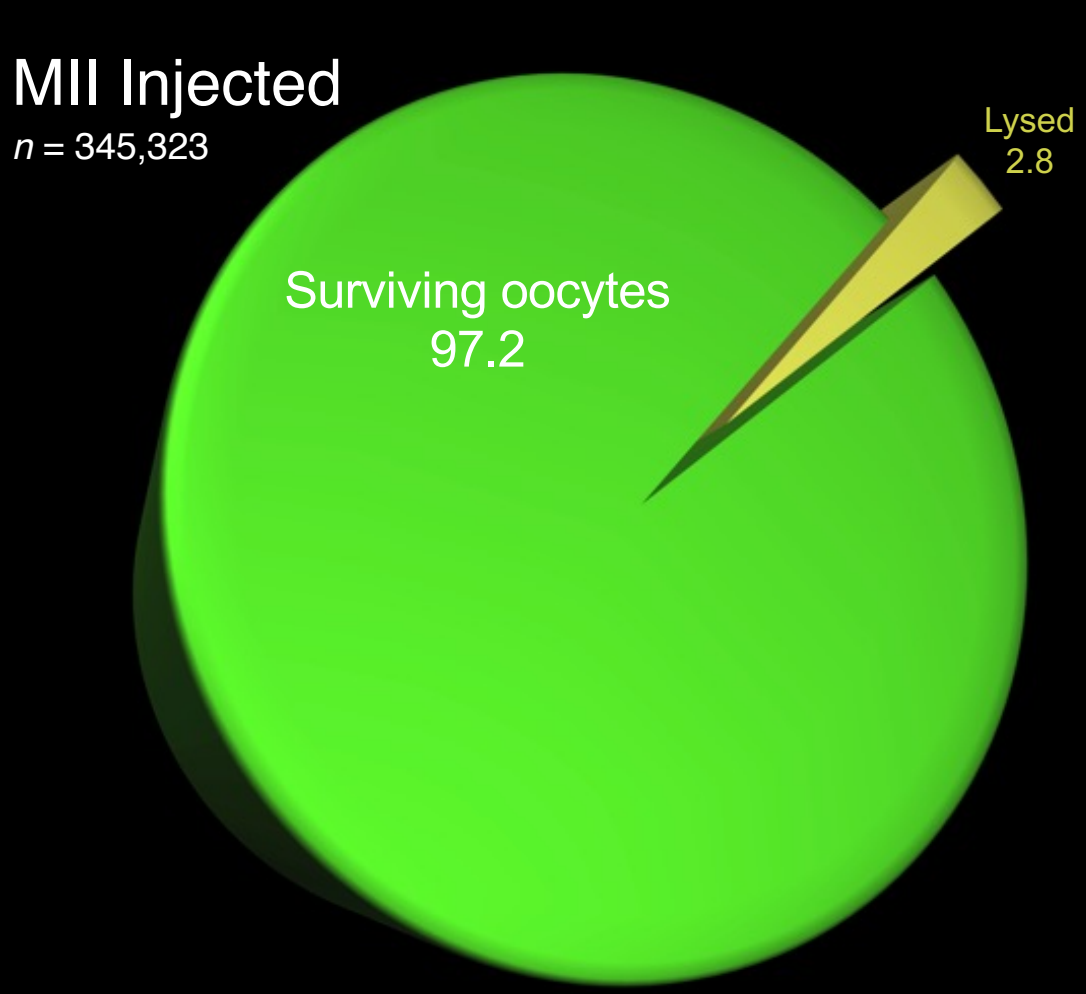
Ejaculated Spermatozoa

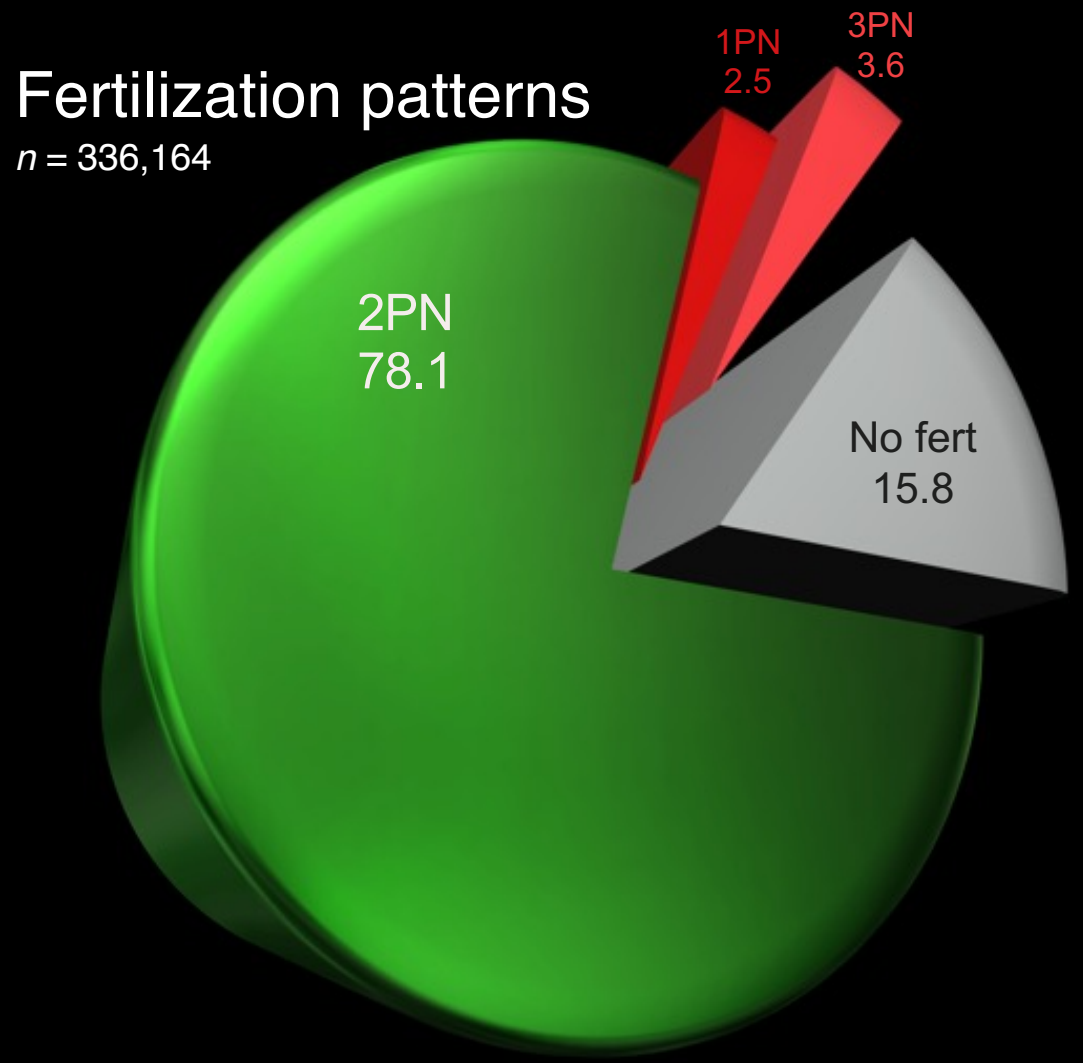
Cycles		40,547
Mean maternal age (± SD)		38.2 ± 5
Semen parameters	Normal	6,777
	Abnormal*	33,742

*WHO, 2010

Survival and Fertilization Characteristics

Ejaculated Spermatozoa



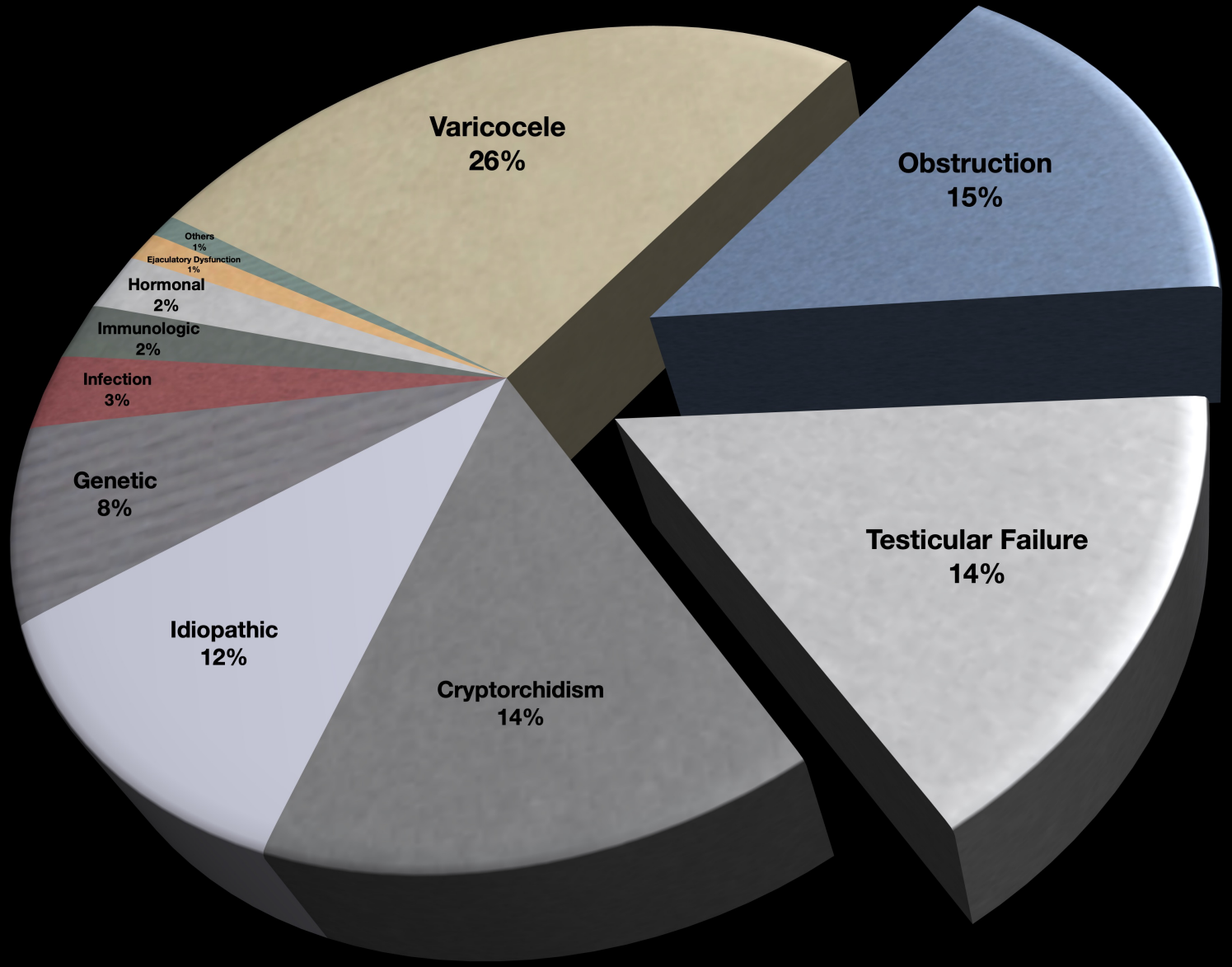


Semen Origin and ICSI Outcome

Semen Origin	Cycles	Fertilization (%)	Clinical Pregnancies (%)*
Ejaculate	37,751	243,768/322,916 (75.5)	13,966 (37.0)
Electroejaculate	88	662/894 (74.0)	40 (46.0)
Retrograde	64	439/575 (76.3)	23 (36.0)

*Includes only cycles with fresh transfer

Male Infertility Indications



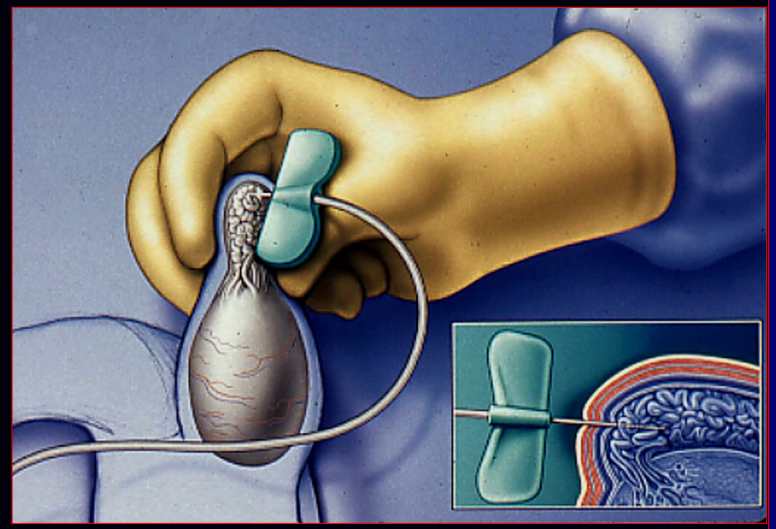
Esteves, et al., Clinics, 2011

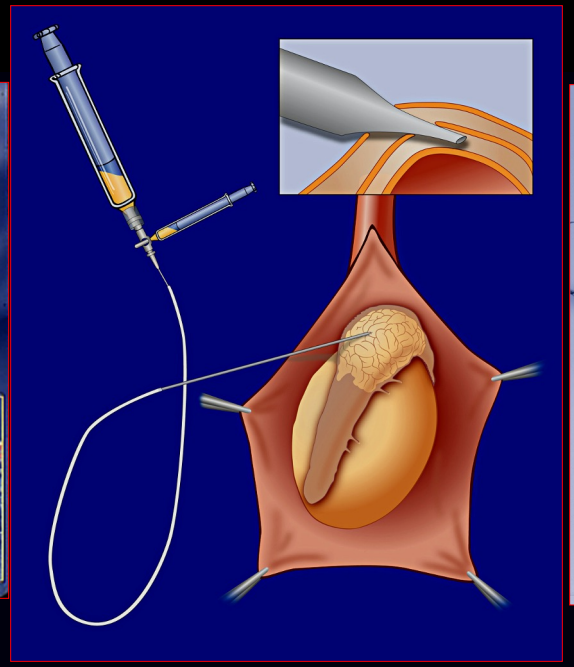
ICSI and Azoospermia

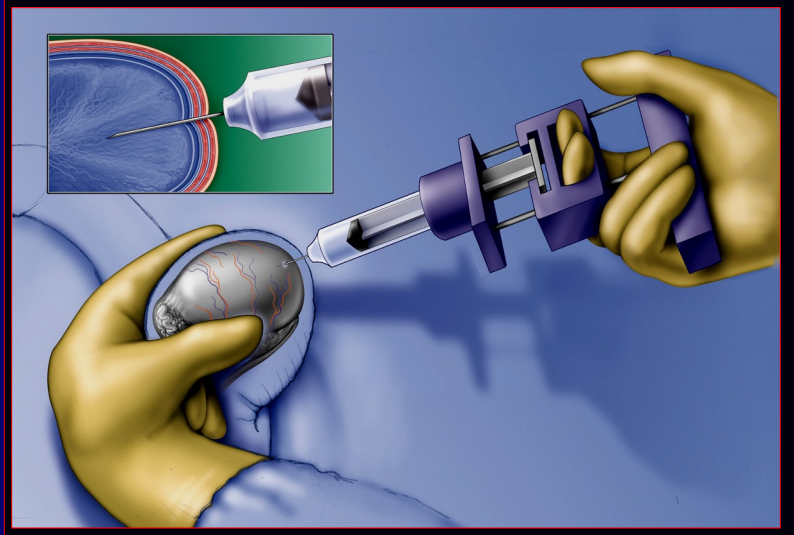
Cycles	3,417
Maternal age (M ± SD)	36.4 ± 5
Epididymal spermatozoa	1,364
Testicular spermatozoa	2,053

Obstructive Azoospermia

- Congenital (e.g., CBAVD)
- Acquired (e.g., infection, trauma)





