PRECONGRESS COURSE 13

Eight technical innovations designed to improve reproductive outcome: Promising or sobering facts?

Middle East fertility Society Exchange Course
Helsinki – Finland, 3 July 2016

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Eight technical innovations designed to improve reproductive outcome: promising or sobering facts?

Helsinki, Finland
3-6 July 2016

Organised by
The Paramedical Group

Contents

Course coordinator

Johnny Awwad (Lebanon) and Mohammad Aboulghar (Egypt)

Course type

Basic and advanced

Course description

Developments in medical technology have led to numerous interventions designed to improve human fertility. Innovations such as Time-lapse embryo imaging, Intra Cytoplasmic Morphology Selected Sperm Injection (IMSI), Pre-implantation Genetic Aneuploidy Screening (PGS), Sperm DNA fragmentation, Adherence compounds in embryo transfer media, Gene profiling in endometrium, Micro-dissection Testicular Sperm Extraction (Micro TESE) and many others, have been introduced to enhance the reproductive outcome of women undergoing assisted reproduction. These breakthrough technologies have largely been the outcome of extensive research and exciting findings in various experimental models before making their way into human reproduction. In addition to advancing our ability to alter reproductive pathways, such technologies have also greatly expanded our understanding of the biology of reproduction. Many however have been hastily introduced into clinical practice with little evidence of improved reproductive outcome, often driven by couples' eagerness to try any promising innovation before the evidence is available to support their use.

This pre-congress course discusses some of these technical innovations introduced into the practice of assisted reproduction over the past several years, with the prime focus of evaluating their clinical relevance to improving live births in view of emerging scientific evidence.

Target audience

- Reproductive Endocrinologists and Fertility Specialists
- Biologists involved in Assisted Reproductive Technologies
- Policy Regulators and Representatives of Third Party Payers

Educational needs and expected outcomes

At the completion of this pre-congress course, participants should be able to:

- Describe the biologic pathways relevant to human reproduction
- Understand the working hypotheses for introducing the innovations described into assisted reproduction
- Evaluate the merits of each described breakthrough in improving live births in women undergoing assisted reproduction
- Formulate an evidence-based decision on whether to offer any one of these technologies in the context of infertility management in women

Scientific programme

09:00 - 09:30	Time-lapse embryo imaging: Does the use of morphokinetics improve embryo implantation? ***Johnny Awwad - Lebanon***
09:30 - 09:45	Discussion
09:45 - 10:15	Preimplantation Genetic Aneuploidy Screening (PGS): Is it delivering on its promise? ***Elias Dahdouh - Canada***
10:15 - 10:30	Discussion
10:30 - 11:00	Coffee break
11:00 - 11:30	Intra Cytoplasmic Morphology Selected Sperm Injection (IMSI): Between Hope and Hype? ***Sherman J. Silber - U.S.A.***
11:30 - 11:45	Discussion
11:45 - 12:15	Adherence compounds in embryo transfer media (fibrin sealant and hyaluronic acid): The evidence ***William H. Kutteh - U.S.A.***
12:15 - 12:30	Discussion
12:30 - 13:30	Lunch break
13:30 - 14:00	Gene profiling in endometrium: Does personalized embryo transfer correct for implantation failure? ***Carlos Simon Valles - Spain***
14:00 - 14:15	Discussion
14:15 - 14:45	Immunologic Testing in Reproduction: Do these tests predict successful implantation ***William H. Kutteh - U.S.A.***
14:45 - 15:00	Discussion
15:00 - 15:30	Coffee break
15:30 - 16:00	Sperm DNA fragmentation: Does it impact live birth rate after IVF or ICSI? ***Yacoub Khalaf - United Kingdom***
16:00 - 16:15	Discussion
16:15 - 16:45	Microdissection Testicular Sperm Extraction (Micro TESE): Does it improve localization of sperm compared with conventional TESE in non-obstructive azoospermia? ***Sherman J. Silber - U.S.A.***
16:45 - 17:00	Discussion

TIME-LAPSE EMBRYO IMAGING: DOES THE USE OF MORPHOKINETICS IMPROVE EMBRYO IMPLANTATION?

Johnny Awwad, MD

Professor of Obstetrics and Gynecology

Head, Division of Reproductive Endocrinology and Infertility

American University of Beirut Medical Center

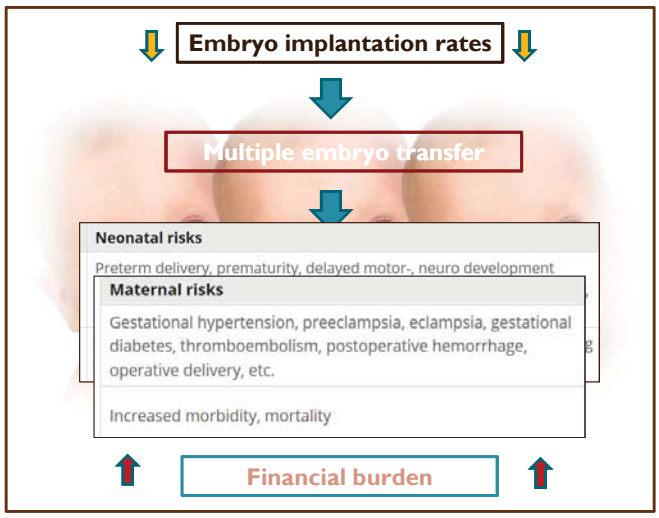
Non Disclosure Statement

NO CONFLICT OF INTEREST

Time Lapse Imaging

A. THE PROBLEM

TRADITIONAL RATIONAL



All SART Member Clinics

Provide feedback on SART's new Clinic Summary Report

Preliminary CSR for 2014 1.4 % of cycles reported as Delayed Outcome

	< 35	35 - 37
Number of cycle starts	41534	21692
Singletons	**31.9 %**	**26.5 %**
Twins	10.4 %	7.1 %
Triplets or more	0.3 %	0.3 %
Live Births	42.6 %	33.9 %
(Confidence Range)	(42.1 - 43.1)	(33.3 - 34.5)

> 42
8786
3.6 %
0.3 %
0.0 %
4.0 %
(3.6 - 4.4)

PROPOSED SOLUTION

SINGLE EMBRYO TRANSFER

Cochrane

The chances of multiple birth are also significantly reduced after one cycle of fresh single embryo transfer than after one cycle of fresh multiple embryo transfer.

Number of embryos for transfer following in vitro fertilisation

Single compared to multiple embryo transfer (in a single cycle) following in vitro fertilisation or intra-cytoplasmic sperm injection

Population: women having embryo transfer following in vitro fertilisation or intra-cytoplasmic sperm injection
Settings: Assisted reproduction
Intervention: Single embryo transfer
Comparison: Multiple embryo transfer (in a single cycle)

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk Multiple	Corresponding risk Single				
Multiple pregnancy	144 per 1000	20 per 1000 (12 to 32)	OR 0.12 (0.07 to 0.20)	1612 (10 Studies)	⊕⊕⊕⊕ HIGH [2]	
Live birth	450 per 1000	282 per 1000 (242 to 329)	OR 0.48 (0.39 to 0.60)	1564 (9 Studies)	⊕⊕⊕⊕ HIGH [1]	

CHALLENGE

SINGLE EMBRYO TRANSFER

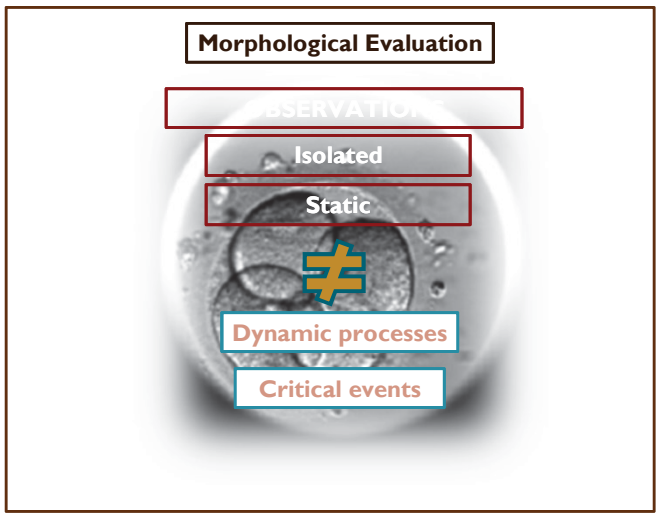
LIVE BIRTH

Time Lapse Imaging

B. WHAT IS ALREADY AVAILABLE

Johnny Awwad, MD

CONVENTIONAL METHODOLOGY



Johnny Awwad, MD

Human Reproduction, Vol.26, No.6 pp. 1270–1283, 2011
Advanced Access publication on April 18, 2011 doi:10.1093/humrep/der037

human reproduction ORIGINAL ARTICLE *ESHRE pages*

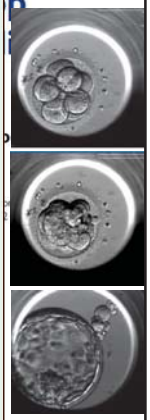


Table IV Timing of observation of fertilized oocytes and embryos, and expected stage of development at each time point.

Type of observation	Timing (hours post-insemination)	Expected stage of development
Fertilization check	17 ± 1	Pronuclear stage
Syngamy check	23 ± 1	Expect 50% to be in syngamy (up to 20% may be at the 2-cell stage)
Early cleavage check	26 ± 1 h post-ICSI 28 ± 1 h post-IVF	2-cell stage
Day-2 embryo assessment	44 ± 1	4-cell stage
Day-3 embryo assessment	68 ± 1	8-cell stage
Day-4 embryo assessment	92 ± 2	Morula
Day-5 embryo assessment	116 ± 2	Blastocyst

ICSI, intracytoplasmic sperm injection.

Human Reproduction, Vol.24,
Advanced Access publication on June

human reproduction OR

Is ng early
e ay?

Table I List of embryonic features included in the study

Day	Feature	Comments (including coding of nominal data)
1	Time of evaluation	17.8–29 h after fertilization (target 25 h)
1	No. cells	Range: 1–6
1	Pronuclei	Code (day 1 stage): 0 = 1 cell + 2PN;
3	No. cells	Range: 1–14
3	Fragmentation*	Code: 0 = 0%; 1 = 1–9%; 2 = 10–25%; 3 = 26–50%; 4 = >50%
3	Symmetry	Code: 1 = No asymmetry; 2 = Some asymmetry; 3 = Severe asymmetry

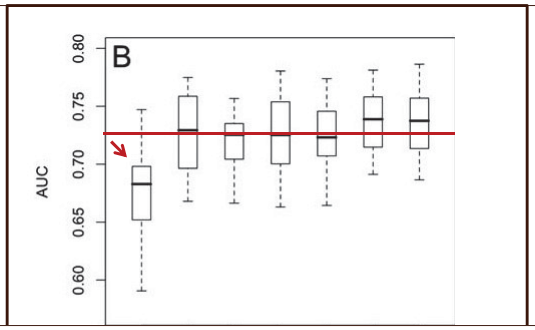
*Due to small numbers in groups having scores of three and four, these groups were combined with those having a score of two.

Table III Candidate models

Model	Formula
Day 1	fetus12wk = age + age^2 + isEggDonor + day1stage + cell1 + dev1
Day 2	fetus12wk = age + age^2 + isEggDonor + cell2 + dev2 + frg2 + sym2 + isAllSingleDay2
Day 3	fetus12wk = age + age^2 + isEggDonor + cell3 + dev3 + frg3 + sym3
Day 1,2	fetus12wk = age + age^2 + isEggDonor + day1stage + cell1 + dev1 + cell2 + dev2 + frg2 + sym2 + isAllSingleDay2
Day 1,3	fetus12wk = age + age^2 + isEggDonor + day1stage + cell1 + dev1 + cell3 + dev3 + frg3 + sym3
Day 2,3	fetus12wk = age + age^2 + isEggDonor + cell2 + dev2 + frg2 + sym2 + isAllSingleDay2 + cell3 + dev3 + frg3 + sym3
Days 1,2,3	fetus12wk = age + age^2 + isEggDonor + day1stage + cell1 + dev1 + cell2 + dev2 + frg2 + sym2 + isAllSingleDay2 + cell3 + dev3 + frg3 + sym3

Note: dev1, 2 or 3 = cell counts on Day 1, 2 or 3—count with maximum yield of fetuses on the respective day.

A single day morphological evaluation on either Days 2 or 3 provides similar predictive value to multi-day scoring.



In view of the demonstrated efficacy of models based on morphology, the putative superiority of any new method should demonstrate an AUC > 0.7.