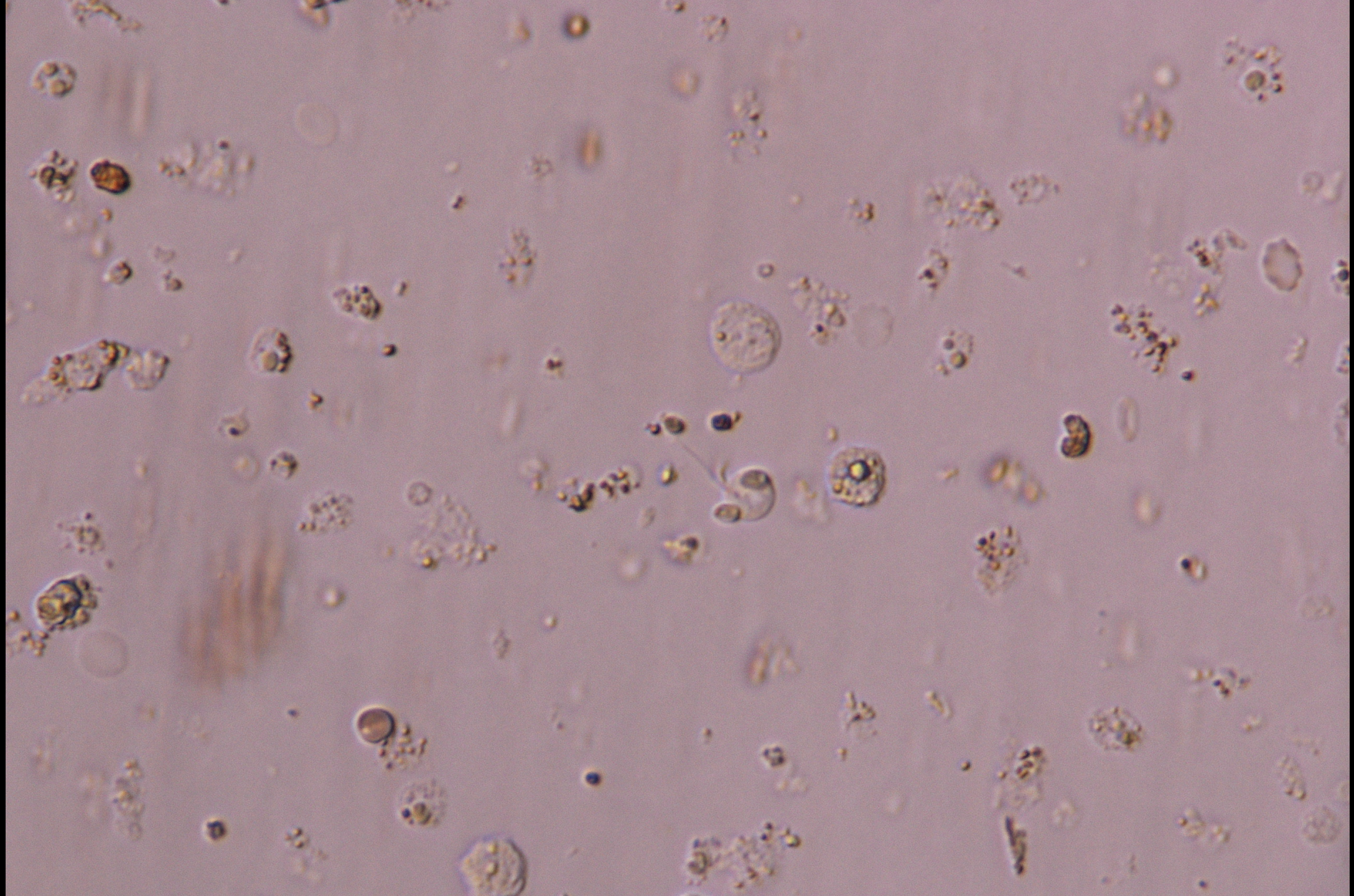


MESA

	Obstruction	
	Congenital	Acquired
Cycles	604	643
Density (x 10^6/ml ± SD)	27.6 ± 45	16.8 ± 26
Motility (M ± SD)	8.2 ± 12	18.3 ± 15
Morphology (M ± SD)	1.2 ± 2	1.0 ± 2
Fertilization (%)	4,411/6,118 (72.1)*	3,991/5,720 (69.8)*
Clinical pregnancies (%)	319 (52.8)†	268 (41.7)†

*χ^2 , 2x2, 1 *df*, Effect of the etiology on fertilization rate, $P = 0.01$

†χ^2 , 2x2, 1 *df*, Effect of the etiology on clinical pregnancy rate, $P = 0.0005$



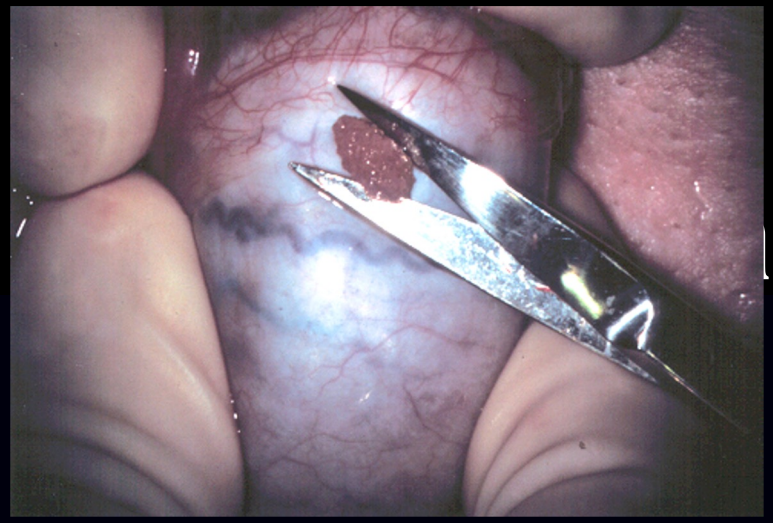
Testicular Sampling

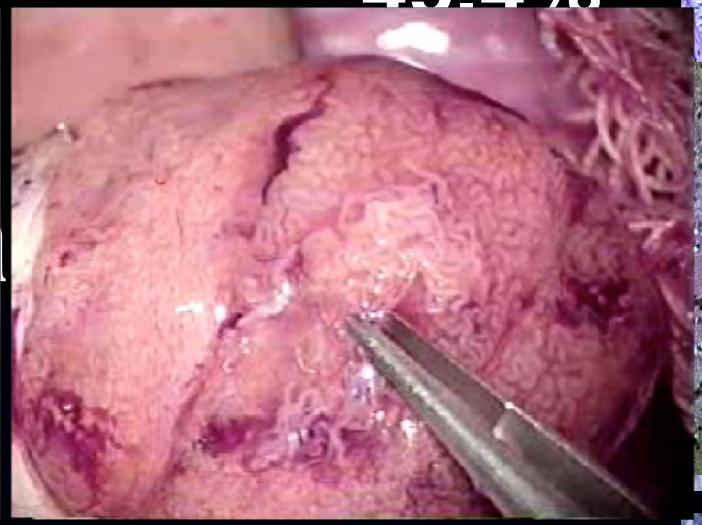
	No. of biopsies
• Obstructive Azoospermia	311
• Non-Obstructive Azoospermia	2,693
Spermatozoa present (%)	1664 (61.8)

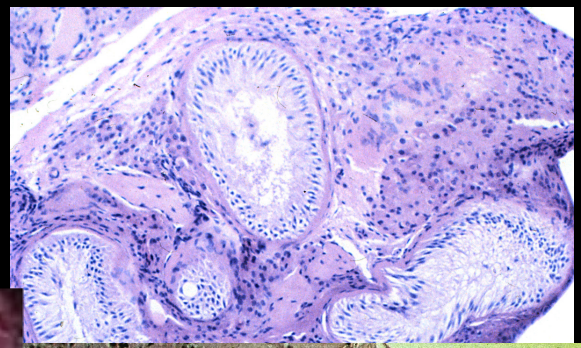
Non-Obstructive Azoospermia

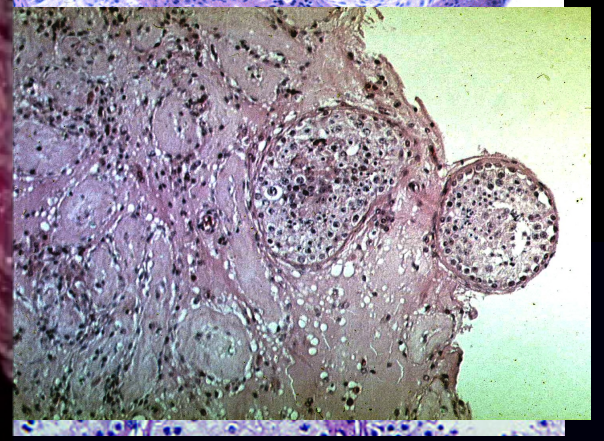
- Hypospermatogenesis

49.4%



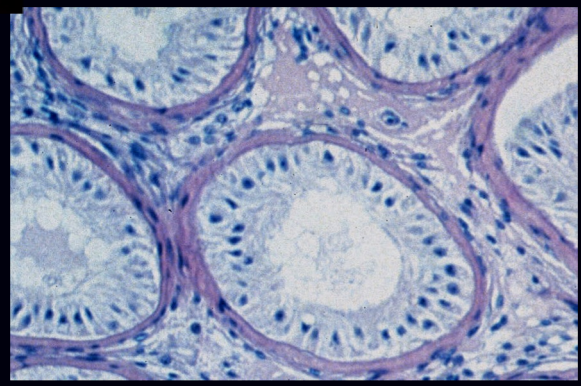






- Sertoli cell only

26.6%



Su et al., 1999

TESE

	Azoospermia	
	Obstructive	Non-obstructive
Cycles	310	1,677
Density (x 10^6/ml ± SD)	2.2 ± 7	0.8 ± 7
Motility (M ± SD)	3.3 ± 8	3.6 ± 13
Morphology (M ± SD)	0	0
Fertilization (%)	1,841/2,782 (66.2)*	8,279/17,287 (47.9)*
Clinical pregnancies (%)	130 (42.5)†	598 (35.7)†

*χ^2, 2x2, 1 *df*, Effect of etiology of azoospermia on fertilization rate, $P < 0.00001$

†χ^2 , 2x2, 1 *df*, Effect of the etiology on clinical pregnancy rate, $P < 0.05$

Klinefelter Syndrome

No. of (%)	
Testicular biopsies	348
with sperm retrieved	215 (61.8)
Oocyte fertilized/injected	1,269/2,651 (47.9)
Deliveries	81 (39.5)
Children	99

53 boys, 46 girls

Updated from Palermo et al., 1998

Fertility and Sterility®

Identification and treatment of men with phospholipase Cζ–defective spermatozoa

Stephanie Cheung, B.Sc., Philip Xie, B.Sc., Alessandra Parrella, M.Sc., Derek Keating, B.A., Zev Rosenwaks, M.D., and Gianpiero D. Palermo, M.D., Ph.D.

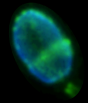
Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, New York

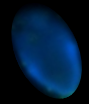
Cheung et al., 2020 Fertil Steril

Study Design

Low ICSI Fertilization (<10%)

PLCζ Assessment

PLCζ presence 

PLCζ absence 

Confirmatory MOAT

Modified Superovulation Protocol

Assisted Gamete Treatment

Genome Assessment

Cheung et al., 2020 Fertil Steril
Cheung et al., 2022 Fertil Steril in press

Oocyte-Related OAD PLCζ presence

No. of (%)	Control		Modified Superovulation
Couples		52	
ICSI Cycles	52		105
Maternal age (M yrs±SD)		33.4±3	
Oocytes Retrieved	456		1120
Paternal age (M yrs±SD)		35.7±5	
MII Oocytes	334 (73.2)		796 (71.1)
Fertilization	7 (2.1)*		470 (59.0)*
Cycles with ET	6		91
Clinical Pregnancy (+FHB)	0		30 (32.9)
Deliveries	–		25

*χ^2, 2x2, 1 *df*, *P*<0.0001

Cheung et al., 2020 Fertil Steril
Cheung et al., 2022 Fertil Steril in press

Spe

absence

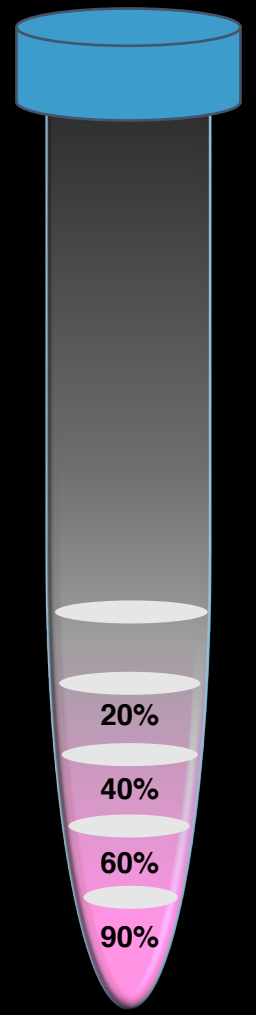
No. of (%)	AGT
Couples	
ICSI Cycles	43
Maternal age (M	
Oocytes Retriev	404
Paternal age (M	
MII Oocytes	323 (79.9)
Fertilization	136 (42.1)*
Cycles with ET	25
Clinical Pregnan	9 (36.0)
Deliveries	6

*χ^2, 2x2, 1 *df*, $P<0.05$

Cheung et al., 2020 Fertil Steril
Cheung et al., 2022 Fertil Steril in press

Sperm Gender Selection

Semen Parameters (selected)	
Concentration (M x10^6/ml±SD)	24.3±14
Motility (M%±SD)	94.5±3
Morphology (%±SD)	3.8±1
Sperm Gender Enrichment	
X-bearing spermatozoa (%±SD)	81.6±1
Y-bearing spermatozoa (%±SD)	80.8±2



Cheung et al., 2022 PLOS in press

Pregnancy Outcome *Female*

Couples/Cycles	52/70
Maternal age (M yrs±SD)	38.9±3
Paternal age (M yrs±SD)	41.7±4

Cheung et al., 2022 PLOS in press

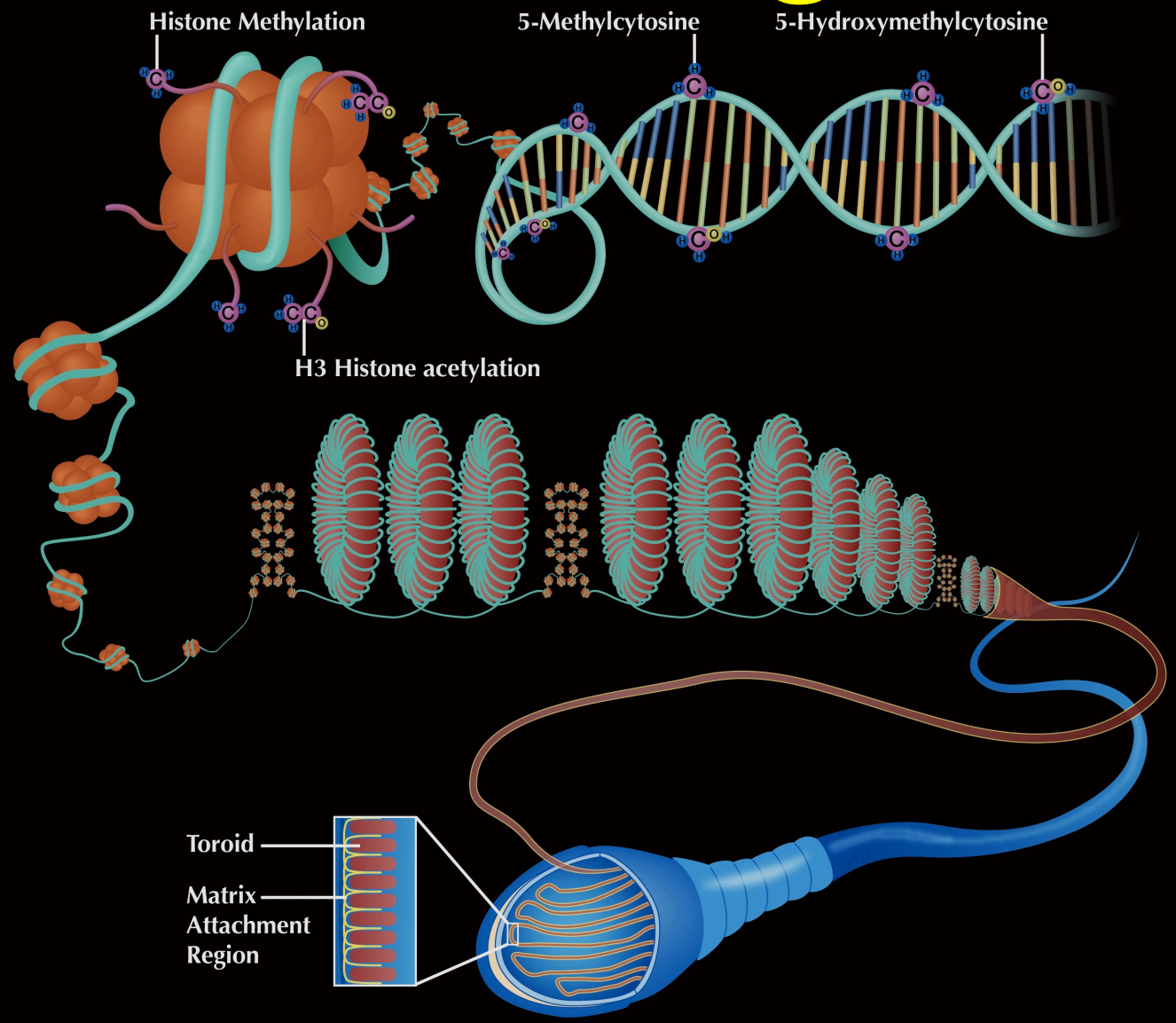
Pregnancy Outcome *Male*

Couples/Cycles	**46/50**
Maternal age (M yrs±SD)	**37.3±4**
Paterna	

Clinicaltrials.gov NCT05500573

Cheung et al., 2022 PLOS in press

Sperm DNA Organization



Adapted from Doug Carrell, 2012

Fertility and Sterility®

Perspectives on the assessment of human sperm chromatin integrity

Gianpiero D. Palermo, M.D., Ph.D., Queenie V. Neri, M.Sc., Tyler Cozzubbo, B.Sc., and Zev Rosenwaks, M.D.