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Accuracy of a combined score of zygote and embryo morphology for selecting the best embryos for IVF*

Yu-li QIAN, Ying-hui YE†‡, Chen-ming XU, Fan JIN, He-feng HUANG
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Table 2 Embryo grading and score for cumulative embryo score (CES) system

Grade	Score	Morphology
1	4	≥5 cells; blastomeres of equal size; no cytoplasmic fragments
2	3	≥5 cells; blastomeres of equal size; <30% cytoplasmic fragments
3	2	≥5 cells; blastomeres of distinctly unequal size; no cytoplasmic fragments
4	1	≥5 cells; blastomeres of equal or unequal size; 30%~50% cytoplasmic fragments if equal or 1%~50% if unequal
5	0	<5 cells of any size, or any pre-embryos with >50% cytoplasmic fragments

*If the embryo was not cleaved at 25~27 h, grading of fragmentation should be carried out during the 64~67 h evaluation. **Grade I: Symmetrical blastomeres and no fragmentation; Grade II: Slightly uneven blastomeres and <20% fragmentation; Grade III: Uneven blastomeres and >20% fragmentation

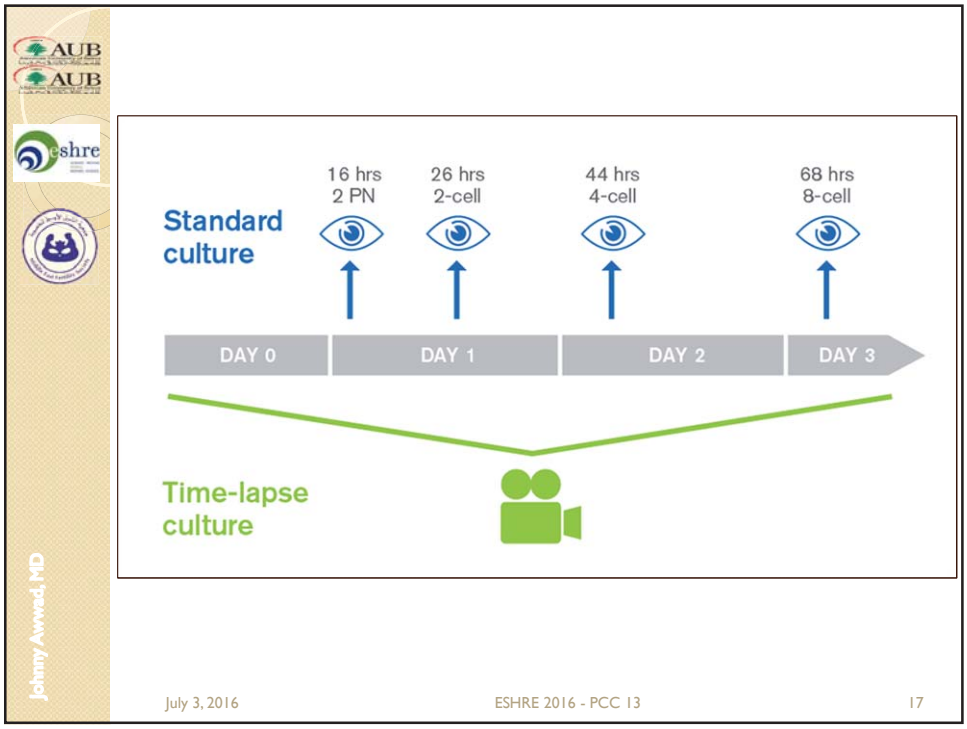
Multi-step grading system was more predictive of IVF outcomes for blastocyst transfers.

Table 6 Statistical measures of the two embryo grading systems

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
CSS≥70	94.7	55	66.7	91.7
CES≥20	94.7	13.3	50.9	72.7

Time Lapse Imaging

C. THE NEW TECHNOLOGY


16 hrs
2 PN
26 hrs
2-cell
44 hrs
4-cell
68 hrs
8-cell
Standard culture
DAY 0
DAY 1
DAY 2
DAY 3
Time-lapse culture

HYPOTHESIS

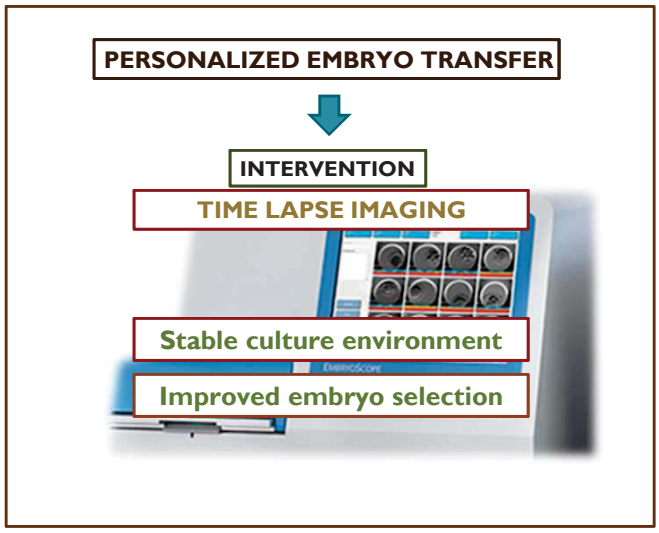
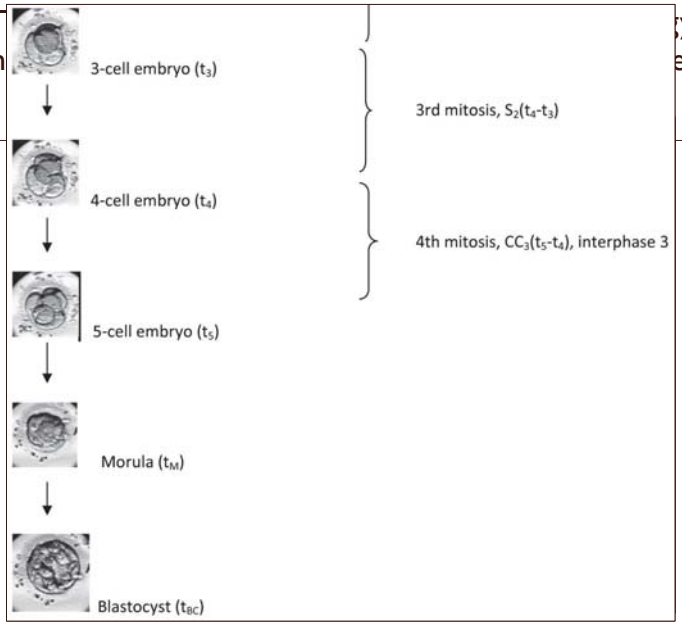
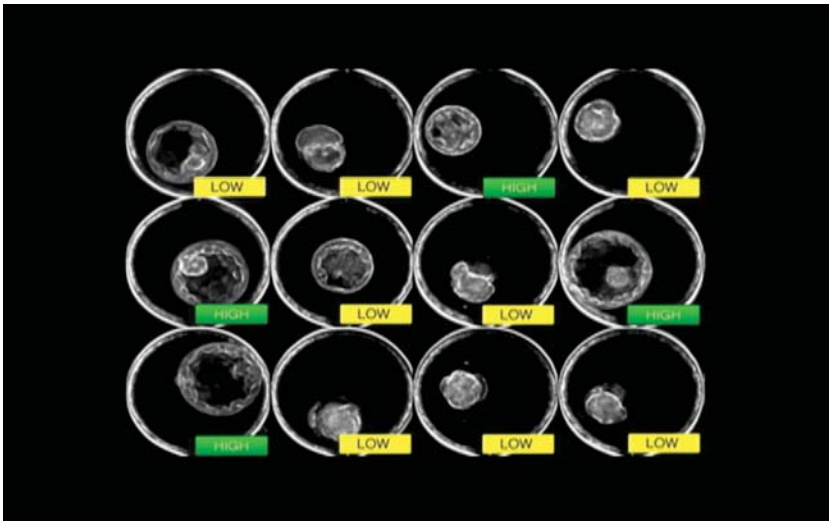

PERSONALIZED EMBRYO TRANSFER
INTERVENTION
TIME LAPSE IMAGING
Stable culture environment
Improved embryo selection

Table 2
Comparison of the technical parameters of three commercially available time-lapse systems

	Embryoscope	Primo vision	EEVA
Illumination	Bright field, low intensity red LED	Bright field, low intensity green LED	Dark field
Microscope/incubator	Incubator with integrated time-lapse system	Microscope that can be placed in standard incubators	Microscope that can be placed in standard incubators
Culture dish	Embryoslide	9-16 well Primo vision embryo culture dish	EEVA dish
Embryo culture	Single culture	Group culture	Group culture
Planes of view	7 focal planes	11 focal planes	Single plain
Max.# of embryos monitored	72	96	Depends on the dish
Other	Comes with software	Comes with software	Automated, software scores blastocyst formation potential





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Time Lapse Imaging

D. STUDY QUESTIONS

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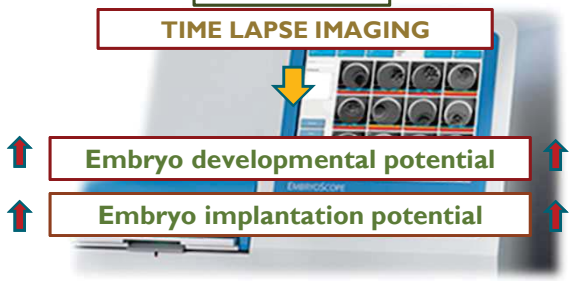
HYPOTHESIS

PERSONALIZED EMBRYO TRANSFER

INTERVENTION

TIME LAPSE IMAGING



Embryo developmental potential

Embryo implantation potential

Andrea is a 35 year-old woman with secondary infertility of 2 years duration. She has been diagnosed with PCOS and complains of oligomenorrhea, acne and hirsutism. She is otherwise healthy.

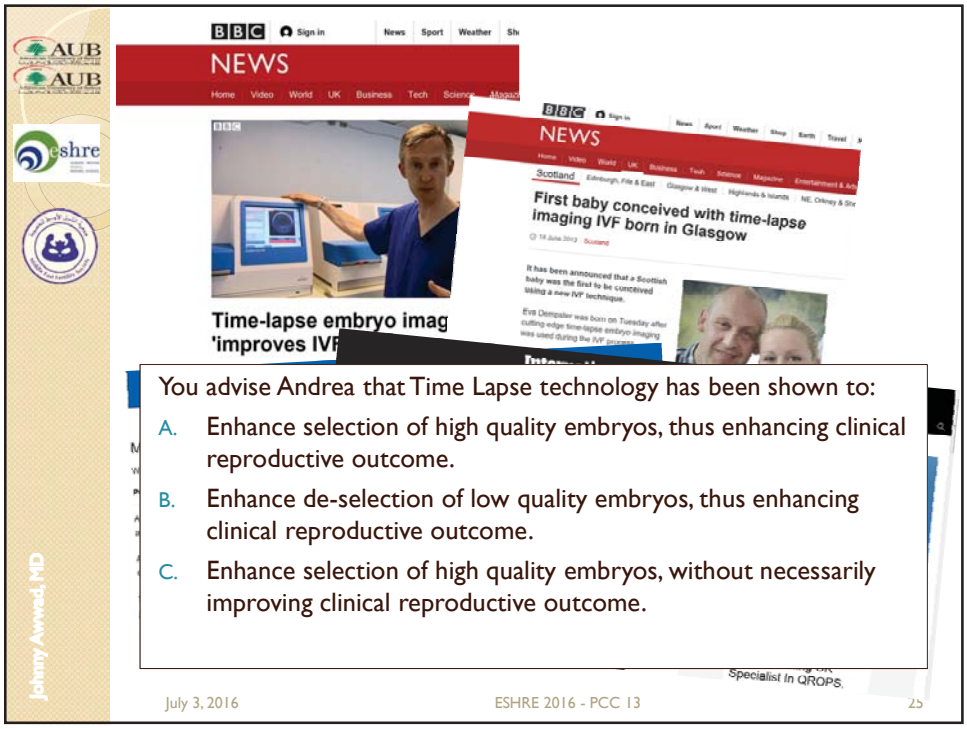
AMH 12 ng/ml. BMI 30 kg/m2. Normal TSH, PRL, FBS, and glucose challenge test. Elevated fasting insulin levels.

HSG bicornuate uterus with patent tubes.

Semen analysis unremarkable.

Because of clomiphene citrate resistance, she received FSH stimulation and conceived with twins. Her pregnancy was complicated with preterm delivery at 25 weeks gestation. Twin A suffered immediate neonatal death. Twin B had a prolonged stay in ICN for 3 months and was discharged home with residual neurodevelopmental injury.

She has heard of In Vitro Fertilization with single embryo transfer. She was also told by her friend about the value of a new innovation: time lapse technology. She is here with a pile of printed web pages supporting these claims.