The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical College, New York, New York

Palermo et al., 2014 Fertil Steril

Apoptosis plays a significant role in regulating germ cell development by removing damaged germ cells from seminiferous tubules, thereby safeguarding the genome of a given species. The unique chromatin-packing process of the spermatozoon has important implications for both the development of male infertility screening tests and understanding of sperm chromatin characteristics, which may affect assisted reproductive technology outcomes. Sperm deoxyribonucleic acid (DNA) integrity tests have been proposed as a means to assess male gamete competence. Although these assays are currently gaining popularity, and are more often used as a supplement to traditional semen analysis, the point at which DNA damage occurs during spermiogenesis, and to what degree, remains to be elucidated. Here, we examined current studies of DNA fragmentation, to understand its origin and import, as well as its impact on pre- and post-implantation development. As the DNA fragmentation index is strongly correlated with the motility characteristics of a semen specimen, controlling for this factor may be helpful. Utilization of more sensitive assays, possibly on the actual spermatozoa used for insemination, may generate healthier conceptuses. (Fertil Steril® 2014;102:1508–17. ©2014 by American Society for Reproductive Medicine.)

Key Words: ICSI, sperm DNA fragmentation, follow-up of children, TUNEL, SCSA, SCD, COMET

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/palermog-human-sperm-chromatin-integrity/

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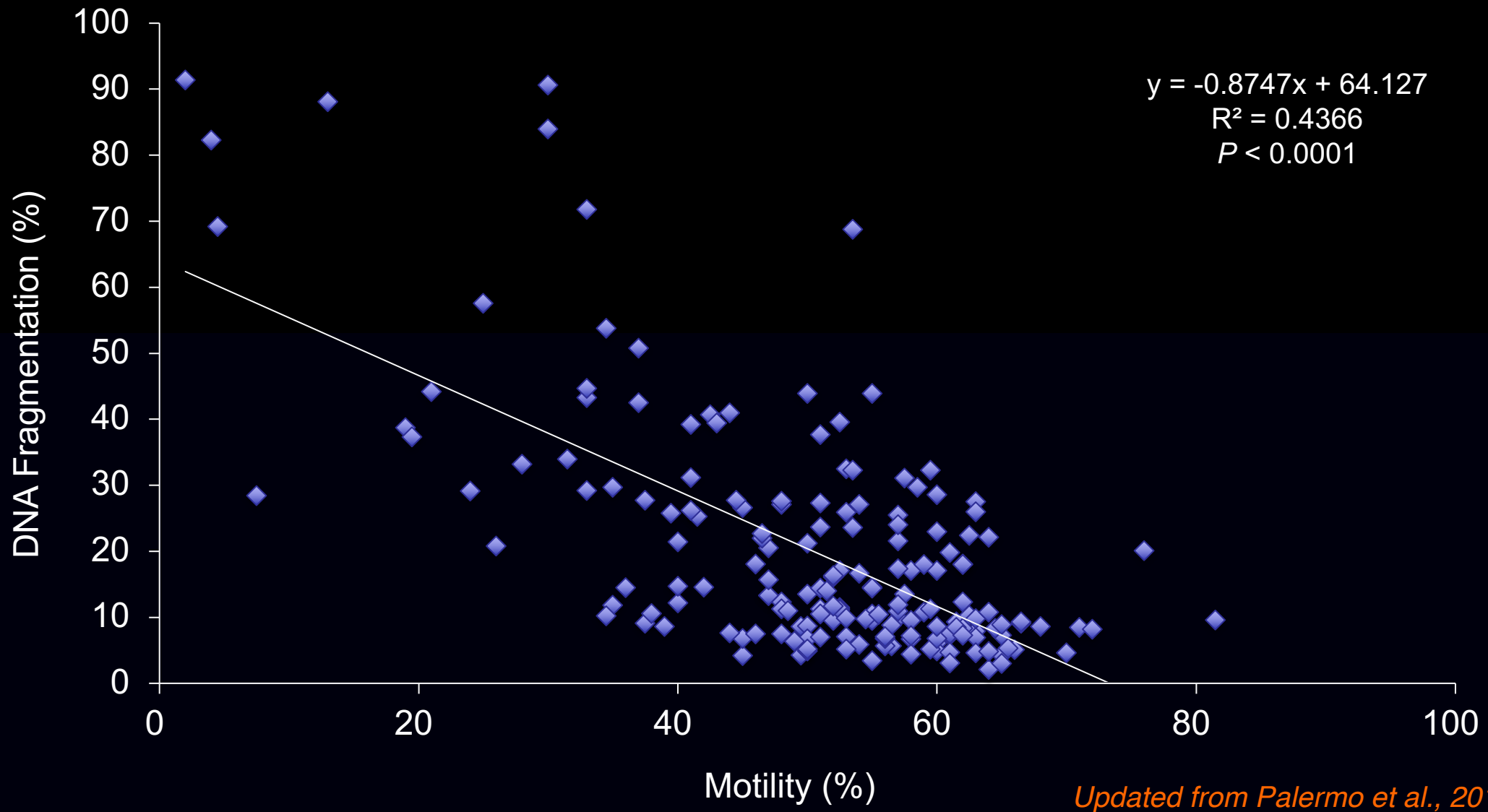
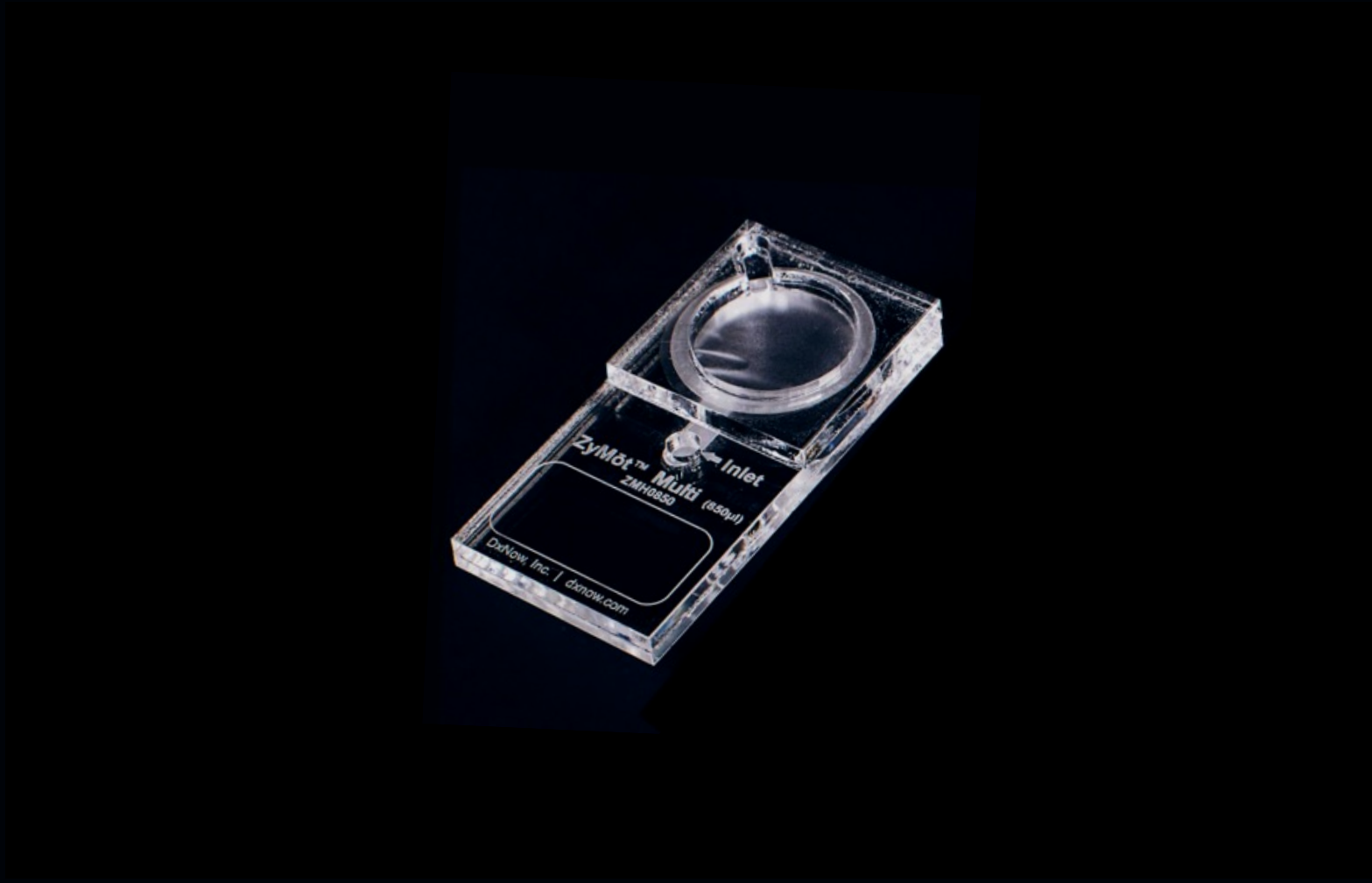
SCSA and Motility

n=179



Updated from Palermo et al., 2014 Fertil Steril

Alternative Treatment



Journal of Assisted Reproduction and Genetics (2019) 36:2057–2066
https://doi.org/10.1007/s10815-019-01543-5

ASSISTED REPRODUCTION TECHNOLOGIES

A treatment approach for couples with disrupted sperm DNA integrity and recurrent ART failure

Alessandra Parrella[1] · Derek Keating[1] · Stephanie Cheung[1] · Philip Xie[1] · Joshua D. Stewart[1] · Zev Rosenwaks[1] · Gianpiero D. Palermo[1]

Received: 3 May 2019 / Accepted: 23 July 2019 / Published online: 16 August 2019

Parrella et al., 2019 JARG

Semen Parameters

69 Patients

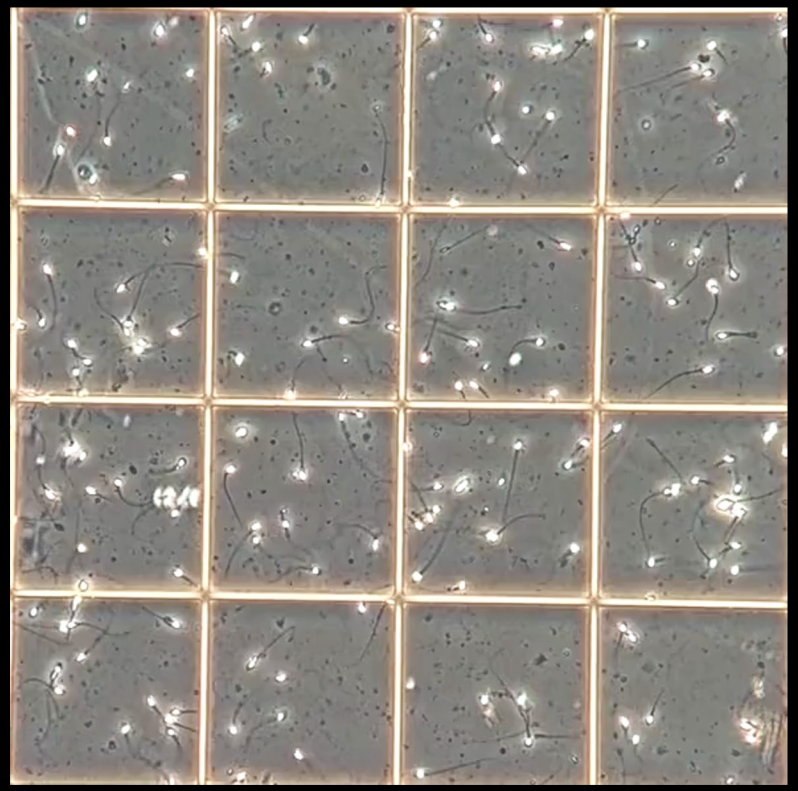
		Selection	
	Raw	Density Gradient	Microfluidics
Volume (mL)	2.2±1*	0.5±0*	0.5±0*
Concentration (M x10^6/ml±SD)	28±34†	17.2±22†	8.4±13†
Motility (M%±SD)	33.6±14‡	59.5±34‡	97.0±1‡
Morphology (M%±SD)	2.1±1∫	1.8±1∫	3.2±1∫