BBC
NEWS
Time-lapse embryo imag
'improves IVF
First baby conceived with time-lapse imaging IVF born in Glasgow
You advise Andrea that Time Lapse technology has been shown to:
A. Enhance selection of high quality embryos, thus enhancing clinical reproductive outcome.
B. Enhance de-selection of low quality embryos, thus enhancing clinical reproductive outcome.
C. Enhance selection of high quality embryos, without necessarily improving clinical reproductive outcome.
Specialist in QROPS
Johnny Awwad, MD
July 3, 2016
ESHRE 2016 - PCC 13
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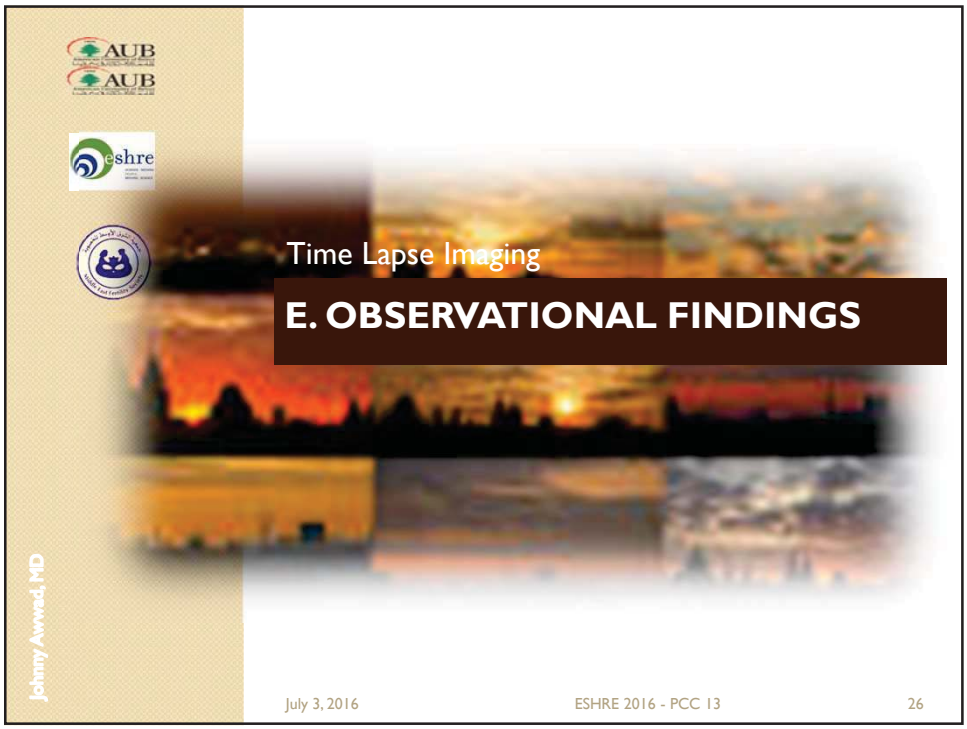
Time Lapse Imaging
E. OBSERVATIONAL FINDINGS
Johnny Awwad, MD
July 3, 2016
ESHRE 2016 - PCC 13
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Human Reproduction, Vol.26, No.10 pp. 2658–2671, 2011
Advanced Access publication on August 9, 2011 doi:10.1093/humrep/der256

The second synchrony s2 is the duration of the transition from a two blastomere embryo to a four blastomere embryo (s2 = t4-t3), i.e. the duration of the period as three blastomere embryo.

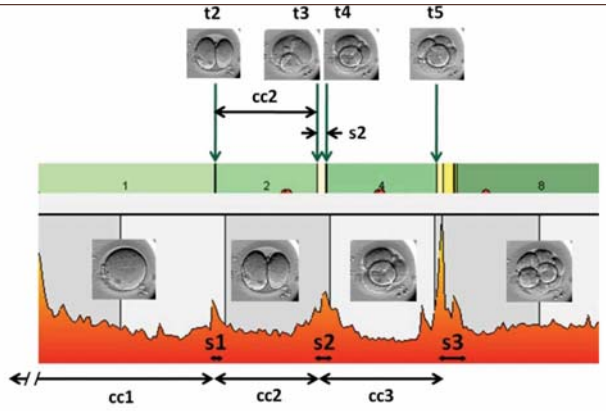


Figure 1 Graphic representation of the considered embryo developmental events t2, t3, t4, t5, cc2 = t3-t2 and s2 = t4-t3. We identified the precise timings and measured them in hours post ICSI microinjection.

There is a tighter distribution of cleavage times for implanting embryos as opposed to non-implanting embryos, with a prominent tail of lagging embryos found for the non-implanting embryos.

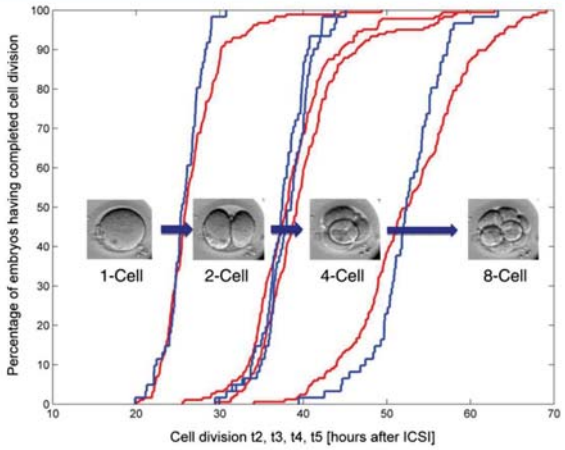


Figure 2 Percentage of embryos having completed a cell division by a given time after insemination. Blue curves represent implanting embryos; red curves represent embryos that did not implant. Four curves of each color represent completion of the four consecutive cell divisions from 1 to 5 cells i.e. t2, t3, t4 and t5.

For both the duration of the second cell cycle, cc2, and the synchrony of cell cleavages in the transition from 2 cell stage to 4 cell stage, s2 (i.e. the duration of the 3 cell stage), embryos cleaving in the two first quartiles had significantly higher implantation rate.

Table I Exact timing of embryo events analysed from transferred implanted and not implanted embryos.

Table II Exact timing of the first cleavages grouped in quartiles (Q1, Q2, Q3 and Q4) from 247 transferred embryos.

Parameter	Q1		Q2		Q3		Q4	
	Limit (h)	Implantation (%)	Limit (h)	Implantation (%)	Limit (h)	Implantation (%)	Limit (h)	Implantation (%)
t2	<24.3	23	**24.3–25.8**	**32**	**25.8–27.9**	**30**	>27.9	15
t3	<35.4	18	**35.4–37.8**	**39**	**37.8–40.3**	**32**	>40.3	11
t4	<36.4	23	**36.4–38.9**	**36**	**38.9–41.6**	**31**	>41.6	10
t5	<48.8	16	**48.8–52.3**	**37**	**52.3–56.6**	**40**	>56.6	14
cc2	**<11.0**	**23**	**11.0–11.9**	**39**	11.9–12.9	18	>12.9	19
s2	**<0.30**	**36**	**0.30–0.76**	**28**	0.76–1.50	20	>1.50	16

Additionally, the percentage of implanting embryos in each quartile is shown. Numbers in bold indicate the two quartiles with the highest implantation percentages.

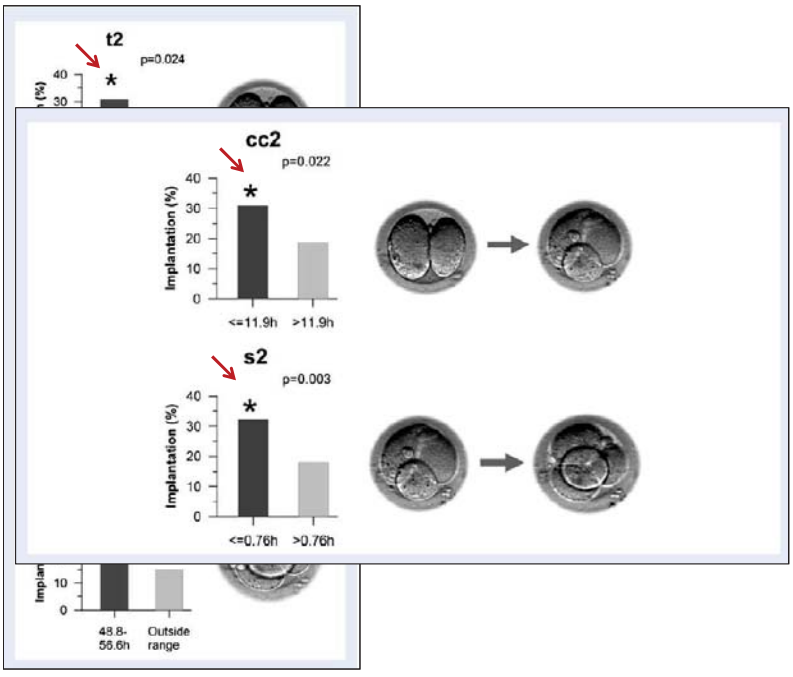


Table III Implantation in the embryo categories of the hierarchical classification tree model.

Embryo category	*n* total	*n* implanted	Implantation (%)	Embryo category	Implantation (%)
A+	29	19	66	A	52
A−	25	9	36		
B+	24	7	29	B	27
B−	25	6	24		
C+	32	8	25	C	19
C−	21	2	10		
D+	10	1	10	D	14
D−					
E					

Logistic regression on the morphology basis gave an AUC of 0.64.
Logistic regression on the simplified time-lapse basis gave an AUC of 0.72.
The higher AUC for the time-lapse categories supports the possibility of improved embryo selection using this approach.

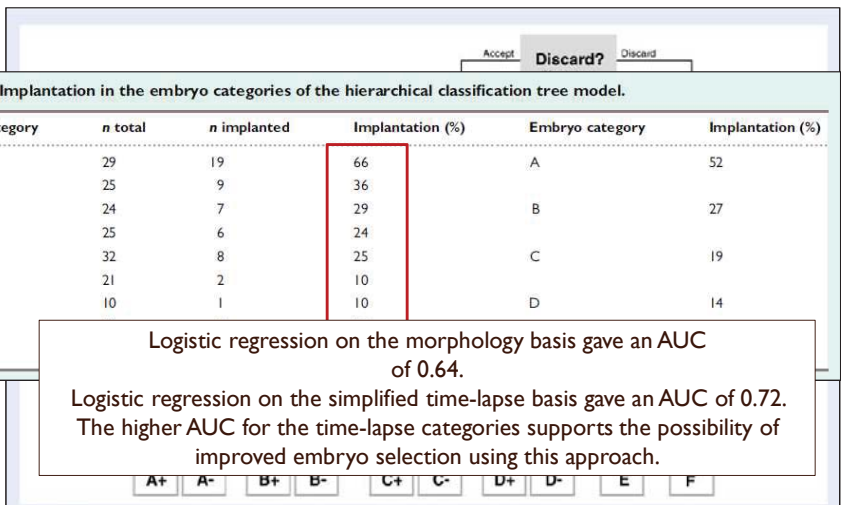
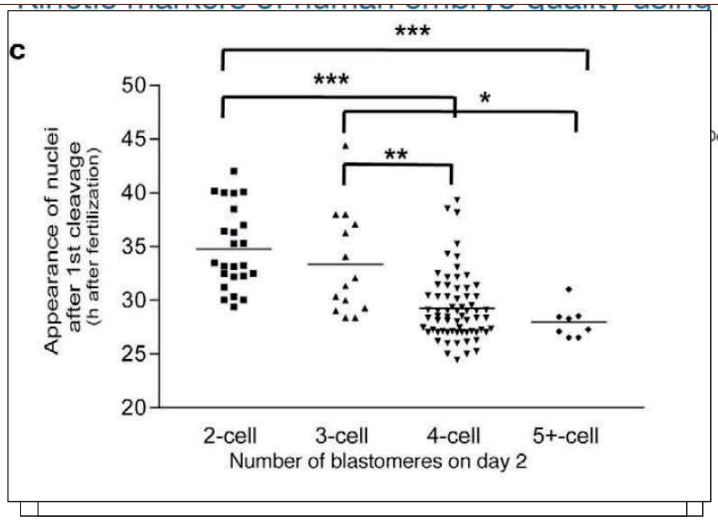


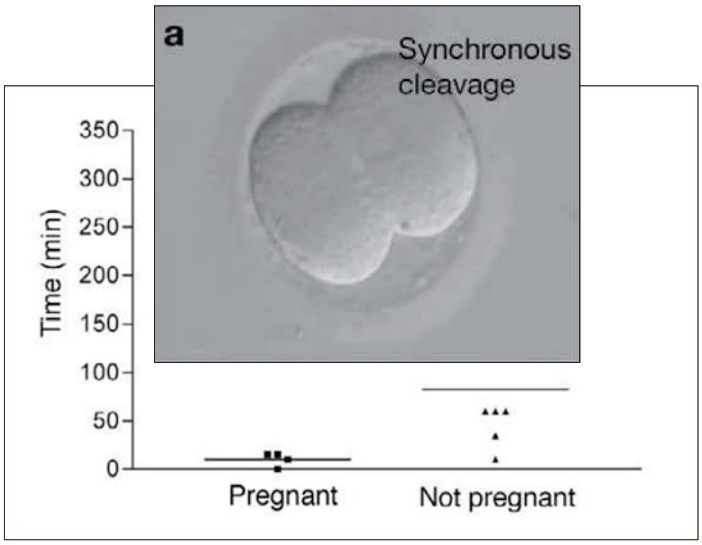
Figure 6 Hierarchical classification of embryos based on: (i) morphological screening; (ii) absence of exclusion criteria; (iii) timing of cell division to 5 cells (t5); (iv) synchrony of divisions from 2 cell to 4 cell stage, s2, i.e. duration of 3 cell stage; (v) duration of second cell cycle, cc2, i.e. the time from division to a two blastomere until divison to a three blastomere embryo. The classification generates 10 categories of embryos with increasing expected implantation potential (right to left) and almost equal number of embryos in each.

RBMOnline - Vol 17 No 3. 2008 385-391 Reproductive BioMedicine Online; www.rbmonline.com/Article/3327 on web 30 July 2008

Fertilized oocytes that developed into ≥4-cell embryos had an earlier pronuclei disappearance and first cleavage than those that developed to 3- or 2-cell embryos.



Synchrony in appearance of nuclei after the first cleavage was significantly associated with pregnancy success.



Limited implantation success of direct-cleaved human zygotes: a time-lapse study

Irene Rubio, Ph.D.,[a] Reidun Kuhlmann,[b] Inge Agerholm, Ph.D.,[c] John Kirk, M.D.,[b] Javier Herrero, Ph.D.,[a] María-José Escribá, Ph.D.,[a] José Bellver, Ph.D.,[a] and Marcos Meseguer, Ph.D.[a]

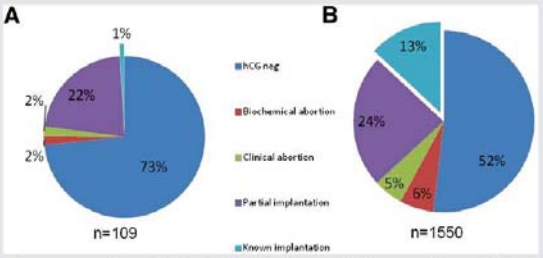
[a] Instituto Valenciano de Infertilidad Valencia, Universidad de Valencia, Valencia, Spain; [b] Maigaard Fertility Clinic, Aarhus, Denmark; and [c] Fertility Clinic Braedstrup, Braedstrup, Denmark

Fertility and Sterility® Vol. 98, No. 6, December 2012 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2012.07.1135

Objective: To evaluate embryos with direct cleavage (≤5 hours) from two to three cells (DC2–3) and correlate this morphokinetic parameter to implantation and ongoing pregnancy.
Design: Clinical multicenter retrospective study.
Setting: Private in vitro fertilization (IVF) centers.
Patient(s): From three clinics, a total of 979 treatments including 5,225 embryos using autologous or donated oocytes, of which 1,659 embryos were transferred.
Intervention(s): None.
Main Outcome Measure(s): Clinical pregnancy confirmed by ultrasound in week 7.

The incidence of direct cleavage was nearly 14% in the total embryonic cohort.



Implantation of (A) direct cleavage two to three cells (DC2–3), 109 (7%) of transferred embryos, and (B) non-DC2–3, 1,550 (93%) of transferred embryos. The classifications are as follows: hCG neg = none of the transferred embryos resulted in a biochemical pregnancy; biochemical abortion = the treatment resulted in a biochemical abortion; clinical abortion = embryo(s) implanted but the treatment resulted in a clinical abortion; partial implantation = some of the transferred embryos implanted, thus the fate (implanted or not) of the specific embryo is unknown; known implantation = all transferred embryos in the treatment implanted.

Embryos with DC2–3 had a statistically significantly lower implantation rate than embryos with a normal cleavage pattern, suggesting that rejection of these embryos for transfer could improve the implantation rate.

Time Lapse Imaging

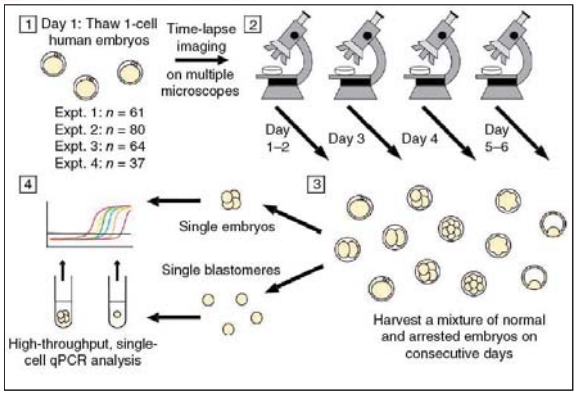
F. PREDICTING DEVELOPMENT

nature biotechnology

Non-invasive imaging of human embryos before embryonic genome activation predicts development to the blastocyst stage

Connie C Wong[1,2,7], Kevin E Loewke[1–3,6,7], Nancy L Bossert[4], Barry Behr[2], Christopher J De Jonge[4], Thomas M Baer[5] & Renee A Reijo Pera[1,2]

NATURE BIOTECHNOLOGY VOLUME 28 NUMBER 10 OCTOBER 2010



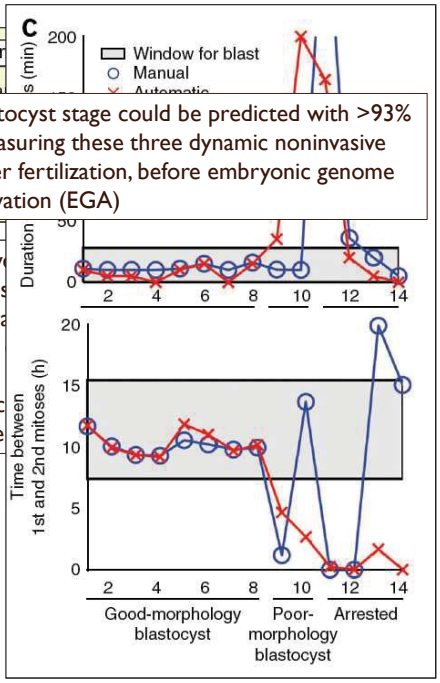
Johnny Awwad, MD

1st mitosis

Success in progression to the blastocyst stage could be predicted with >93% sensitivity and specificity by measuring these three dynamic noninvasive imaging parameters by day 2 after fertilization, before embryonic genome activation (EGA)

Three parameters collectiv
(i) duration of the first cytokines
physically separa
(ii) time interval between the end

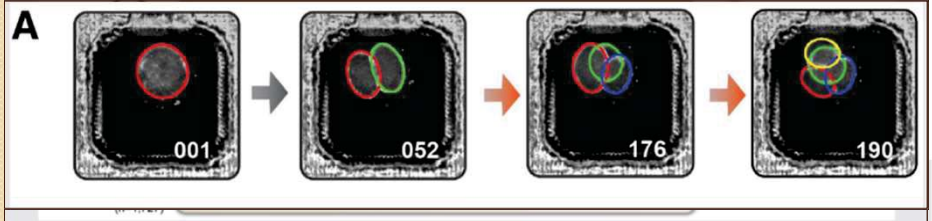
(iii) time interval between the sec
the appearance of the cleavage



Johnny Awwad, MD

Improving embryo selection using a computer-automated time-lapse image analysis test plus day 3 morphology: results from a

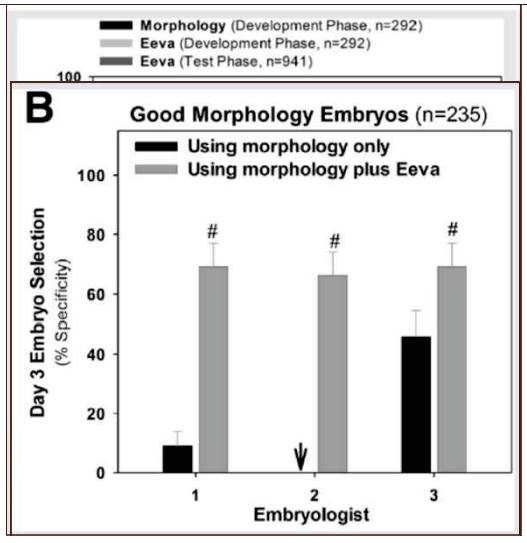
FIGURE 1



Schematic representation of the clinical study workflow at each of five IVF sites. Oocytes were retrieved and fertilized by IVF or ICSI as per each clinic's standard protocol. Successfully fertilized oocytes (2PNs) were cultured in a multiwell dish and imaged in a standard incubator using Eeva, which captured one dark-field image every 5 minutes for 3 days (insets show embryo development and frame numbers from the 1-cell to 8-cell stage). Following imaging, key cell division timing parameters (P1 = duration of first cytokinesis; P2 = time interval between cytokinesis 1 and 2; P3 = time interval between cytokinesis 2 and 3) were manually measured and used to develop and independently validate a model that could predict usable blastocyst outcome at the cleavage stage.

Conaghan. Validation of a time-lapse screening tool. Fertil Steril 2013.

When Eeva was used in combination with D3 morphology, embryologists experienced significant improvement in the likelihood of selecting embryos that would develop to usable blastocysts, with a reduction in inter-observer variability.



Reproductive BioMedicine Online (2012) 25, 371–381

www.sciencedirect.com
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ELSEVIER

ARTICLE

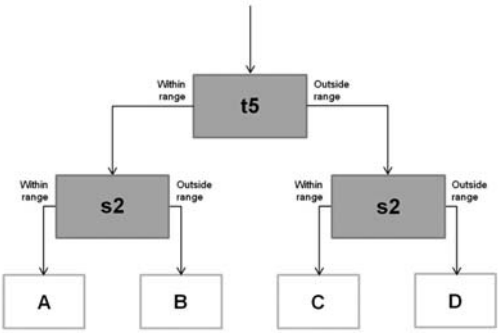
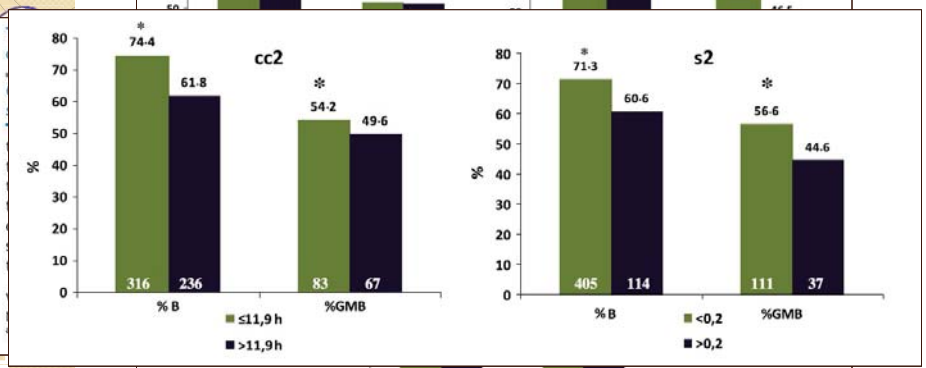


Figure 2 Hierarchical classification of embryos. The system generates a number of categories of embryos with increasing expected implantation potential (right to left), based on the timing of cleavage to 5 cells and the synchrony of the divisions from the 3-cell stage to the 4-cell stage.

Embryos that cleave earlier have a significantly improved chance of continuing development to day 5 when compared with embryos that develop more slowly.



Reproductive BioMedicine Online (2012) 25, 474–480

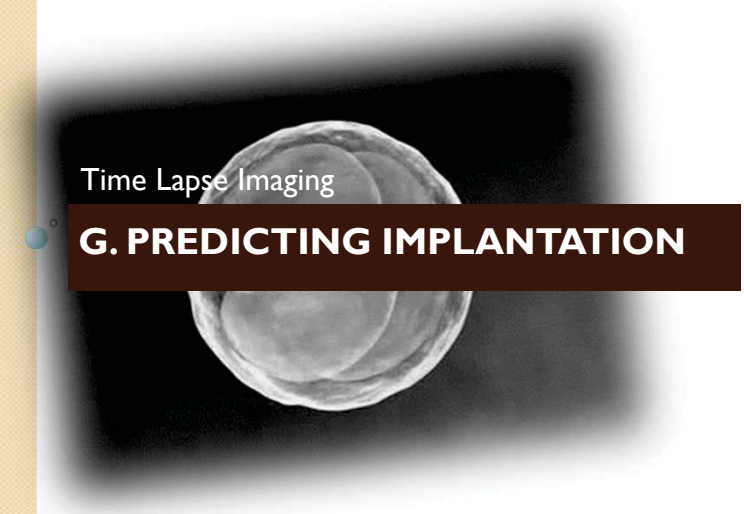
Comparison of times between
divisions did not reveal significant differences.
Only the intervals 4–8-cells and 5–8-cells were significantly shorter
in embryos with the potential to develop to blastocyst stage.