*†‡∫ Paired t-test, 1 *df*, *P<0.0001*

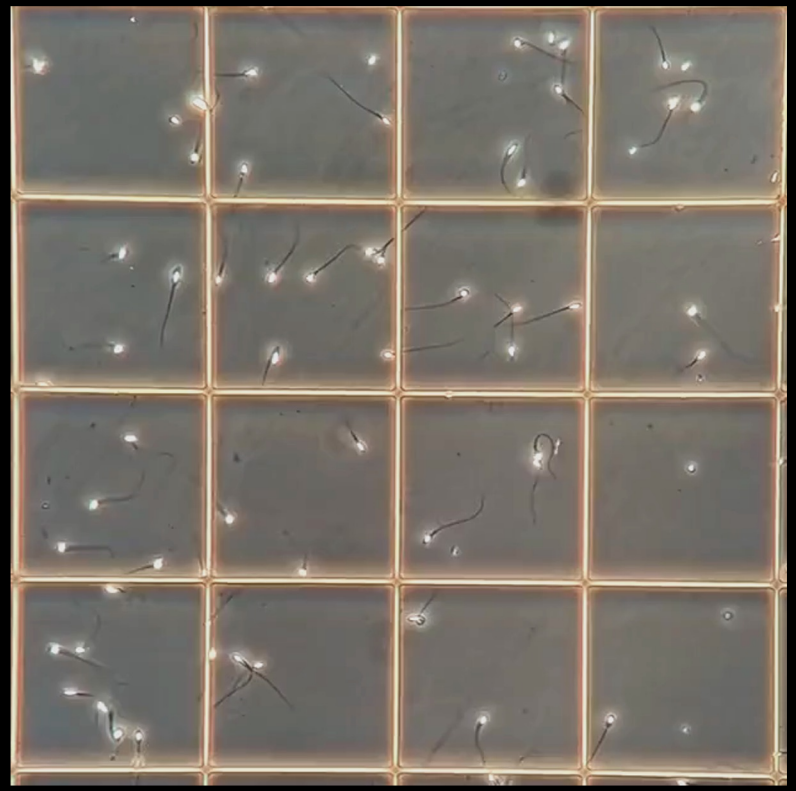
Parrella et al., 2019 JARG

Spermatozoa Motility

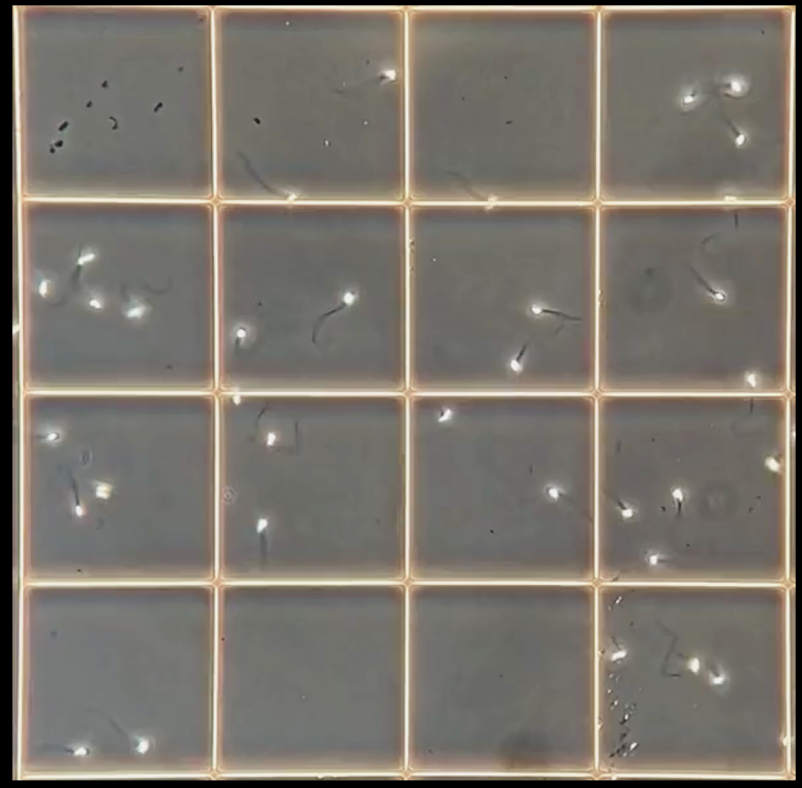
Raw



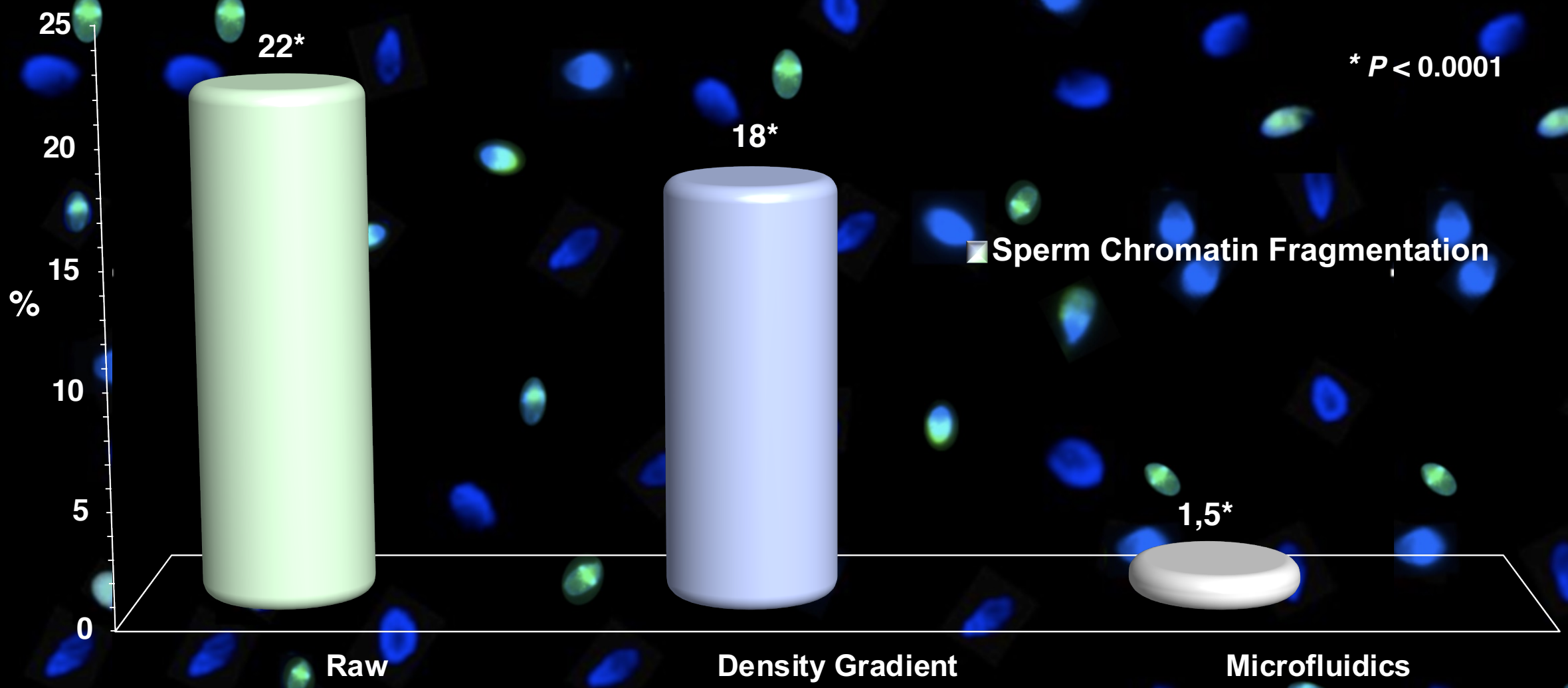
Density Gradient



Microfluidics



Sperm Chromatin Fragmentation



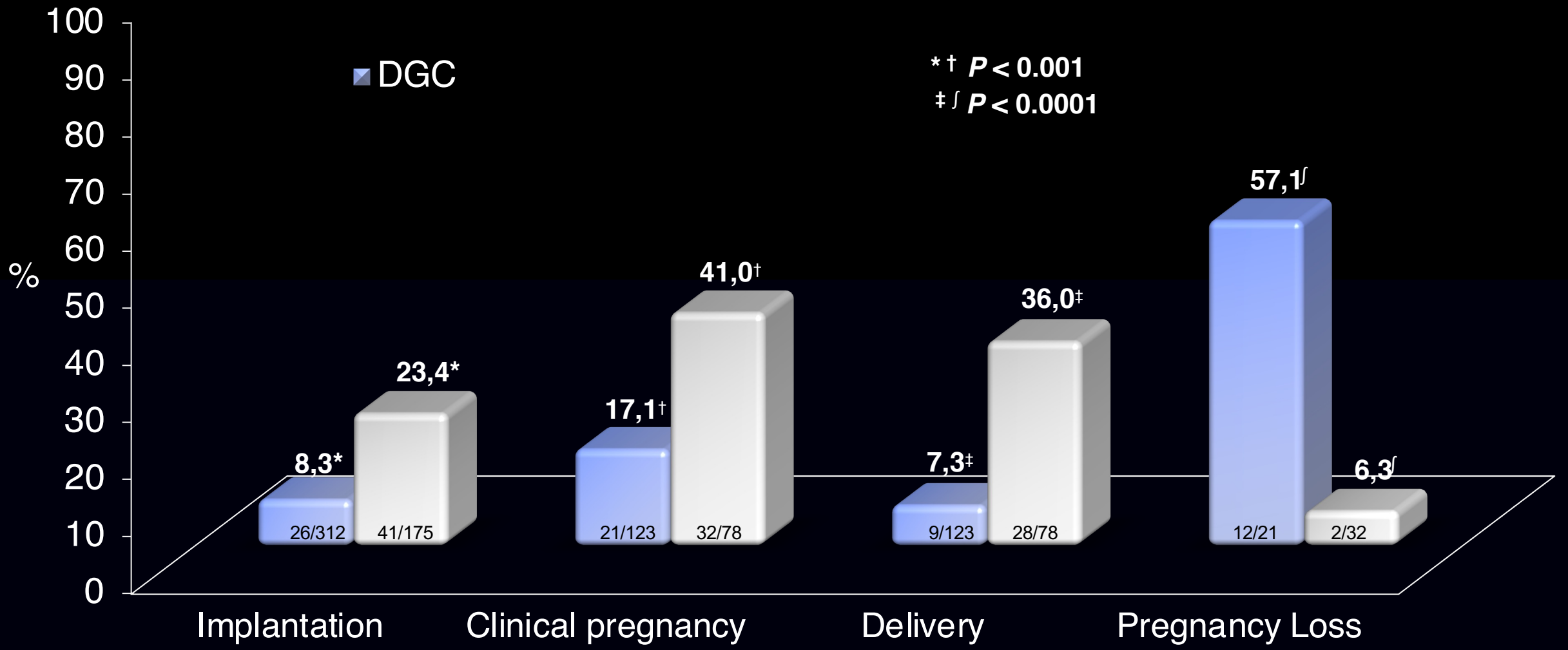
MFSS & Fresh ET

	Selection	
No. of	Density Gradient	Microfluidics
Couples	80	
Cycles	191	90
Maternal Age (M±SD)	37.8±3	37.9±3
Paternal Age (M±SD)	42.8±6	43.2±6
Fertilization rate	1172/1761 (66.6)	523/768 (68.1)

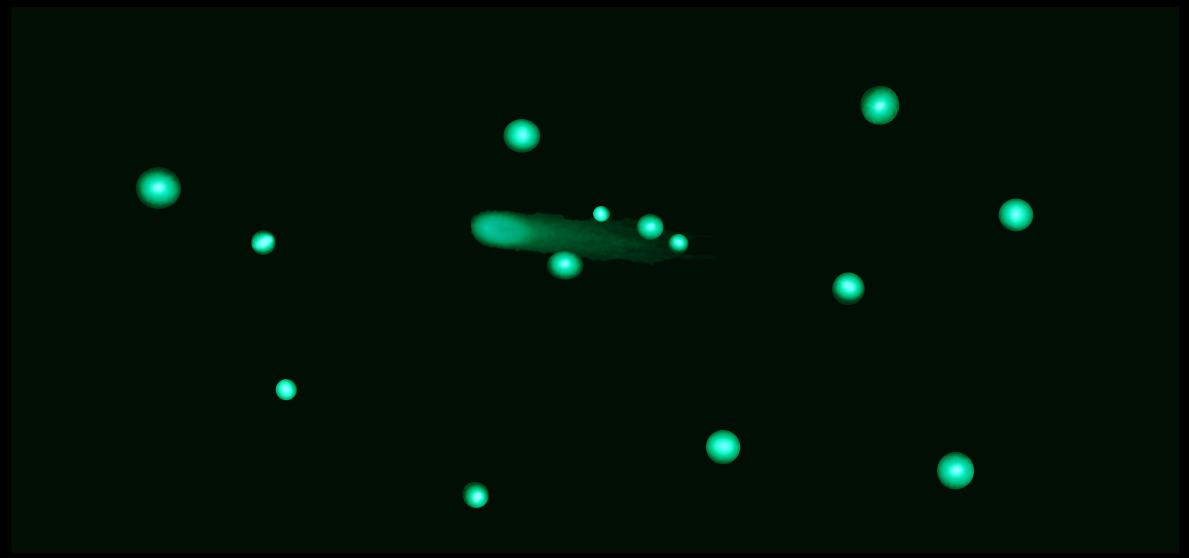
Parrella et al., 2019 JARG
Kocur et al., 2022 F&S Science

Clinical Outcome

Fresh ET



Parrella et al., 2019 JARG
Kocur et al., 2022 F&S Science



Patterns

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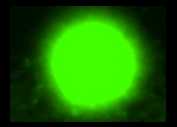

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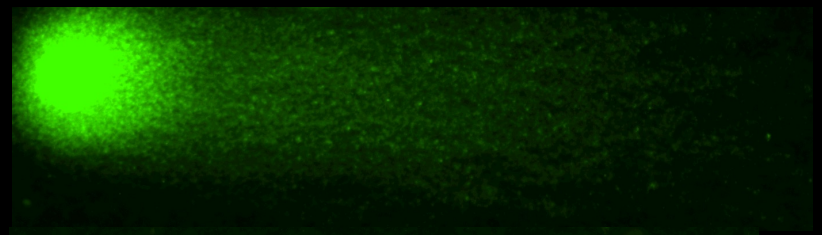

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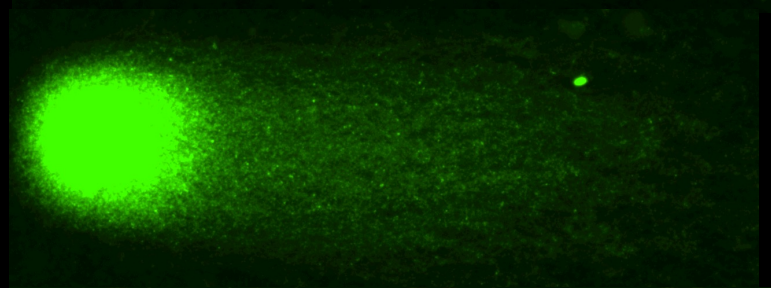

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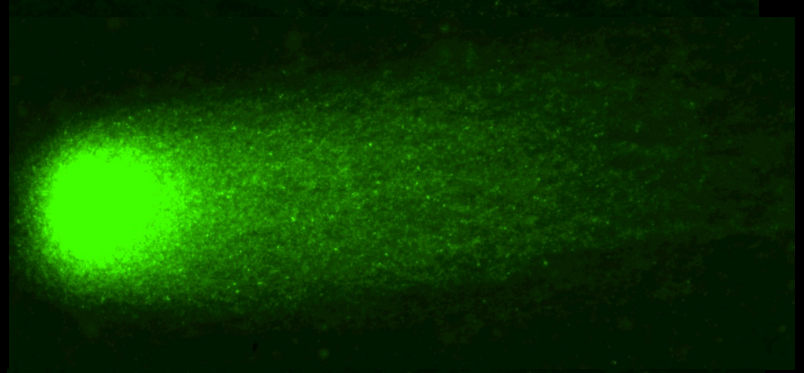

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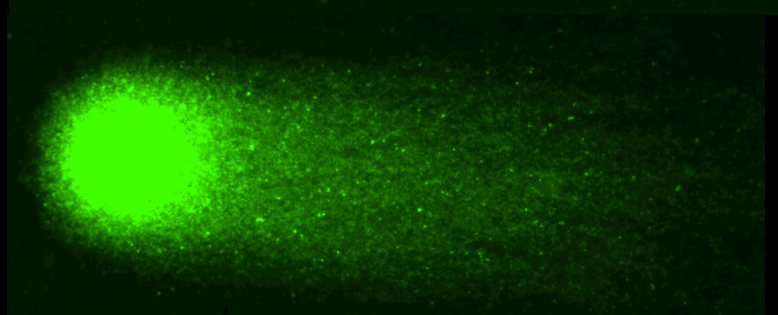
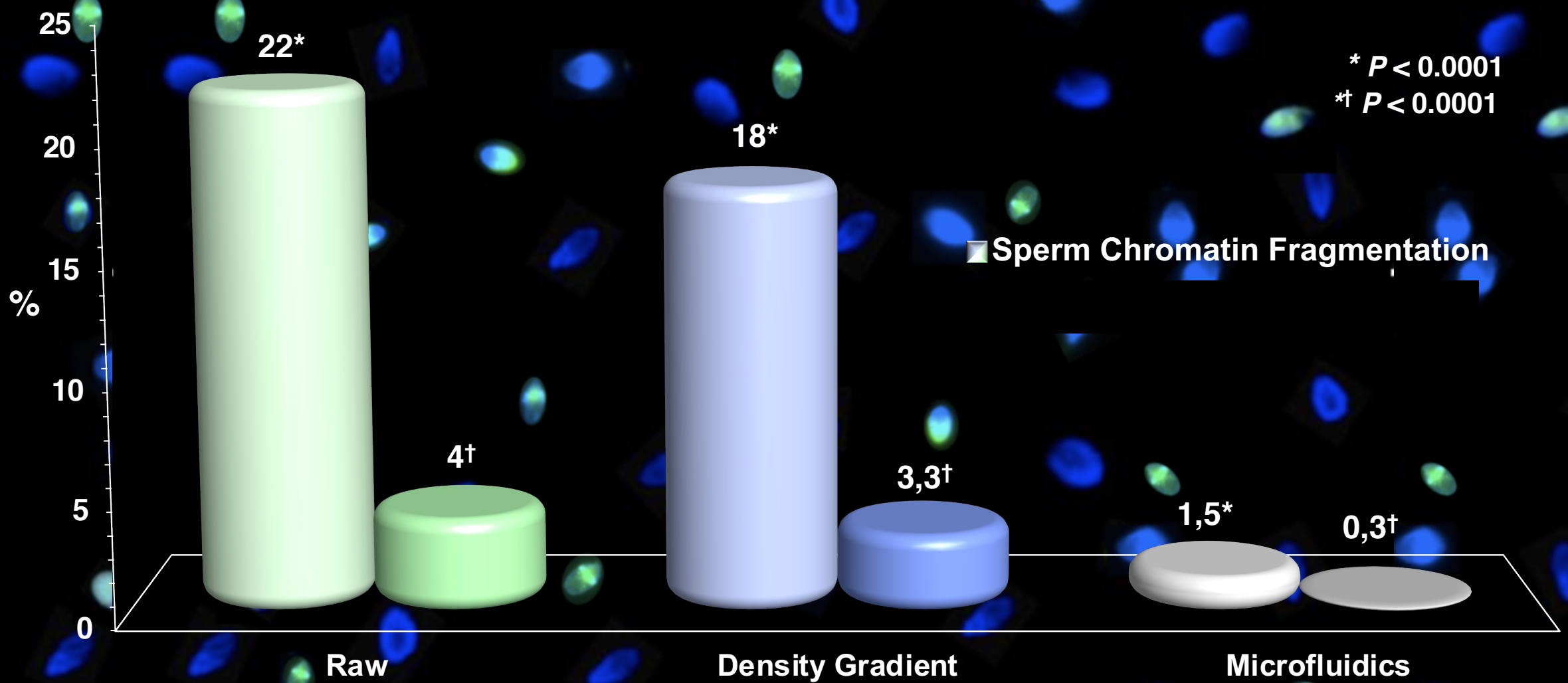


Sperm Chromatin Fragmentation



Parrella et al., 2019 JARG
Kocur et al., 2022 Andrology, Submitted

MFSS & PGT-A

	Selection	
No. of	Density Gradient	Microfluidics
Couples	85	
Cycles	88	86
Maternal Age (M±SD)	36.2±5	36.9±5
Paternal Age (M±SD)	36.9±7	37.5±7
Fertilization rate	848/1274 (66.6)*	949/1223 (77.6)*

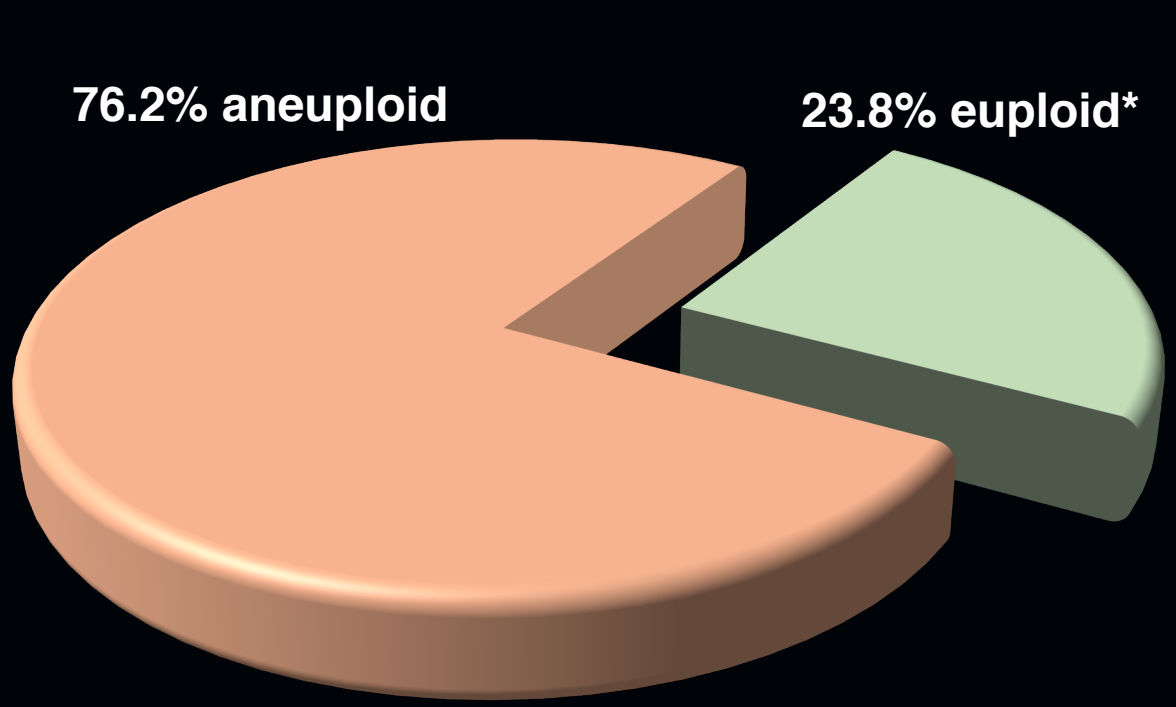
*$P<0.00001$

Parrella et al., 2019 JARG
Kocur et al., 2022 Andrology, Submitted

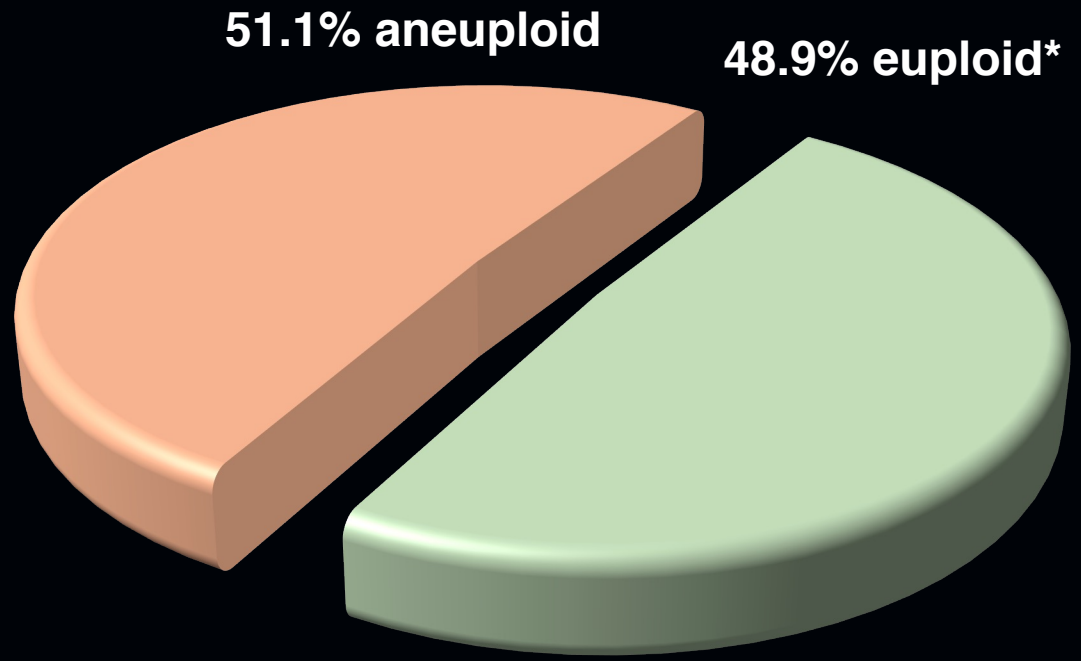
Euploidy Rates

*$P < 0.0001$

Density Gradient

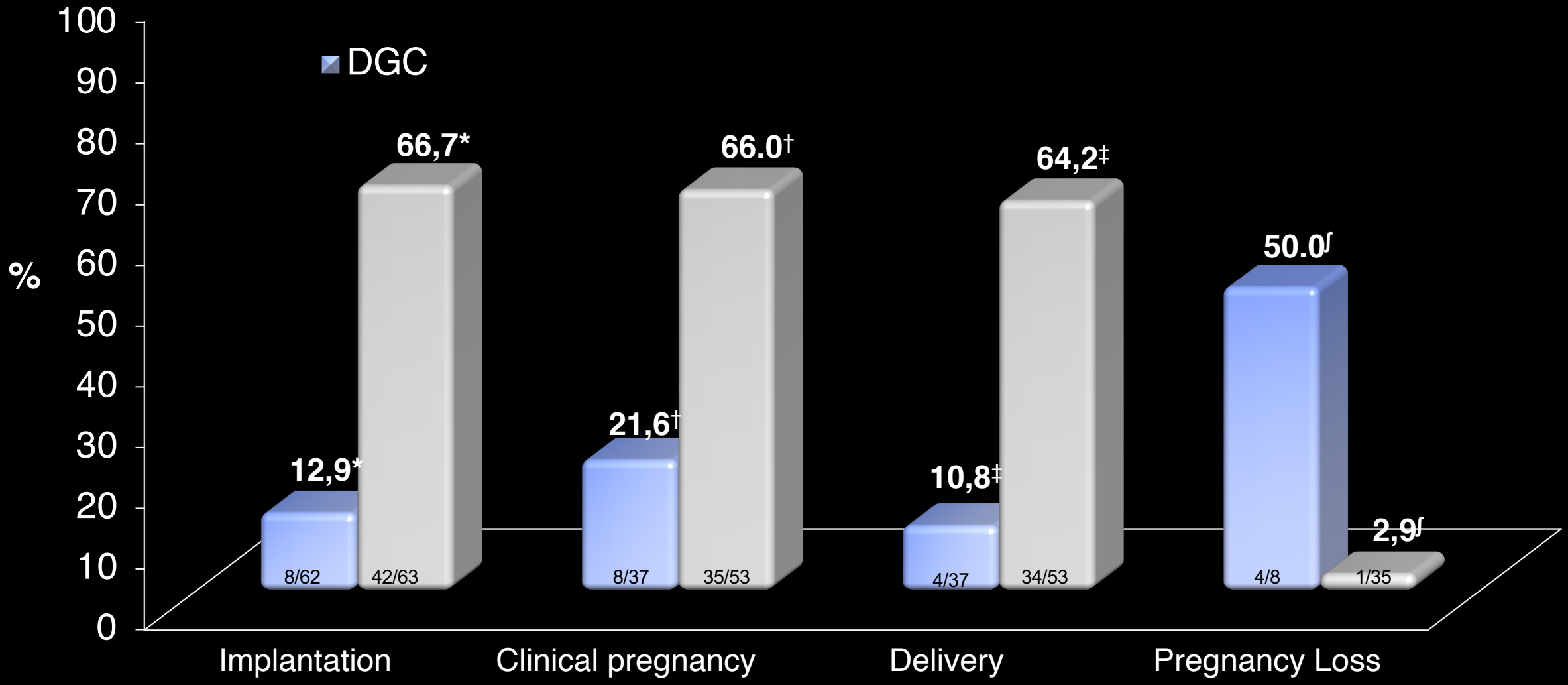


Microfluidics



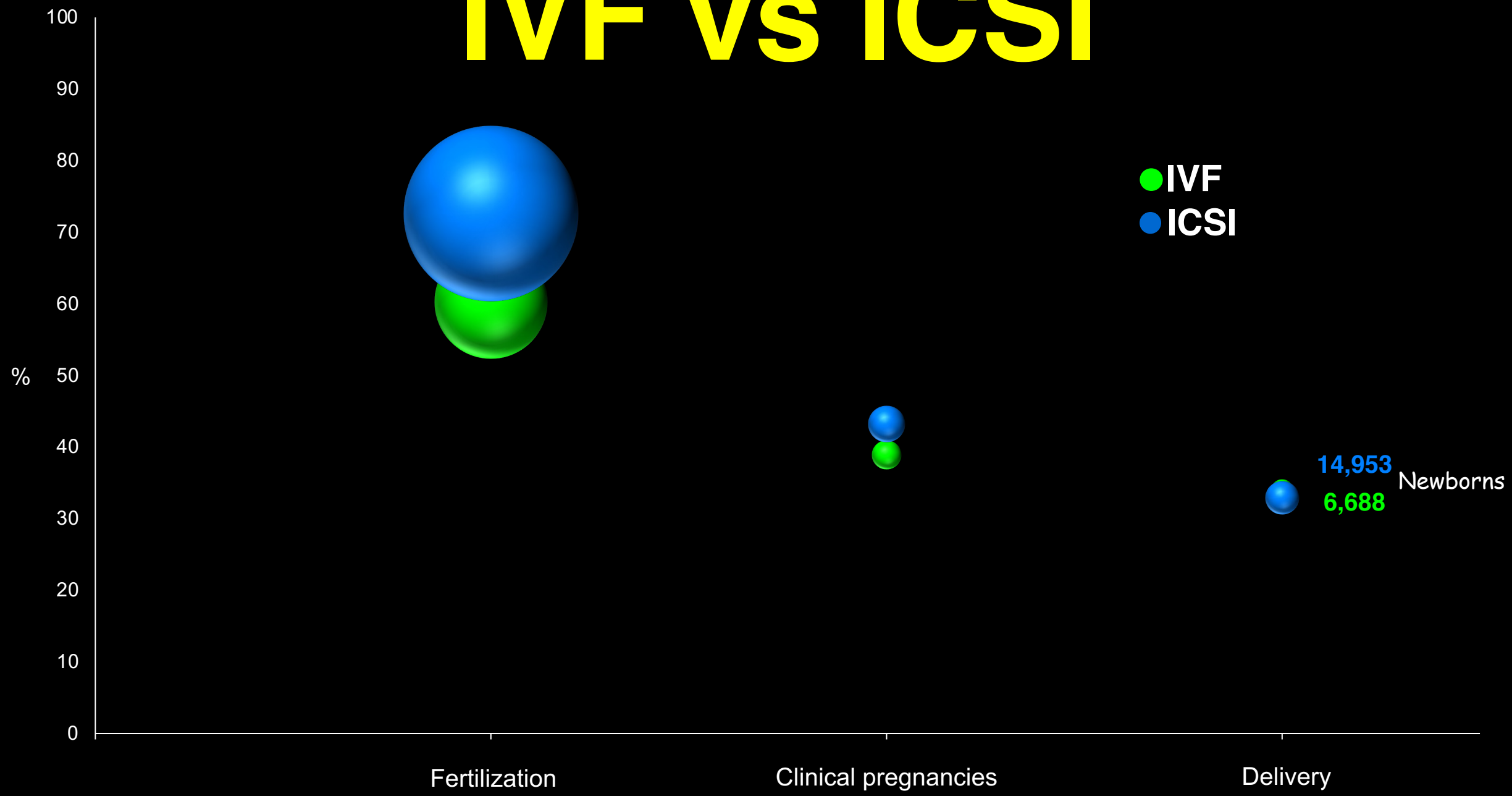
Clinical Outcome

PGT-A

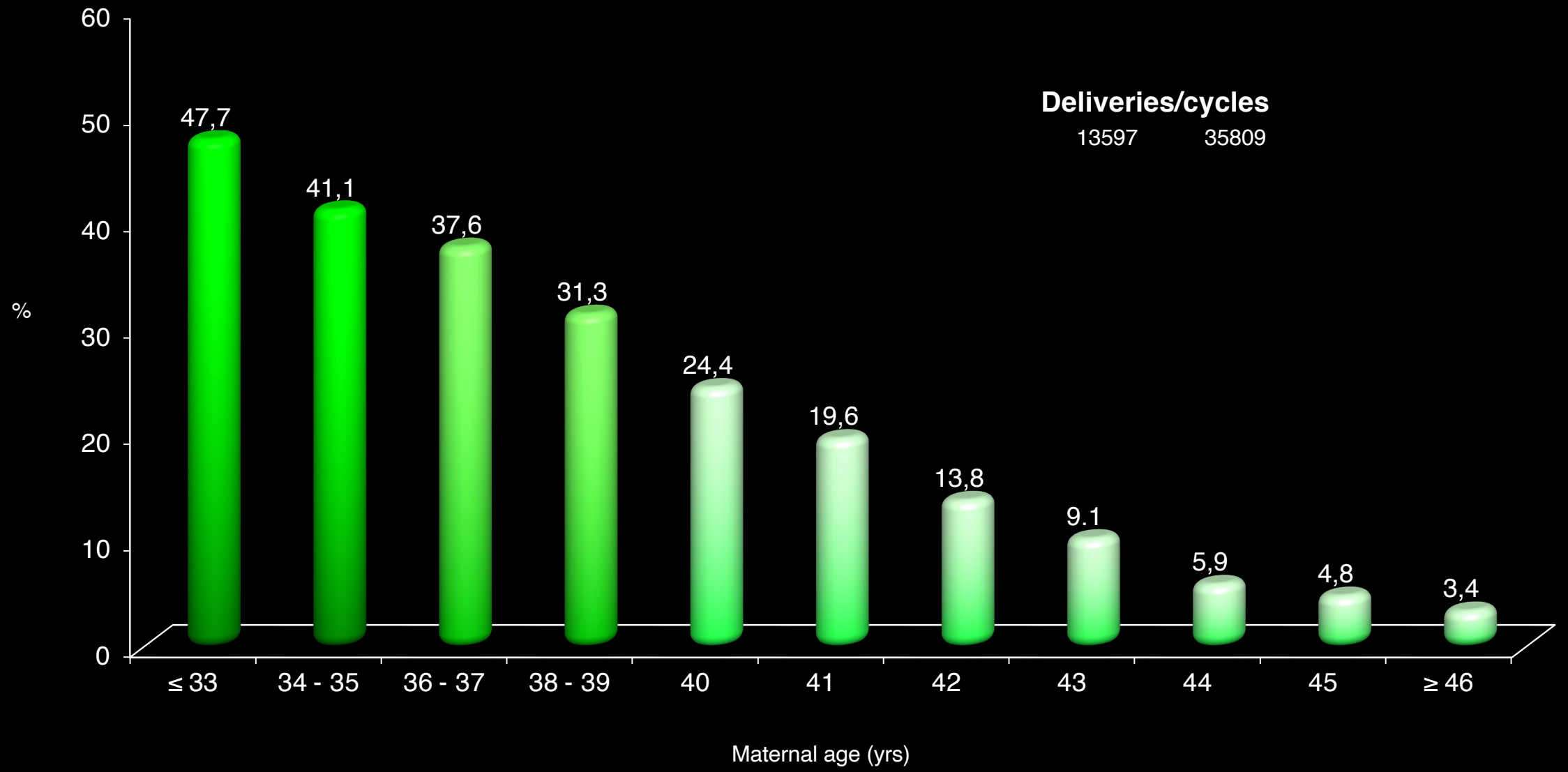


Parrella et al., 2019 JARG
Kocur et al., 2022 Andrology, Submitted

IVF vs ICSI


100
90
80
70
60
%
50
40
30
20
10
0
IVF
ICSI
14,953
6,688
Newborns
Fertilization
Clinical pregnancies
Delivery