Table 2 Cleavage times from the 2- to the 8-cell stage and relative intervals between divisions for embryos developing to the blastocyst stage or arresting at later cleavage stages.

Stage or interval	*Arresting after 8-cell stage (n = 151)*	*Developing to blastocyst (n = 151)*	*P-value*
2-cell	27.9 ± 4.1	27.6 ± 3.0	NS
3-cell	37.9 ± 4.6	38.0 ± 3.5	NS
4-cell	40.2 ± 5.8	39.8 ± 4.5	NS
5-cell	50.2 ± 6.7	50.7 ± 6.1	NS
6-cell	53.9 ± 7.6	53.0 ± 6.8	NS
7-cell	58.8 ± 10.4	56.5 ± 8.1	0.03
8-cell	65.2 ± 13.0	61.0 ± 9.4	0.0008
2–3-cell	10.6 ± 1.8	10.5 ± 1.9	NS
3–4-cell	2.3 ± 3.6	1.8 ± 3.1	NS
2–4-cell	12.3 ± 3.3	12.2 ± 2.6	NS
4–8-cell	25.2 ± 11.2	21.3 ± 8.0	0.0005
5–8-cell	14.9 ± 10.7	10.4 ± 8.2	0.0001

Values are mean ± SD time in hours. NS = not statistically significant.

Johnny Awwad, MD



Time Lapse Imaging

G. PREDICTING IMPLANTATION

Johnny Awwad, MD

Human Reproduction, Vol.28, No.10 pp. 2643–2651, 2013
Advanced Access publication on July 30, 2013 doi:10.1093/humrep/det300

human reproduction

ORIGINAL ARTICLE *Embryology*

Time-lapse parameters as predictors of blastocyst development and pregnancy outcome in embryos from good prognosis patients: a prospective cohort study

K. Kirkegaard[1,2,*], U.S. Kesmodel[1,2], J.J. Hindkjær[1], and H.J. Ingerslev[1,2]

[1]Centre for Preimplantation Genetic Diagnosis, The Fertility Clinic, Aarhus University Hospital, Skejby, Brendstrupgaardsvej 100, Aarhus N 8200, Denmark [2]Health, Aarhus University, Vennelyst Boulevard 9, Aarhus C DK-8000, Denmark

STUDY DESIGN, SIZE, DURATION: A prospective cohort study conducted from February 2011 to June 2012. A total of 571 ICSI embryos from 92 patients were included in the blastocyst development analysis and 84 single embryo transfers were included in the pregnancy outcome analysis.

Duration of the first cytokinesis, duration of the 3-cell stage and direct cleavage to 3 cells predicted development to high quality blastocyst.

blastocyst.

Parameter	OR (95% CI)	*P*-value
PN breakdown	0.94 (0.88; 1.01)	0.09
Duration of the first cytokinesis (h)	0.36 (0.16; 0.83)	0.02
Duration of the 2-cell stage (h)	0.89 (0.77; 1.04)	0.14
Multi-nucleation at the 2-cell stage (yes/no)	0.89 (0.49; 1.59)	0.70
Duration of the 3-cell stage (h)	0.88 (0.80; 0.97)	0.01
Direct cleavage to 3 cells[a] (yes/no)	0.11 (0.02; 0.69)	0.02

The OR was obtained with a single logistic regression analysis where all six parameters were included. OR, odds ratio.
[a]Duration of the 2-cell stage <5 h.

Figure 1 Cohort flowchart. ICSI fertilized embryos cultured at 5% O_2.

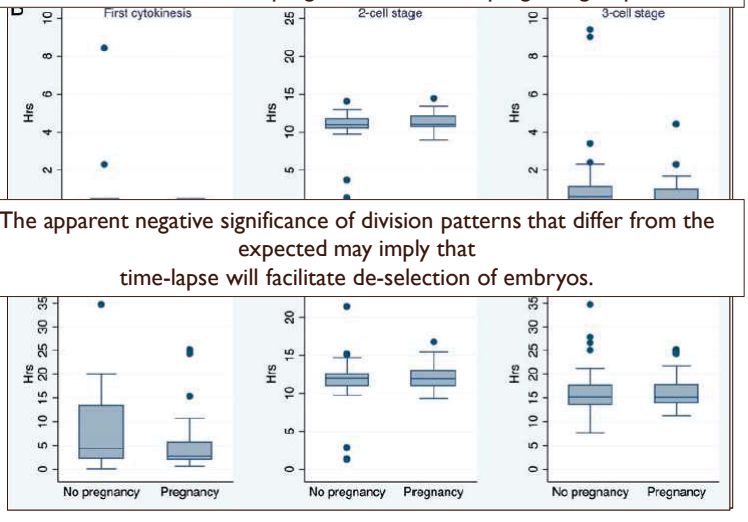
Direct cleavage to 3 cells (duration of the 2-cell stage < 5 h) could not predict pregnancy and non-pregnancy status.

pregnancy.

Parameter	OR (95% CI)	*P*-value
Duration of the first cytokinesis (h)	0.84 (0.45; 1.57)	0.59
Duration of the 3-cell stage (h)	0.84 (0.59; 1.22)	0.36
Age (years)	0.84 (0.73; 0.98)	0.03
Number of previous cycles	1.2 (0.62; 2.4)	0.56
Number of GQE on Day 2	1.0 (0.78; 1.3)	0.98
Number of GQE on Day 3	1.1 (0.83; 1.4)	0.57
Total FSH dose(100 IU)	0.99 (0.93; 1.1)	0.82
Cause of infertility (categorical)	0.34 (0.05; 2.2)	0.25

OR were obtained by performing a logistic regression analysis with the two time-lapse parameters and one of the parameters listed below them. This analysis was repeated for each parameter. GQE, good quality embryo; OR, odds ratio.

None of the mean time points of cellular divisions or embryonic stages differed between the pregnant and the non-pregnant groups.



The apparent negative significance of division patterns that differ from the expected may imply that
time-lapse will facilitate de-selection of embryos.

ORIGINAL ARTICLES: ASSISTED REPRODUCTION

Clinical validation of embryo culture and selection by morphokinetic analysis: a randomized, controlled trial of the EmbryoScope

Irene Rubio, Ph.D.,[a] Arancha Galán, Ph.D.,[a] Zaloa Larreategui, Ph.D.,[b] Fernando Ayerdi, Ph.D.,[b] Jose Bellver, M.D.,[a] Javier Herrero, Ph.D.,[a] and Marcos Meseguer, Ph.D.[a]

[a] Instituto Universitario IVI Valencia, University of Valencia, Valencia; and [b] IVI Bilbao, Bilbao, Spain

Objective: To determine whether incubation in the integrated EmbryoScope time-lapse monitoring system (TMS) and selection supported by the use of a multivariable morphokinetic model improve reproductive outcomes in comparison with incubation in a standard incubator (SI) embryo culture and selection based exclusively on morphology.
Design: Prospective, randomized, double-blinded, controlled study.
Setting: University-affiliated private in vitro fertilization (IVF) clinic.
Patient(s): Eight hundred forty-three infertile couples undergoing intracytoplasmic sperm injection (ICSI).
Intervention(s): No patient intervention; embryos cultured in SI with development evaluated only by morphology (control group) and embryos cultured in TMS with embryo selection was based on a multivariable model (study group).
Main Outcome Measure(s): Rates of embryo implantation, pregnancy, ongoing pregnancy (OPR), and early pregnancy loss.

Incubation and selection in the Time Lapse group improved ongoing pregnancy and implantation rate and reduced early pregnancy loss.

Descriptive characteristics of the embryo development and fate in the time-lapse and control groups.

Outcome results per intention to treat, per cycle, per transfers and per embryo transferred.

Outcome	TMS group	Control group	RR	*P* value
All cycles with oocyte retrieval	438	405		
Pregnancy (% of all treated cycles)	61.6 (56.9–66.0)	56.3 (51.4–61.0)	1.09 (0.98–1.23)	.12
Ongoing pregnancy (% of all treated cycles)	51.4 (46.7–56.0)	41.7 (37.0–46.6)	1.23 (1.06–1.43)	.005
All transfers	415	373		
Pregnancy (% of all transfers)	65.3 (60.6–69.7)	61.1 (56.1–65.9)	1.07 (0.95–1.19)	.22
Ongoing pregnancy (% of all transfers)	54.5 (49.6–59.2)	45.3 (40.3–50.4)	1.20 (1.04–1.39)	.01
All pregnant cycles	271	228		
Early pregnancy loss (% of all pregnancies)	16.6 (12.6–21.4)	25.8 (20.6–31.9)	0.64 (0.45–0.91)	.01
All transferred embryos	775	699		
Implantation rate (% of all transferred embryos)	44.9 (41.4–48.4)	37.1 (33.6–40.7)	1.43 (1.05–1.39)	.02

Note: Results shown as proportion with 95% confidence limits in brackets, relative risk (RR) with 95% confidence limits in brackets and the corresponding *P* value (Fisher's exact test). Total number of cycles are also presented in brackets.

Rubio. Clinical validation of EmbryoScope. Fertil Steril 2014.

Physiol. Res. 61: 513-525, 2012

Time-Lapse Cleavage Rating Predicts Human Embryo Viability

D. HLINKA[1], B. KAĽATOVÁ[2], I. UHRINOVÁ[2], S. DOLINSKÁ[2], J. RUTAROVÁ[3],

Cleavage timeliness:	Timely (T)	Untimely (U)	Total
(A) Abnormalities in morphology	**14** (7.8 %)	**52** (28.9 %)	66 (36.7 %)
(B) Blastocysts, no pregnancy	**78** (43.3 %)	**8** (4.4 %)	86 (47.7 %)
(BP) Blastocysts giving pregnancy	**28** (15.6 %)	**0** (0 %)	28 (15.6 %)
Total	120 (66.7 %)	60 (33.3 %)	180 (100 %)

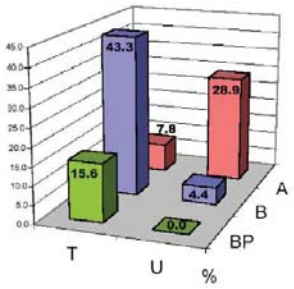


Fig. 1. Counts and percentage ratios of embryos in relation to their cleavage (un)timeliness, morphology, and uterine nidation success.

The specificity of time lapse imaging in determining how well embryo cleavage rating selects from all blastocysts for those that would surely nidate and yield clinical pregnancies was 9 %.

Table 4. Specificity and sensitivity of recognizing timely blastocysts yielding pregnancies.

N = 180	Blastocyst formed: NO Timely cleavage: any	Blastocyst formed: YES Timely cleavage: NO	Blastocyst formed: YES Timely cleavage: YES
No pregnancy	66	8 (true negatives, TN)	78 (false positives, FP)
Pregnancy proven	n/a (no embryo transfer)	0 (false negatives, FN)	28 (true positives, TP)
Total embryos	66	8	106

$SE_{BT/P}$ = TP / (TP+FN) = 28 / (28+0) = **1.00** $SP_{BT/P}$ = TN / (TN+FP) = 8 / (8+78) = **0.09**

There was no conclusive evidence of a difference in live birth rate per couple randomly assigned to the TLS and conventional incubation arms.

Cochrane Database of Systematic Reviews

Cochrane Database of Systematic Reviews 2015, Issue 2. Art. No.: CD011320.