Maternal Age & ICSI Deliveries



ICSI Indications

Palermo et al., 2015 Semin Reprod Med

Global ART in 2014

	76 Countries		
	IVF	ICSI	Total
Cycles	374,843 (37.7)	619,811 (62.3)	994,654
Newborns	165,518	273,521	439,039

ICMART, Chambers, et al., 2021 Human Reproduction

PLOS ONE

RESEARCH ARTICLE

Genetic and epigenetic profiling of the infertile male

Stephanie Cheung, Alessandra Parrella, Zev Rosenwaks, Gianpiero D. Palermo*

The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, New York, United States of America

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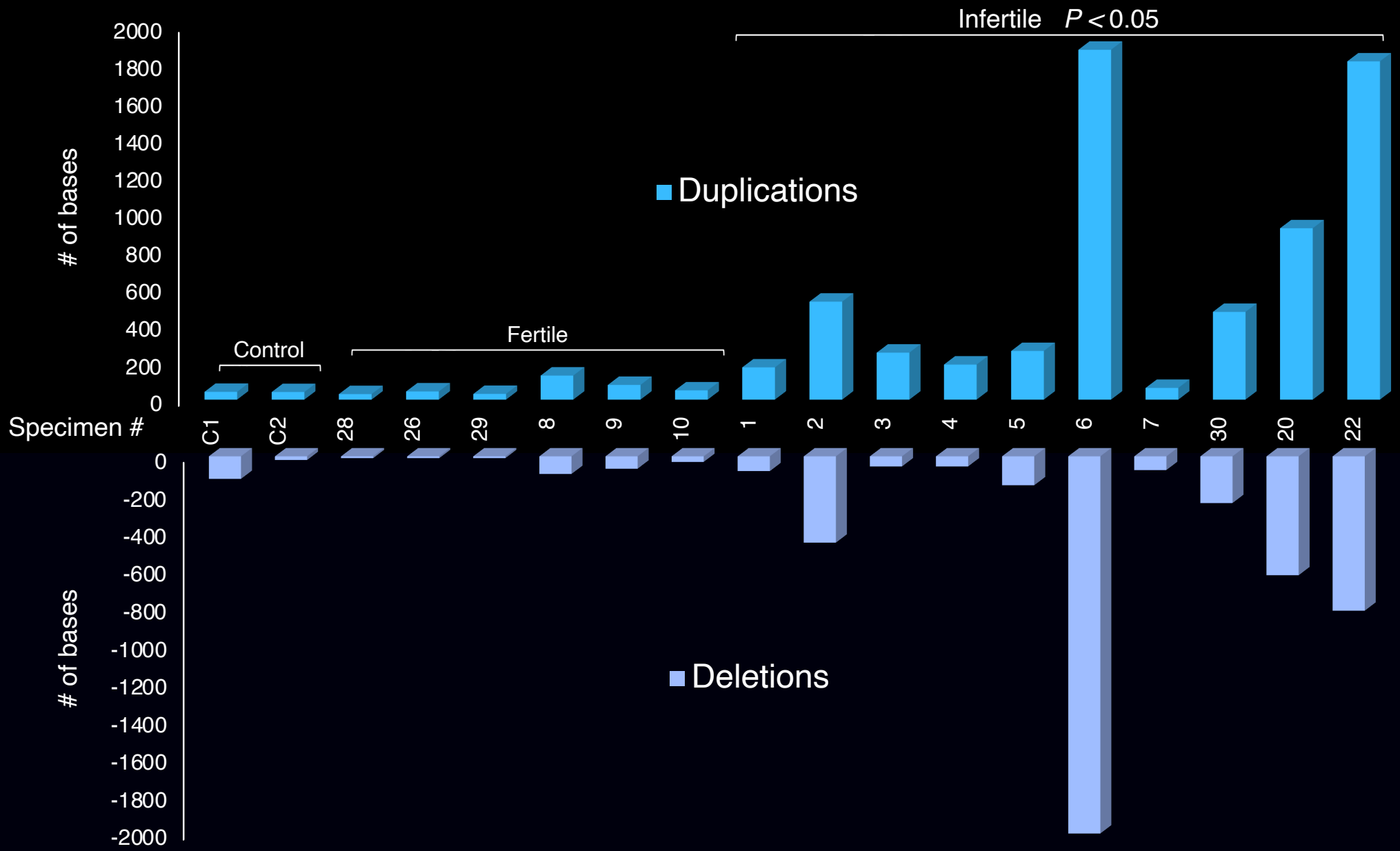
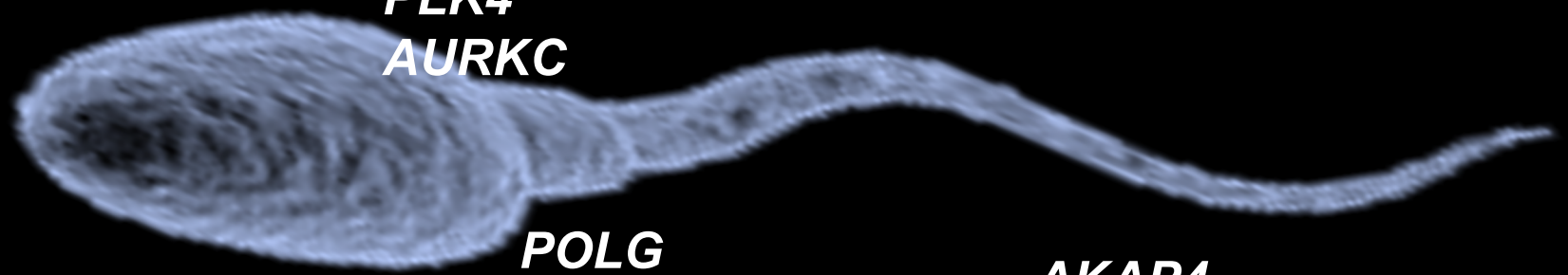
Abstract

Evaluation of reproductive quality of spermatozoa by standard semen analysis is often inadequate to predict ART outcome. Men may be prone to meiotic error and have higher proportion of spermatozoa with fragmented chromatin, capable of affecting the conceptus' health. In men with unexplained infertility, supplementary tests may be pivotal to gain insight into the paternal contribution to the zygotic genome. A total of 113 consenting men were



Cheung et al., 2019 PLOS

Spermiogenesis



PLK4
AURKC

POLG
SMCP

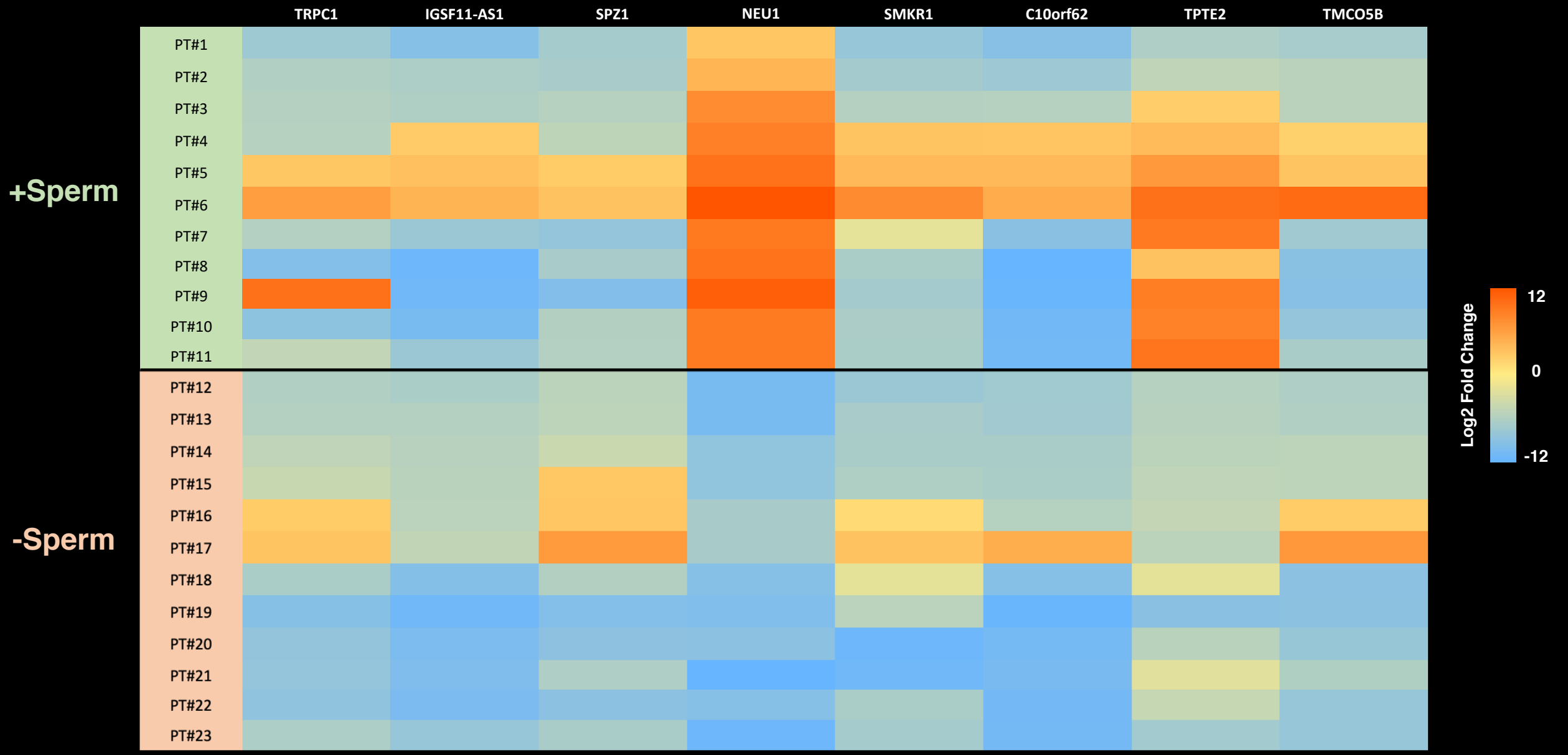
DPYL19L2
SPATA16
PICK1
ADCY10
TSSK6
H1FNT

AKAP4
CATSPER
SPAG16
DNAH11

Cheung et al., 2019 PLOS

Spermatogenesis Prediction

RNAseq



Ng et al., 2022 personal communication

Spermatogenesis Prediction

DNAseq

		TRPC1	IGSF11-AS1	SPZ1	NEU1	SMKR1	C10orf62	TPTE2	TMCO5B
+Sperm	PT#1	--	ns	--	syn	--	ms	--	--
	PT#2	--	ns	--	syn	--	ms	--	--
	PT#3	--	ns	--	syn	--	ms	--	--
-Sperm	PT#4	ms	ns	fs	fs	fs	ms	fs	--
	PT#5	ms	ns	fs	fs	fs	ms	fs	--
	PT#6	ms	ns	fs	fs	fs	ms	fs	--
	PT#7	ms	ns	fs	fs	fs	ms	fs	--
	PT#8	ms	ns	fs	fs	fs	ms	fs	--
	PT#9	ms	ns	fs	fs	fs	ms	fs	--
	PT#10	ms	ns	fs	fs	fs	ms	fs	--

ms: missense ns: nonsense fs: frameshift syn: synonymous

Gene	Ch	Function
TRPC1	3	Transient receptor potential non voltage-channel 1, expressed in adult heart, brain, testis, ovaries (Wes et al., 1995 PNAS)
IGSF11-AS1	3	Long non-coding RNA, downregulated in infertile male (Zhou and Wang, 2020 JIMR)
SPZ1	5	Regulation of cell proliferation/differentiation during spermatogenesis (Horowitz et al., 2005 Mol Hum Reprod)
NEU1	6	Neuraminidase, acrosomal reaction and capacitation (Ma et al., 2012 J Biol Chem)
SMKR1	7	Spermatid development, testis-specific in ovine species (Hodge et al., 2021 Genes)
C10orf62	10	Spermatid development, testis-specific (Djureinovic et al., 2014 Hum Reprod)
TPTE2	13	Acts as a lipid phosphatase, candidate genes for severe sperm motility disorders (Oud et al., 2021 Hum Reprod)
TMCO5B	15	Pseudogene, Testis-specific (Hong et al., 2018 BMC Genomics) & spermatid development in mouse (Yamase et al., 2019 PLoS One)

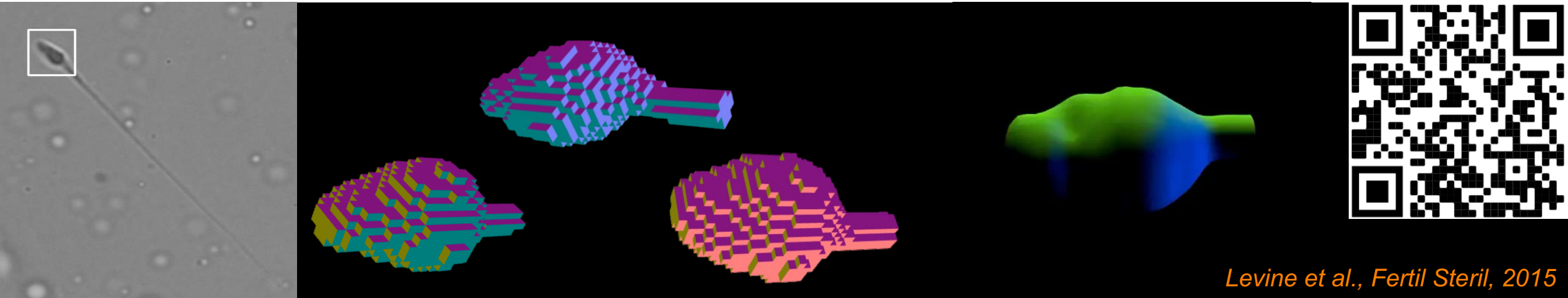
Cheung et al., 2022 personal communication

Fertility and Sterility®

Three-dimensional sperm surface reconstruction: a novel approach to assessing sperm morphology

Brian A. Levine, M.D., M.S.,[a] Jeremy Feinstein, M.Eng.,[b] Queenie V. Neri, M.Sc.,[a] Dan Goldschlag, M.D.,[a] Zev Rosenwaks, M.D.,[a] Serge Belongie, M.S., Ph.D.,[c] and Gianpiero D. Palermo, M.D., Ph.D.[a]

[a] The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical College, New York; [b] Department of Computer Science, College of Engineering, Cornell University, Ithaca; and [c] Cornell Tech, Cornell University, New York, New York



Levine et al., Fertil Steril, 2015

Rheotaxis-based separation of sperm with progressive motility using a microfluidic corral system

Meisam Zaferani[a], Soon Hon Cheong[b], and Alireza Abbaspourrad[a,1]

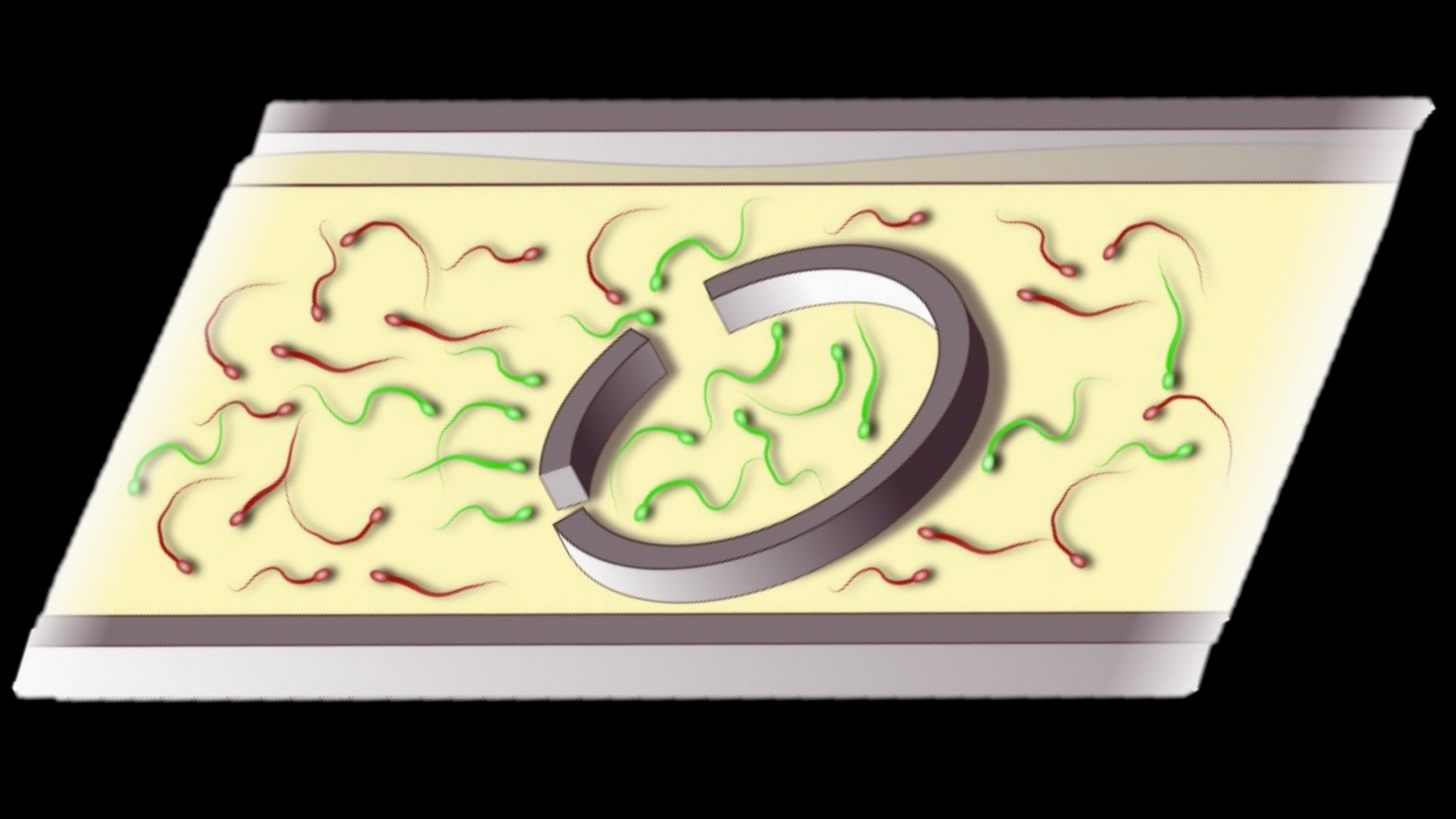
Zaferani et al., 2018 PNAS

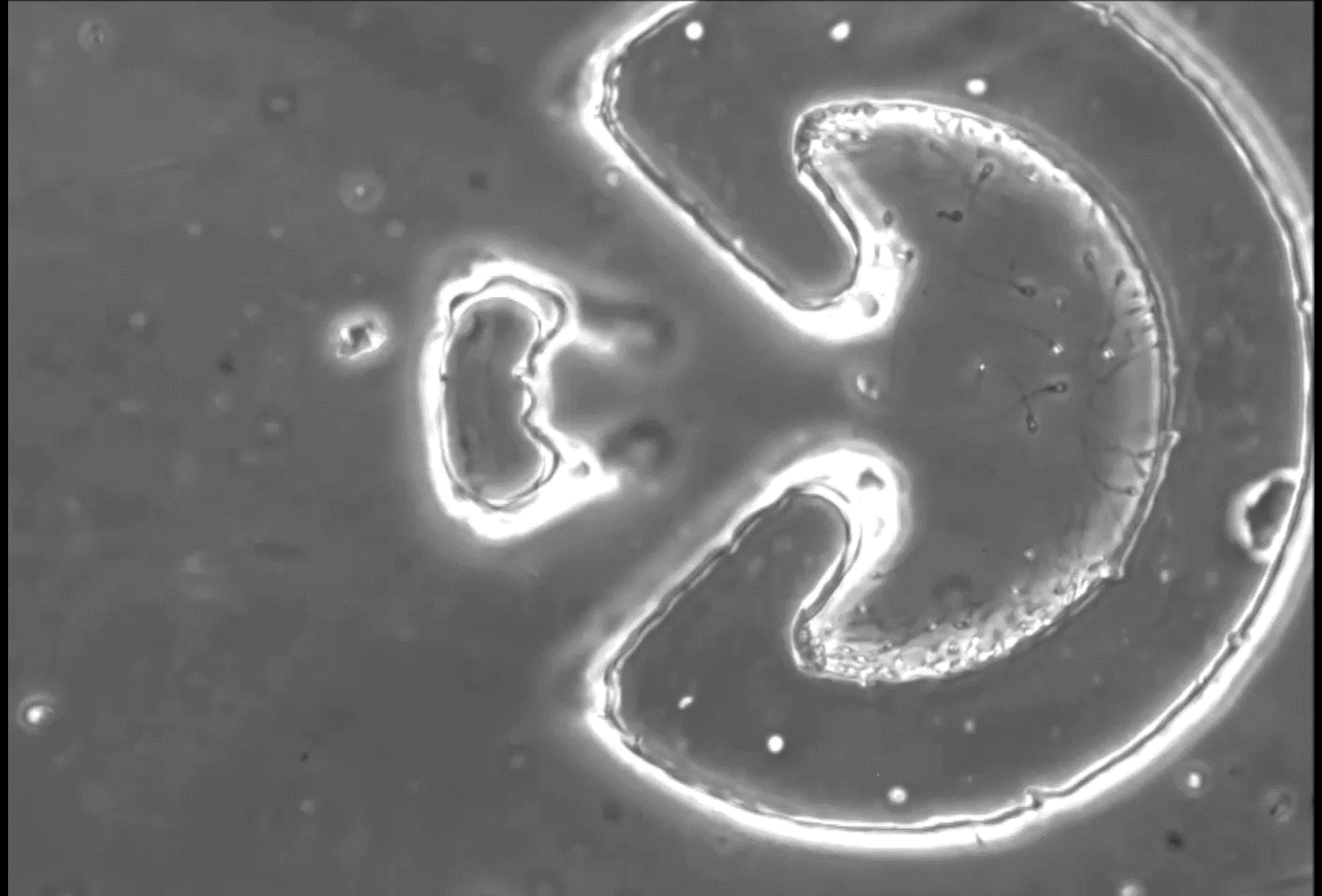
[a]Department of Food Science, College of Agriculture and Life Sciences, Cornell University, Ithaca, NY 14853; and [b]Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853

Edited by Howard A. Stone, Princeton University, Princeton, NJ, and approved July 3, 2018 (received for review January 15, 2018)

The separation of motile sperm from semen samples is sought after for medical infertility treatments. In this work, we demonstrate a high-throughput microfluidic device that can passively isolate motile sperm within corrals inside a fluid channel, separating them from the rest of the diluted sample. Using finite element method simulations and proposing a model for sperm motion, we investigated how flow rate can provide a rheotaxis zone in front of the corral for sperm to move upstream/downstream depending on their motility. Using three different flow rates that provided shear rates above the minimum value within the rheotaxis zone, we experimentally tested the device with human and bovine semen. By taking advantage of the rheotactic behavior of sperm, this microfluidic device is able to corral motile sperm with progressive velocities in the range of 48–93 $\mu m \cdot s^{-1}$ and 51–82 $\mu m \cdot s^{-1}$ for bovine and human samples, respectively. More importantly, we demonstrate that the separated fractions of both human and bovine samples feature 100% normal progressive motility. Furthermore, by extracting the sperm swimming distribution within the rheotaxis zone and sperm velocity distribution inside the corral, we show that the minimum velocity of the corralled sperm can be adjusted by changing the flow rate; that is, we are able to control the motility of the separated sperm.

biology. The factors that influence the journey of a sperm cell, which starts with ejaculation and ends with egg fertilization, are poorly characterized. Some efforts have investigated the response of sperm to external stimuli, like chemical gradients and fluid flow; such responses are generally referred to as "taxis" (10, 11). Researchers have also investigated the tail-beating patterns of sperm in different situations (12–14), as well as the molecular interactions between sperm and the female reproductive tract (1, 14–16). However, since the study of sperm in vivo is complicated by the existence of many environmental variables, such as pH, chemical gradients, and fluid flow (2, 10, 11), many questions about sperm behavior remain unanswered. The concurrent existence of these variables impedes our ability to gain better insight into sperm motion itself, which is a complex topic (17). Thus, the isolation of motile sperm in vitro (eliminating all external hydrodynamic velocity fields and dead sperm) could further assist the study of sperm locomotion. Additionally, isolating sperm in a particular region would enable the evaluation of an individual sperm's biological and physiological responses to a specific chemical or physical factor (18). To summarize, any



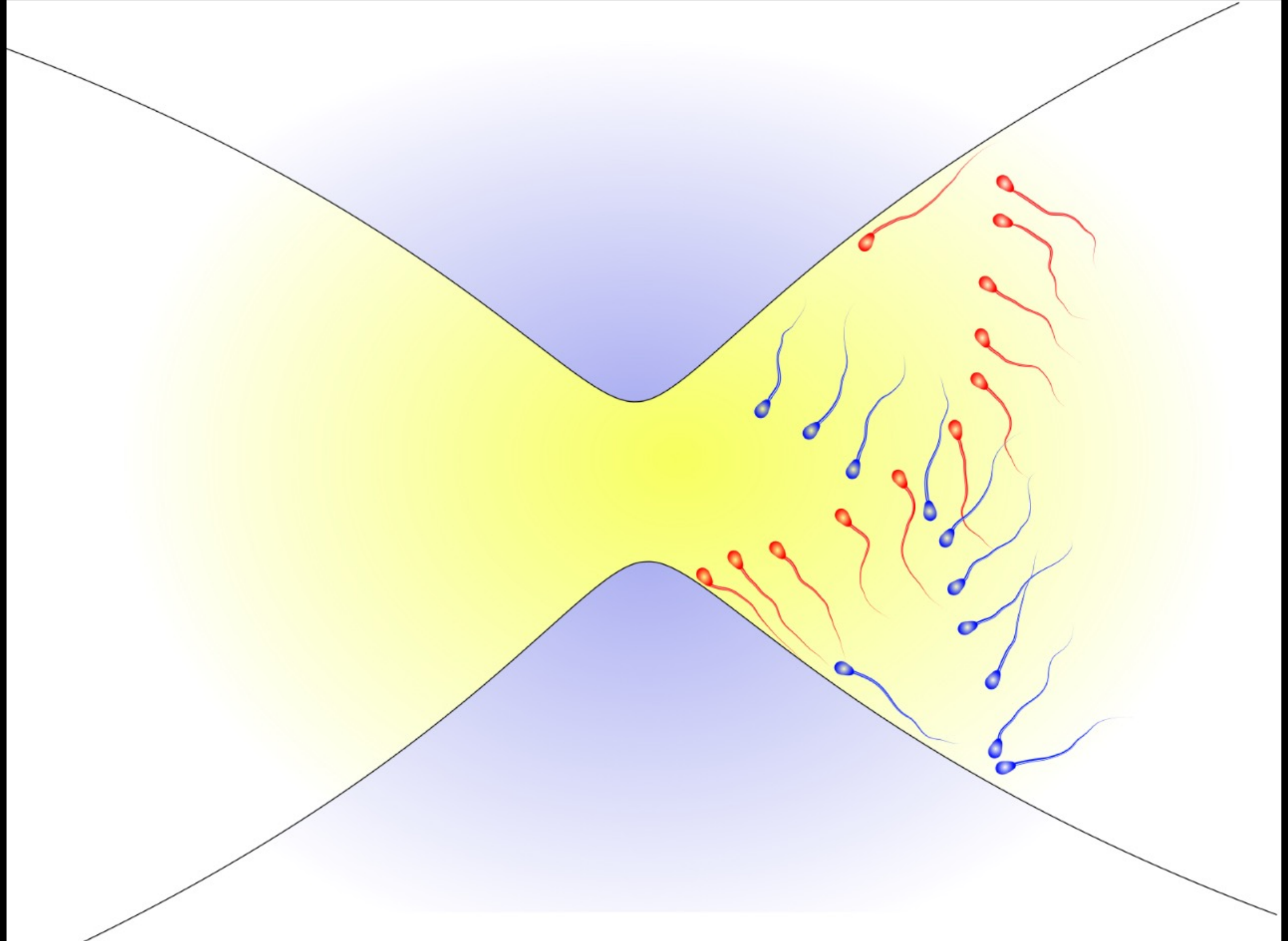


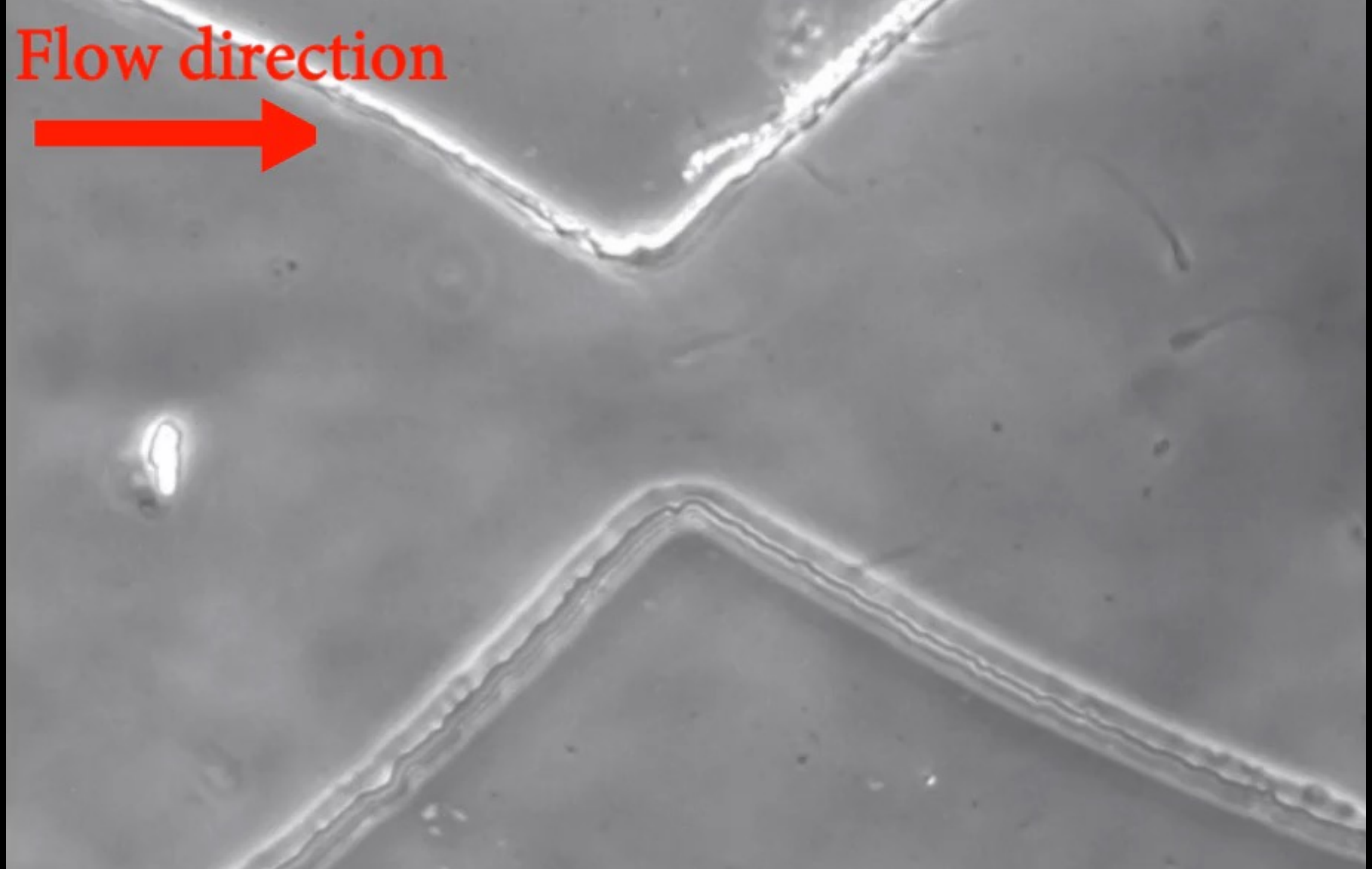
BIOPHYSICS

Strictures of a microchannel impose fierce competition to select for highly motile sperm