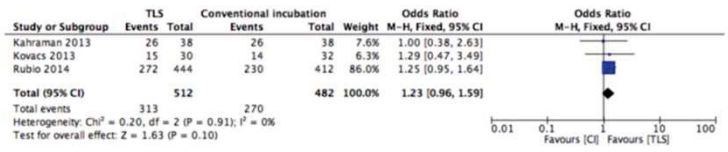
TLS with or without cell-tracking algorithms versus conventional incubation for embryo incubation in assisted reproduction

Patient or population: embryo incubation in assisted reproduction
Settings:
Intervention: TLS with or without cell-tracking algorithms
Comparison: conventional incubation

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk conventional incubation	Corresponding risk TLS with or without cell-tracking algorithms				
Live birth	500 per 1000	526 per 1000 (310 to 732)	OR 1.11 (0.45 to 2.73)	76 (1 RCT)	⊕⊕⊕○ MODERATE [1]	
Miscarriage	143 per 1000	105 per 1000 (73 to 148)	OR 0.7 (0.47 to 1.04)	994 (3 RCTs)	⊕⊕○○ LOW [2,3,4]	
Stillbirth	53 per 1000	53 per 1000 (7 to 294)	OR 1 (0.13 to 7.49)	76 (1 RCT)	⊕⊕⊕○ MODERATE [1]	
Clinical pregnancy	558 per 1000	609 per 1000 (548 to 668)	OR 1.23 (0.96 to 1.59)	994 (3 RCTs)	⊕⊕○○ LOW [2,3,4]	

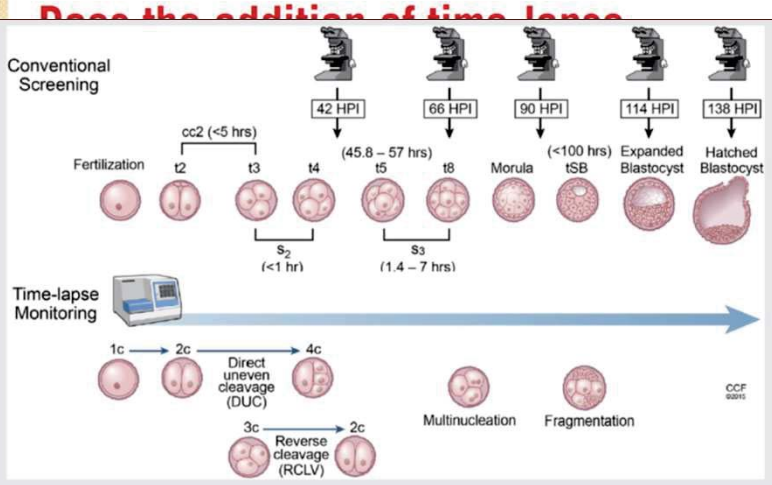
There was no conclusive evidence of
a difference in clinical pregnancy rate per couple randomly assigned
to the Time Lapse Imaging and conventional incubation arms.

Figure 5. Forest plot of comparison: 1 TLS with or without cell-tracking algorithms versus conventional incubation, intervention : 1.3 Clinical pregnancy.



There is insufficient evidence of differences in live birth, miscarriage, stillbirth or clinical pregnancy to choose between Time Lapse Imaging and conventional incubation.

Does the addition of time-lapse



Embryo growth and development over time. The top of the figure depicts the conventional screening standard viewing points ± 1 hour (HPI = hours after insemination). Time-lapse monitoring occurred continuously. Dysmorphisms that were evaluated are pictured at the bottom. Established time frames incorporated into the morphokinetic score (cc2, s2, t5, s3, and tSB) are shown.

Goodman. Time lapse-assisted embryo selection. Fertil Steril 2016.

Kinetic data of embryos with known implantation data.

Dysmorphic events and timing of events in embryos with known implantation status.

Embryo characteristic	Implanted (n = 140)	Did not implant (n = 156)	*P* value
Multinucleation	32 (22.9%)	55 (35.3%)	.02
Reverse cleavage	6 (4.3%)	11 (7.0%)	.33
Direct uneven cleavage	0 (0%)	9 (5.7%)	<.01
Irregular division	23 (8.6%)	23 (14.7%)	.11
cc2 <5 h	120 (96.0%)	111 (88.8%)	.05
t5 45.8–57 h	94 (73.4%)	94 (68.1%)	.35
s2 0–1 h	100 (80.7%)	85 (66.4%)	.02
s3 1.4–7 h	77 (62.1%)	71 (62.3%)	.98
tSB <100 h	106 (75.7%)	84 (53.8%)	<.01
Morphokinetic score	2.36 ± 1.15	1.55 ± 1.40	<.01

Note: All data is n (%) or mean ± SD. Abbreviations as in Supplemental Table 4.

Goodman. Time lapse–assisted embryo selection. Fertil Steril 2016.

Note: Abbreviations as in Supplemental Table 4.

Goodman. Time lapse–assisted embryo selection. Fertil Steril 2016.

Pregnancy and implantation rates were not significantly different between TLM and CS groups.

Outcome results in cycles with selection, stratified by day of transfer and age.

Clinical outcome	TLM	CS	*P* value
All transfers (day 3 and 5)	n = 119	n = 116	
CPR	81/119 (68.1%)	73/116 (62.9%)	.41
IR	122/239 (51.0%)	100/221 (45.2%)	.21
All transfers, <40 y old	n = 110	n = 110	
CPR	79/110 (71.8%)	72/110 (65.5%)	.10
IR	119/211 (56.4%)	99/205 (48.3%)	.31
Blastocyst transfers	n = 91	n = 89	
CPR	67/91 (73.6%)	61/91 (67.0%)	.33
IR	96/173 (55.5%)	83/162 (51.2%)	.44
Pregnancy outcomes	n = 81	n = 73	.23
Viable singleton pregnancy	48 (59.3%)	48 (65.8%)	
Viable twin pregnancy	29 (35.8%)	21 (28.8%)	
Viable triplet pregnancy	2 (2.5%)	1 (1.4%)	
Spontaneous abortion	2 (2.5%)	3 (4.1%)	

Note: Patients with 1–3 embryos were excluded from analysis. CPR = clinical pregnancy rate; IR = implantation rate; other abbreviations as in Supplemental Table 1.

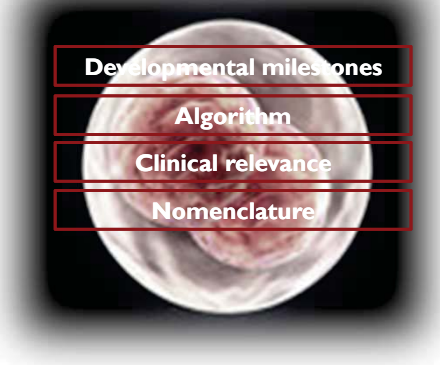
Goodman. Time lapse–assisted embryo selection. Fertil Steril 2016.

Time Lapse Imaging

H. REASONS FOR CAUTION

LACK OF CONSENSUS

Putative Kinetic Markers



- Developmental milestones
- Algorithm
- Clinical relevance
- Nomenclature

Human Reproduction Update, Vol.20, No.5 pp. 617–631, 2014
Advanced Access publication on June 2, 2014 doi:10.1093/humupd/dmu023

Few data indicate whether TLM effectively distinguishes between embryos with high and low implantation potential.

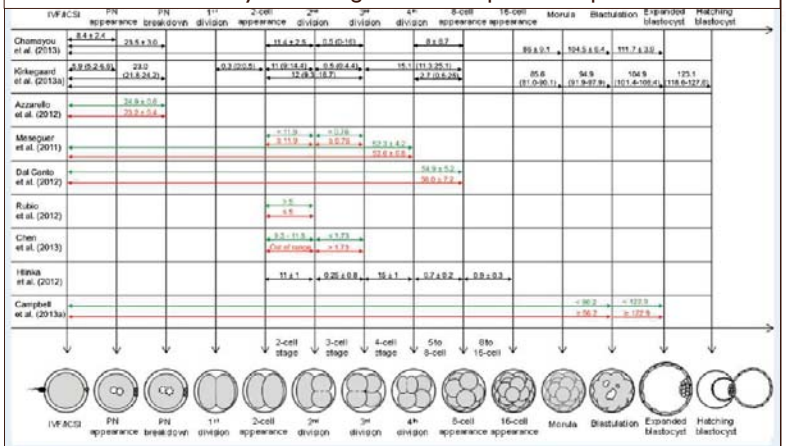


Figure 2 Schematic of preimplantation embryo development with corresponding time-lapse markers from 9 of the 13 studies with time values reported. When there was no significant difference observed between 'implanters' and 'non-implanters', only the value for the implanted embryos is shown (in black). When significant differences were reported, the 'implanter' values are shown in green, and 'non-implanters' are in red. All values are expressed in hours, as mean ± standard deviation or mean (95% confidence interval) for normally distributed variables, and median (minimum:maximum) for non-normally distributed variables. PN, pronuclei. Modified from Chen *et al.* (2013).

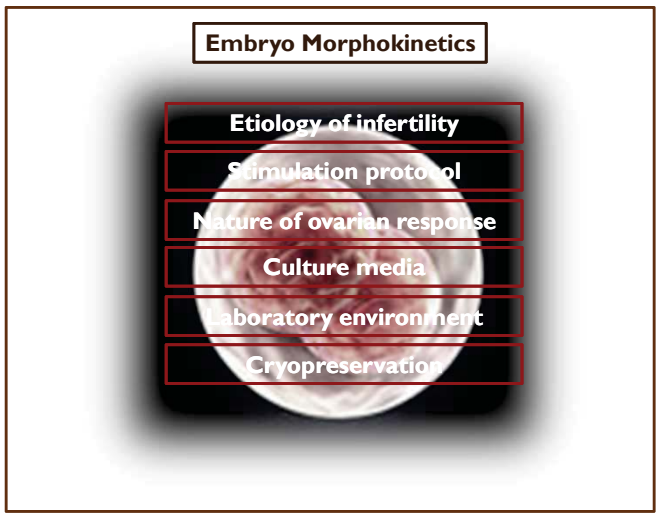
Human Reproduction, Vol.27, No.5 pp. 1277–1285, 2012
Advanced Access publication on March 14, 2012 doi:10.1093/humrep/des079

human reproduction REVIEW *Embryology*

Table II Inconsistencies in the current nomenclature for time-lapse markers.

Developmental milestone	Name of time-lapse marker	Description of time-lapse marker
Duration in the 2-cell stage	DC2-3	Direct cleavage (Rubio *et al.*, 2012)
	P2	Parameter 2 (Wong *et al.*, 2010; Chen *et al.*, 2013; Conaghan *et al.*, 2013)
	i2	Interphase 2 (Hlinka *et al.*, 2012)
	cc2	Cleavage cycle 2 (Meseguer *et al.*, 2011, 2012; Chamayou *et al.*, 2013)
Duration in the 3-cell stage	s2	Synchronicity 2 (Meseguer *et al.*, 2011, 2012; Chamayou *et al.*, 2013; Kirkegaard *et al.*, 2013a)
	c2	Cleavage 2 (Hlinka *et al.*, 2012)
	P3	Parameter 3 (Wong *et al.*, 2010; Chen *et al.*, 2013; Conaghan *et al.*, 2013)
Duration in the 4-cell stage	cc3	Cleavage cycle 3 (Meseguer *et al.*, 2011, 2012)
	i3	Interphase 3 (Hlinka *et al.*, 2012)
Time from the 2-cell to 4-cell stage	cc2	Cleavage cycle 2 (Hlinka *et al.*, 2012; Kirkegaard *et al.*, 2013a)
Time from the 3-cell to 5-cell stage	cc3	Cleavage cycle 3 (Chamayou *et al.*, 2013)
Time from the 4-cell to 8-cell stage	cc3	Cleavage cycle 3 (Hlinka *et al.*, 2012; Kirkegaard *et al.*, 2013a)
	s3	Synchronicity 3 (Freour *et al.*, 2013)
Time from the 5-cell to 8-cell stage	s3	Synchronicity 3 (Chamayou *et al.*, 2013)
	c3	Cleavage 3 (Hlinka *et al.*, 2012)
Time from the 5-cell to 9-cell stage	cc4	Cleavage cycle 4 (Chamayou *et al.*, 2013)
		(2011)
Time point of the 5-cell stage	Meseguer *et al.* (2011)	Pregnancy 228 Yes[b]

CONFOUNDING FACTORS



Gurbuz et al. Journal of Ovarian Research (2016) 9:22

The percentage of optimal embryos according to kinetic markers were significantly higher in GnRH agonist group than hCG triggering group.

GnRH agonist triggering affects the kinetics

Table 2 Embryo developmental kinetics according to the type of oocyte maturation triggering agent

Table 3 Percentages of optimal embryos whose cleavages are included in optimal timing ranges with a predicted higher implantation potential (Meseguer et al. 2011) according to type of triggering

Embryo category	GnRHa triggering	hCG triggering	*p*
T5 (%)	15.4	17.4	NS
S2 (%)	42.0	24.8	0.000
CC2 (%)	52.3	43.1	0.005

Data are presented as % (*n*) for each category. The proportions of optimal embryos in each category were compared using the χ^2 test. NS. no statistically significant differences were found

t time, *h* hour, t_{PB2} appearance of second polar body t_{PNF} both pronuclei faded, *cc* cell cycle *s*, synchrony

http://informahealthcare.com/gye
ISSN: 0951-3590 (print), 1473-0766 (electronic)

The use of a total FSH dose more than 2500 IU was accompanied by prolongation of kinetic time parameters.

ORIGINAL ARTICLE

Analysis of … embryos in ART cyc…

Mykola Grygorie…

V.I. Gryshchenko Clini…

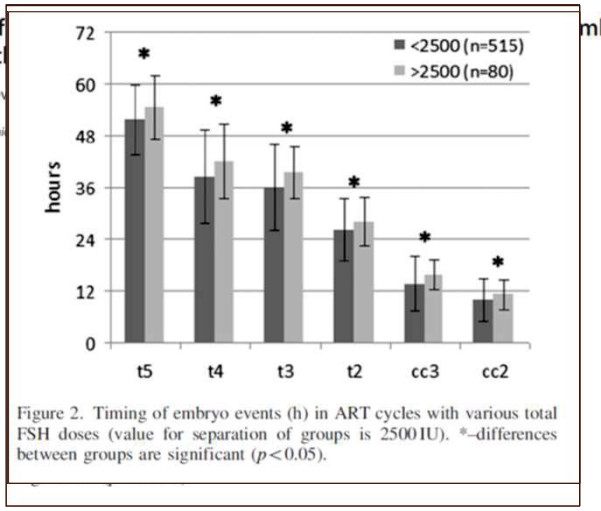


Figure 2. Timing of embryo events (h) in ART cycles with various total FSH doses (value for separation of groups is 2500 IU). *–differences between groups are significant ($p<0.05$).

Reproductive BioMedicine Online (2012) 25, 474–480

Embryos generated by standard IVF
underwent the first and second cleavage considerably later
and had a shorter cleavage time intervening between the
2- and 3-cell stages.

Cleavage ki… predicts de…ion

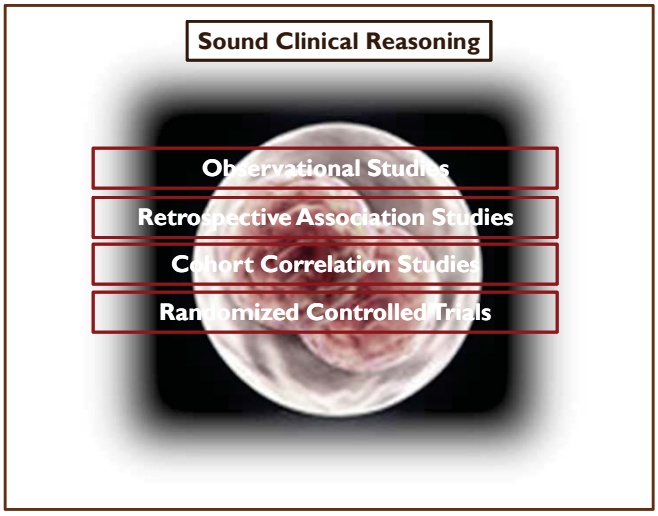
Mariabeatrice D… [a],
Elena De Ponti [b],
Ruggero Comi [a],

Table 1 Cleavage times from the 2- to the 8-cell stage and relative intervals between divisions for standard IVF and ICSI embryos developing to the blastocyst stage.

Stage or interval	Standard IVF (n = 73)	ICSI (n = 78)	P-value
2-cell	28.6 ± 2.6	27.0 ± 3.1	0.0005
3-cell	38.7 ± 3.3	37.6 ± 3.7	0.02
4-cell	40.2 ± 3.1	39.8 ± 5.5	NS
5-cell	51.4 ± 4.8	50.3 ± 7.2	NS
6-cell	53.2 ± 4.8	53.2 ± 8.6	NS
7-cell	56.6 ± 6.1	56.6 ± 9.9	NS
8-cell	60.8 ± 7.9	60.9 ± 10.6	NS
2–3-cell	10.3 ± 1.9	10.6 ± 2.0	0.002
3–4-cell	1.5 ± 2.5	2.2 ± 3.8	NS
2–4-cell	11.5 ± 1.5	12.8 ± 3.3	NS
4–8-cell	20.7 ± 6.9	21.4 ± 8.4	NS
5–8-cell	9.8 ± 6.5	10.6 ± 9.2	NS

Values are mean ± SD time in hours. NS = not statistically significant.

PREMATURE ADOPTION OF INNOVATION



Human Reproduction, Vol.27, No.5 pp. 1277–1285, 2012
Advanced Access publication on March 14, 2012 doi:10.1093/humrep/des079

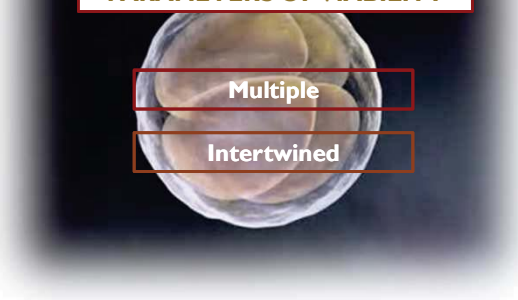
Table III Summary of ongoing studies registered with the National Institutes of Health (http://clinicaltrials.gov) using time-lapse monitoring for embryo selection.

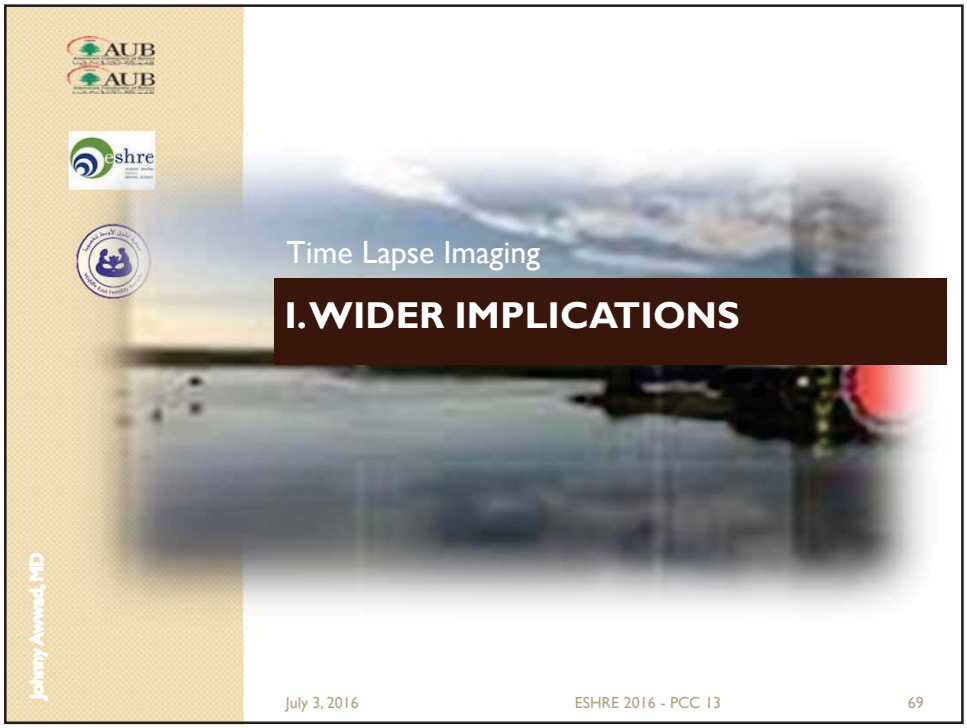
Study title	Year registered	Clinicaltrials.gov identifier	Sponsor	Location (s)	Principal investigator	Status	Design	Purpose
Correlating Time-Lapse Parameters Detected by the Eeva™ System With Comprehensive Chromosome Screening Results, Implantation and Live Birth	2012	NCT01635049	Auxogyn, Inc.	Reproductive Medicine Associates (USA)	Richard Scott, Jr., MD	Active, not recruiting	Prospective observational	To determine if there is a correlation between time-lapse parameters and comprehensive chromosome screening results.
Assessment of Implantation Potensial of Embryos by Time-Lapse Technology	2012	NCT01760278	Bloom IVF and Fertility Center	Lilavati Hospital and Research Center (India)	Hrishikesh Pai, MD	Active, not recruiting	RCT	To compare implantation potential of embryos selected by time-lapse to those selected by conventional morphology.
Embryo Selection by Time-Lapse Monitoring for Single Embryo Transfer	2012	NCT01694641	Kaali Institute IVF Center	Kaali Institute IVF Center (Hungary)	Peter Kovacs, MD	Recruiting	RCT	To determine whether clinical pregnancy rates using TLM are superior to conventional morphology for single blastocyst transfer.
Clinical Validation of Embryo Culture and Selection by Morphokinetic Analysis	2012	NCT01549262	Instituto Valenciano de Infertilidad, Spain	IVI Valencia (Spain)	Marcos Meseguer, PhD	Recruiting	RCT	To determine whether the hierarchal time-lapse model for embryo selection (Meseguer et al., 2012) improves ongoing pregnancy rates compared with conventional morphology.
US Eeva™ Pregnancy Investigational Clinical Study (US EPIC)	2012	NCT01671657	Auxogyn, Inc.	Fertility Physicians of Northern California (USA)	Shehua Shen, MD	Recruiting	Case–control	To compare implantation rates for Day 3 embryo transfers using TLM plus conventional morphology versus conventional morphology alone.
Eeva™ Pregnancy Investigational Clinical Study: A Postmarket Follow-Up Study	2012	NCT01671644	Auxogyn, Inc.	Gent University Hospital (Belgium) and VU University Medical Center (Netherlands)	Shehua Shen, MD	Recruiting	Case–control	To evaluate the impact of TLM plus conventional morphology on clinical pregnancy rates, compared with a matched control group using conventional morphology alone.
MERGE: MultcEmbryo ReGistry With Eeva™	2013	NCT01816802	Auxogyn, Inc.	Multiple private and academic centers in California, Connecticut, Illinois, New York, Ohio and Texas (USA)	Shehua Shen, MD	Recruiting	Prospective observational (non-comparative study)	To record the clinical pregnancy rates following embryo selection with conventional morphology plus TLM.

FALSE ASSUMPTIONS

KINETICS AS THE SOLE PREDICTOR OF EMBRYO VIABILITY

PARAMETERS OF VIABILITY



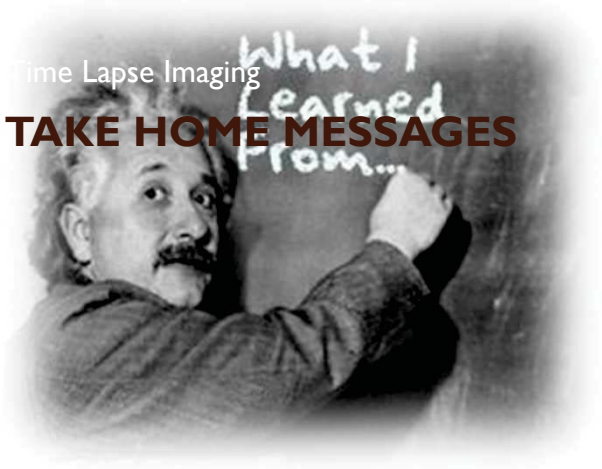


- As the steps of embryo development can be precisely standardized, Time Lapse Imaging could be useful for:
  - Research and Development purposes by
    - Improving our understanding of early embryonic development in vitro
    - Using kinetic markers as surrogate measures of optimal embryo development for innovative developments in the field
  - Laboratory Quality Control

- The identification of embryos with high developmental potential through monitoring of early kinetic events
  - May allow embryos to be selected for early day 3 transfer, thus avoiding extended in vitro culture.
  - Could pre-select the best-cleaving embryos before blastomere biopsy for Pre-Implantation Genetic Diagnosis PGD, thus reducing the diagnostic time and resources.

Time Lapse Imaging

TAKE HOME MESSAGES



You advise Andrea that Time Lapse technology has been shown to:

A. Enhance selection of high quality embryos, thus enhancing clinical reproductive outcome.

B. Enhance de-selection of low quality embryos, thus enhancing clinical reproductive outcome.

C. Enhance selection of high quality embryos, without necessarily improving clinical reproductive outcome.

Time Lapse Imaging appears to improve blastocyst prediction compared with conventional morphology.

While there appears to be a correlation between abnormal morphokinetics and poor implantation, normal cleavage kinetics do not guarantee post-implantation viability.

While Time Lapse Imaging has the potential to revolutionize clinical embryology,

there are currently no high-quality data to support its usefulness for the selection of human embryos on the basis of their implantation potential.