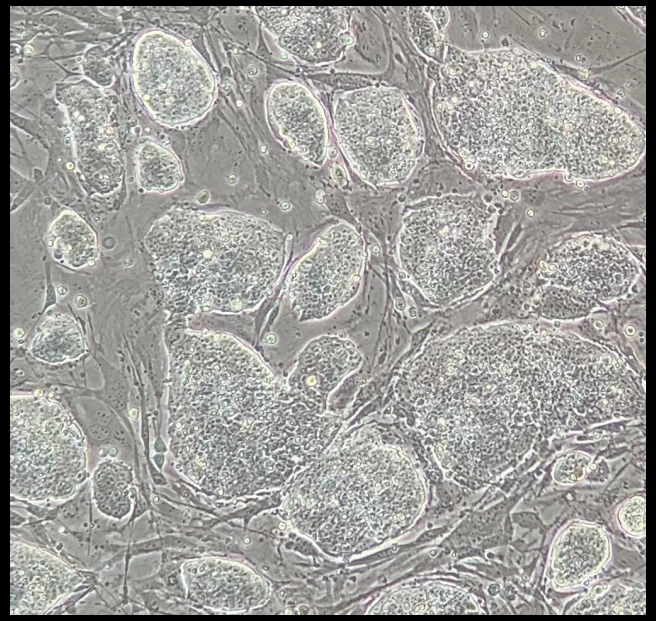
Meisam Zaferani[1], Gianpiero D. Palermo[2], Alireza Abbaspourrad[1]*

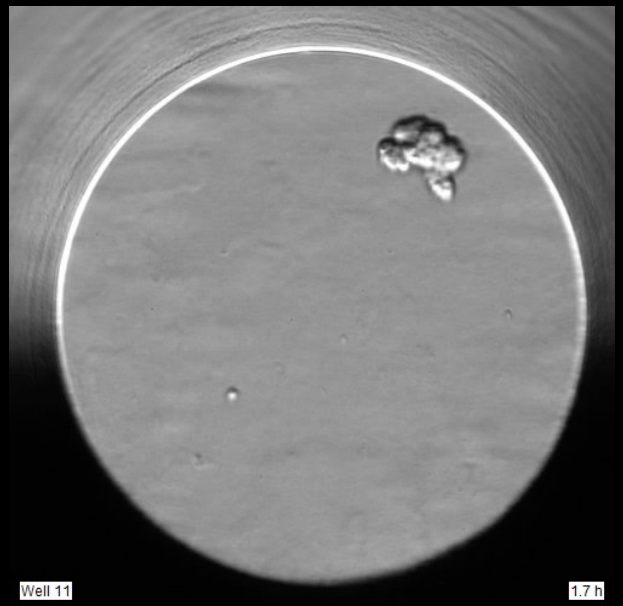
Investigating sperm locomotion in the presence of external fluid flow and geometries simulating the female reproductive tract can lead to a better understanding of sperm motion during fertilization. Using a microfluidic device featuring a stricture that simulates the fluid mechanical properties of narrow junctions inside the female reproductive tract, we documented the gate-like role played by the stricture in preventing sperm with motilities below a certain threshold from advancing through the stricture to the other side (i.e., fertilization site). All the slower sperm accumulate below (i.e., in front of) the stricture and swim in a butterfly-shaped path between the channel walls, thus maintaining the potential for penetrating the stricture and ultimately advancing toward the fertilization site. Accumulation below the stricture occurs in a hierarchical manner so that dense concentrations of sperm with higher velocities remain closer to the stricture, with more sparsely distributed arrays of lower-velocity sperm lagging behind.

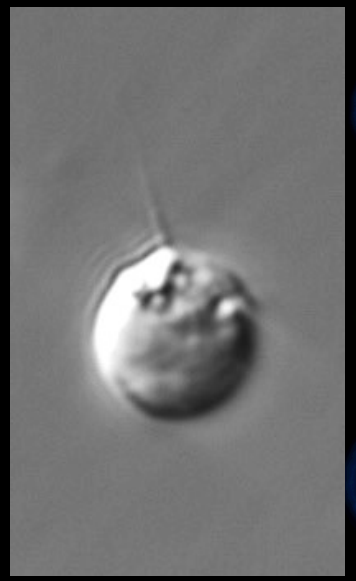


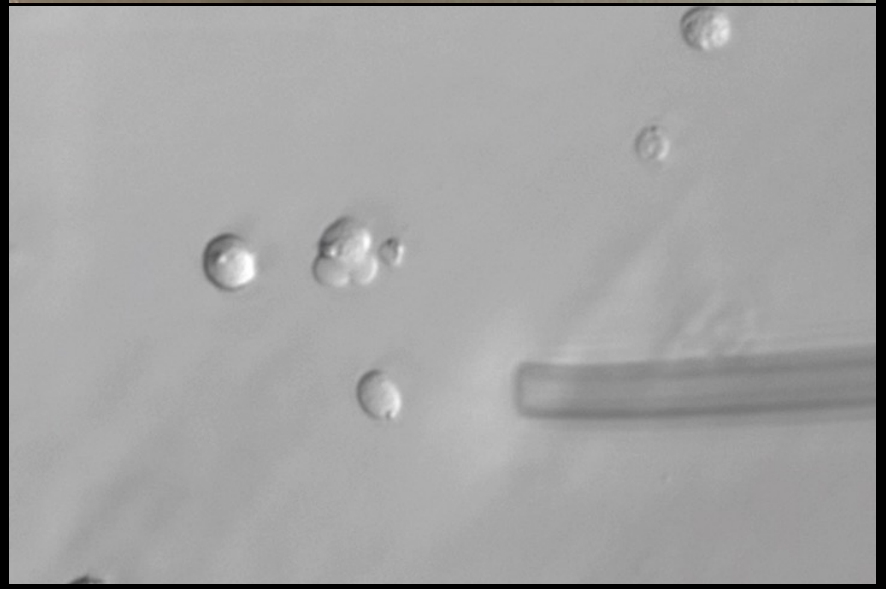

Flow direction

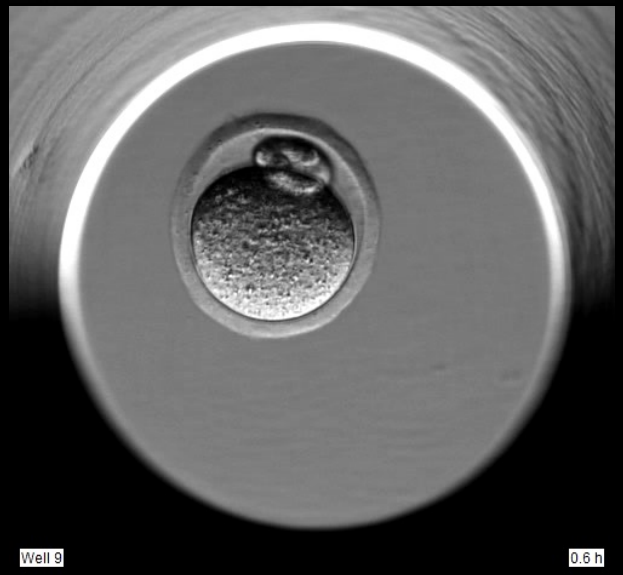
Spherification





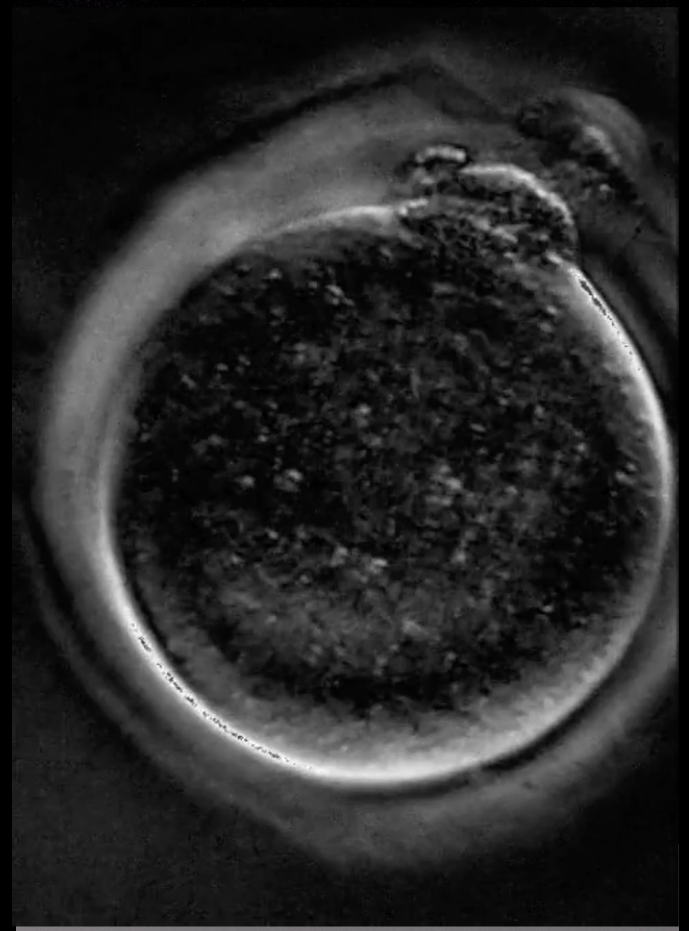


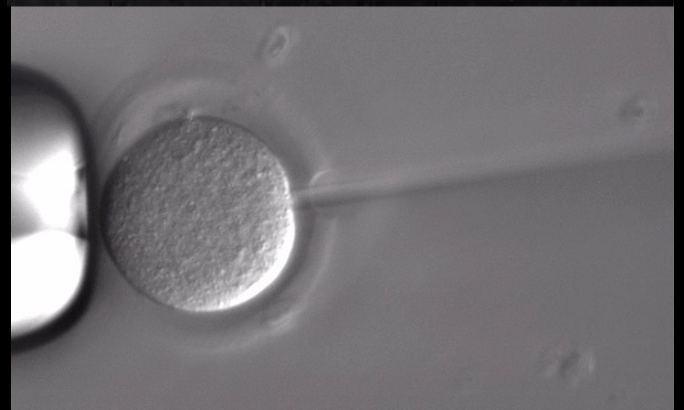


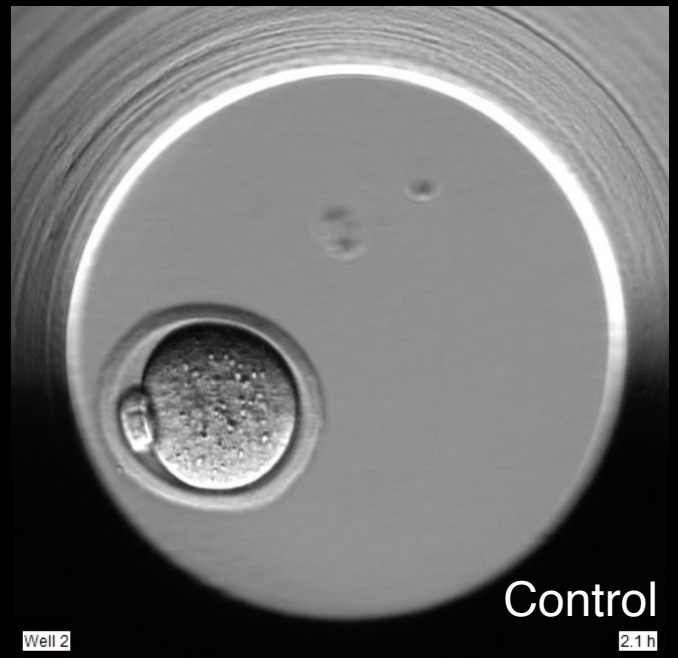


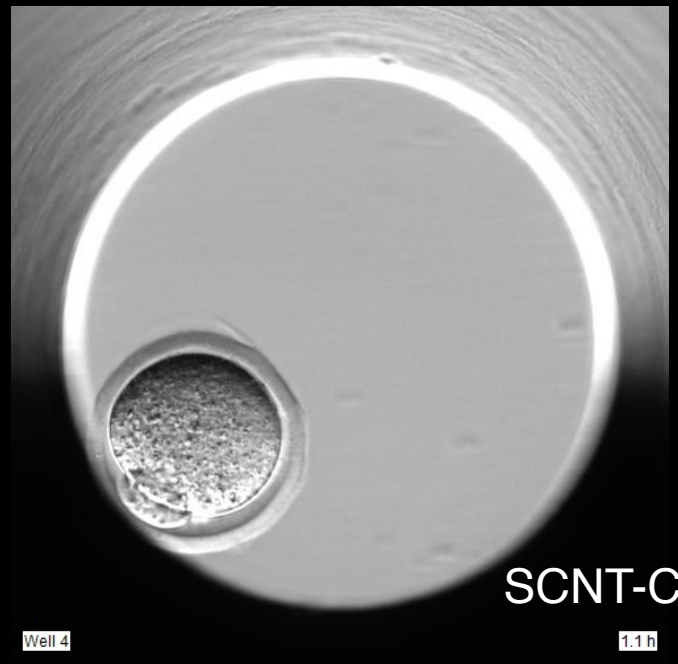
McKnight et al., 2022 ASRM

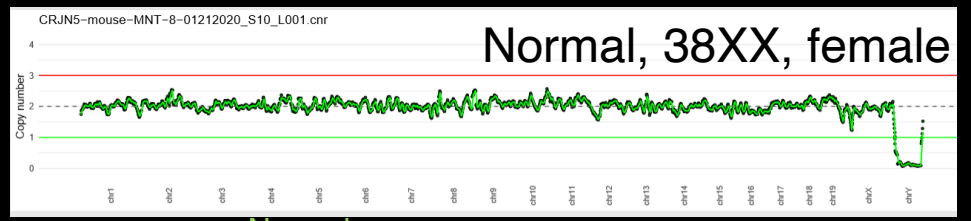
Somatic Cell Nuclear Transfer and Haploidization



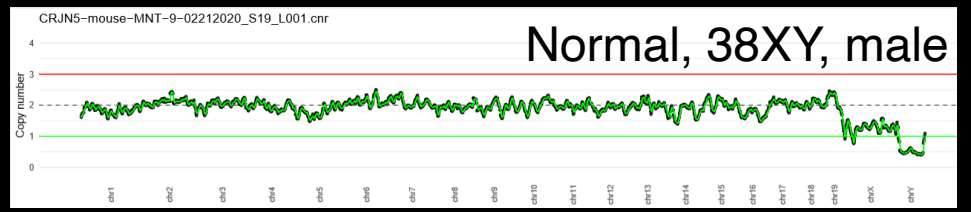








Cumulus 4 Normal 38XY



Lee et al., 2022 Nature Commun Biol

Conclusions

- ICSI is the ultimate treatment for male infertility
- ICSI is versatile and consistent
- ICSI remains a popular insemination method
- More information on the male gamete genome
- *In-Vitro* Gametogenesis will certainly require ICSI
- *AI* may present the next chapter

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The ICSI Story
To Infinity and Beyond

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