Bibliography

- Wong CC1, Loewke KE, Bossert NL, Behr B, De Jonge CJ, Baer TM, Reijo Pera RA. Non-invasive imaging of human embryos before embryonic genome activation predicts development to the blastocyst stage. Nat Biotechnol. 2010 Oct;28(10):1115-21
- Daniel J. Kaser and Catherine Racowsky. Clinical outcomes following selection of human preimplantation embryos with time-lapse monitoring: a systematic review. Human Reproduction Update, Vol.20, No.5 pp. 617–631, 2014.
- Irene Rubio, Arancha Gal, Zaloa Larreategui, Fernando Ayerdi, Jose Bellver, Javier Herrero, and Marcos Meseguer. Clinical validation of embryo culture and selection by morphokinetic analysis: a randomized, controlled trial of the EmbryoScope. Fertil Steril 2014;102:1287–94.
- Irene Rubio, Reidun Kuhlmann, Inge Agerholm, John Kirk, Javier Herrero, María-Jose Escriba, Jose Bellver, and Marcos Meseguer. Limited implantation success of direct-cleaved human zygotes: a time-lapse study. Fertil Steril 2012;98:1458–63.
- Linnea R. Goodman, Jeffrey Goldberg, Tommaso Falcone, Cynthia Austin and Nina Desai. Does the addition of time-lapse morphokinetics in the selection of embryos for transfer improve pregnancy rates? A randomized controlled trial. Fertil Steril 2016;105:275–85.
- Catherine Racowsky, Lucila Ohno-Machado, Jihoon Kim, and John D. Biggers. Is there an advantage in scoring early embryos on more than one day? Human Reproduction, Vol.24, No.9 pp. 2104–2113, 2009.
- Yu-li QIAN, Ying-hui YE, Chen-ming XU, Fan JIN, He-feng HUANG. Accuracy of a combined score of zygote and embryo morphology for selecting the best embryos for IVF. J Zhejiang Univ Sci B 2008 9(8):649-655.
- Sandrine Chamayou, Pasquale Patrizio, Giorgia Storaci, Venera Tomaselli, Carmelita Alecci, Carmen Ragolia, Claudia Crescenzo, Antonino Guglielmino. The use of morphokinetic parameters to select all embryos with full capacity to implant. J Assist Reprod Genet (2013) 30:703–710.
- Mariabeatrice Dal Canto, Giovanni Coticchi, Mario Mignini Renzini, Elena De Ponti, Paola Vittoria Novara, Fausta Brambillasc, Ruggero Comi, Rubens Fadini. Cleavage kinetics analysis of human embryos predicts development to blastocyst and implantation. Reproductive BioMedicine Online (2012) 25, 474– 480.
- Pandian Z, Marjoribanks J, Ozturk O, Serour G, Bhattacharya S. Number of embryos for transfer following in vitro fertilisation or intra-cytoplasmic sperminjection (Review). Cochrane Database of Systematic Reviews 2013, Issue 7.
- Marcos Meseguer, Irene Rubio, Maria Cruz, Natalia Basile, Julian Marcos, Antonio Requena. Embryo incubation and selection in a time-lapse monitoring system improves pregnancy outcome compared with a standard incubator: a retrospective cohort study. Fertil Steril 2012;98:1481–9.

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Bibiography

- Marcos Meseguer, Javier Herrero, Alberto Tejera, Karen Marie Hilligsøe, Niels Birger Ramsing, Jose Remohi. The use of morphokinetics as a predictor of embryo implantation. Human Reproduction, Vol.26, No.10 pp. 2658–2671, 2011.
- JG Lemmen, I Agerholm, S Ziebe. Kinetic markers of human embryo quality using time-lapse recordings of IVF/ICSI-fertilized oocytes. Reproductive BioMedicine Online Vol 17 No 3. 2008 385-391.
- Kirstine Kirkegaard, Inge E. Agerholm, Hans Jakob Ingerslev. Time-lapse monitoring as a tool for clinical embryo assessment. Human Reproduction, Vol.27, No.5 pp. 1277–1285, 2012.
- K. Kirkegaard, U.S. Kesmodel, J.J. Hindkjær, H.J. Ingerslev. Time-lapse parameters as predictors of blastocyst development and pregnancy outcome in embryos from good prognosis patients: a prospective cohort study. Human Reproduction, Vol.28, No.10 pp. 2643–2651, 2013.
- Semra Kahraman, Murat Çetinkaya,, Caroline Pirkevi, Hakan Yelke, Yeşim Kumtepe. Comparison of blastocyst development and cycle outcome in patients with eSET using either conventional or time lapse incubators. A prospective study of good prognosis patients. J Reprod Stem Cell Biotechnol 3(2):55-61.
- Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Human Reproduction, Vol.26, No.6 pp. 1270–1283, 2011.
- D. HLINKA, B. KAĽATOVÁ, I. UHRINOVÁ, S. DOLINSKÁ, J. RUTAROVÁ, J. ŘEZÁČOVÁ, S. LAZAROVSKÁ, M. DUDÁŠ. Time-Lapse Cleavage Rating Predicts Human Embryo Viability. Physiol. Res. 61: 513-525, 2012.
- Ali Sami Gurbuz, Funda Gode, Mehmet Sukru Uzman, Betul Ince, Melek Kaya, Necati Ozcimen Emel Ebru Ozcimen and Ali Acar. GnRH agonist triggering affects the kinetics of embryo development: a comparative study. Journal of Ovarian Research (2016) 9:22.
- Mykola Grygorievich Gryshchenko, Alexey Igorovich Pravdyuk & Valentin Yurievich Parashchyuk. Analysis of factors influencing morphokinetic characteristics of embryos in ART cycles. Gynecological Endocrinology, 30:sup1, 6-8.
- Joe Conaghan, Alice A. Chen, Susan P. WillmanKristen Ivani, Philip E. Chenette, Robert Boostanfar, Valerie L. Baker, G. David Adamson, Mary E. Abusief, Marina Gvakharia, Kevin E. Loewke, Shehua Shen, M. Improving embryo selection using a computer-automated time-lapse image analysis test plus day 3 morphology: results from a prospective multicenter trial. Fertil Steril 2013;100:412–9.
- Marı´a Cruz, Nicolas Garrido, Javier Herrero, Inmaculada Perez-Cano, Manuel Munoz, Marcos Meseguer. Timing of cell division in human cleavage-stage embryos is linked with blastocyst formation and quality. Reproductive BioMedicine Online (2012) 25, 371– 381.
- Armstrong S, Arroll N, Cree LM, Jordan V, Farquhar C. Time-lapse systems for embryo incubation and assessment in assisted reproduction (Review). Cochrane Database of Systematic Reviews 2015, Issue 2.

Johnny Awwad, MD

Preimplantation Genetic Aneuploidy Screening (PGS): Is it delivering on its promise?

Elias M. Dahdouh, M.D., M.Sc.

Founder and Medical Director
Assisted Reproduction Center, CHU Sainte-Justine
Assistant Professor
Gynecologic Reproductive Endocrinology and Infertility
Department of Obstetrics-Gynecology, University of Montreal
Associate Member
Procrea Clinics Montreal

Elias M. Dahdouh, MD, MSc
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has nothing to disclose for this presentation

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Learning Objectives

At the conclusion of this presentation, participants should be able to explain and discuss:

- ✓ **WHY PGS is performed?**
- ✓ **WHEN to biopsy ?**
- ✓ **HOW to test?**
- ✓ **WHAT are the clinical results?**

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PGD versus PGS

	PGD	PGS
Aim	Identify genetically normal embryos	Achieve a live birth
Indication	Monogenic disorder X-linked Chromosome abnormality HLA typing Gender Selection ...	AMA RIF RPL Severe male factor Embryo Selection
Fertility	Often fertile	Infertile
Prenatal diagnosis	Indicated	Indicated for same risk factors as natural conceptions

Harper et al. Hum Genet 2012

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IVF Success depends on

- ✓ Embryo status
  - Normal chromosome complement
- ✓ Endometrial receptivity
  - Negative effect of stimulation (Shapiro et al. Fertil Steril 2011)
  - Transfer in a frozen-thawed cycle?
- ✓ Embryo-endometrial synchronization
- ✓ Embryo transfer procedure

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Aim of PGS

- ✓ PGS: to increase clinical outcomes in IVF
  - Euploid ET
  - Invasive embryo selection
- ✓ Aneuploidies are frequent in IVF cycles
  - High rate of embryonic aneuploidies (30% → 80%)
  - Low IR (30% → 6%)

Munné S. Curr Genomics 2012

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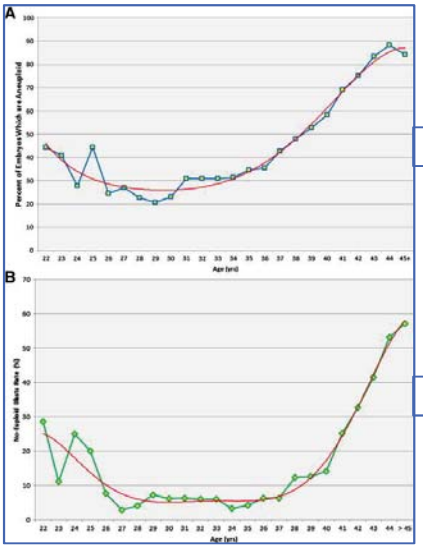

% Aneuploidy

% No Euploid

Franasiak et al. Fert Steril 2014

The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening

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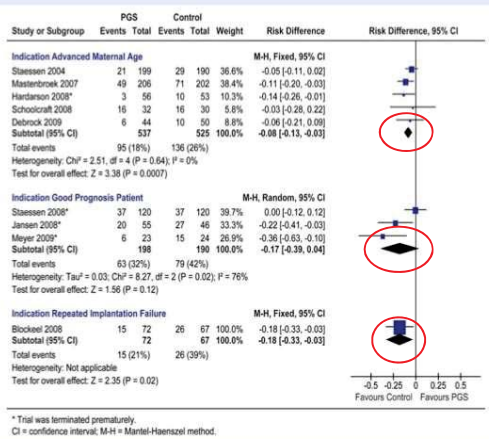
Human Reproduction Update, Vol.17, No.4 pp. 454–466, 2011
Advanced Access publication on April 25, 2011 doi:10.1093/humupd/dmr00

human reproduction update

Preimplantation genetic screening: a systematic review and meta-analysis of RCTs

S. Mastenbroek*, M. Twisk, F. van der Veen, and S. Repping



* Trial was terminated prematurely.
CI = confidence interval; M-H = Mantel-Haenszel method.

Figure 2 The effect of PGS on the live birth rate per patient.

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Drawbacks of PGS-FISH & D3 biopsy

- ✓ Limited number of chromosomes tested
- ✓ Technical problems: subjective, hybridization failure, signal overlap, and splitting
- ✓ Negative impact of Embryo biopsy on development
- ✓ Negative effect with FISH-PGS on IVF program

Mastenbroek et al. N Engl J Med 2007

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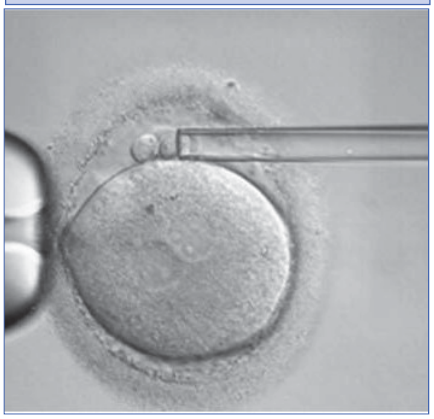
Oocyte biopsy

1st and 2nd Polar Body	Characteristics
	✓ Simultaneous (8-12 h after ICSI) or sequential ✓ Performed where embryo biopsy is considered illegal ✓ Detects only maternal anomalies (aneuploidies) ✓ 30% of postmeiotic anomalies not detected

Montag et al. Fertil Steril 2013

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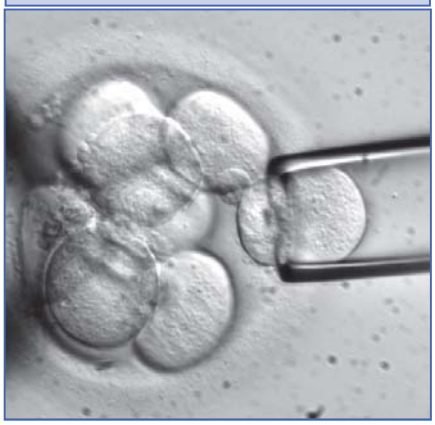
Cleavage-stage biopsy

Day 3: 1 Blastomere



Pros & Cons

PROS
- ✓ Worldwide experience
- ✓ Enough time for fresh ET
- ✓ Suitable for many patients

CONS
- ✓ Less DNA
- ✓ High rate of mosaicism
- ✓ Less implantation

Harton et al. Hum Reprod 2011

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Blastocyst biopsy

Blastocyst: 3-10 trophectoderm Cells

Pros & Cons

PROS
- ✓ More DNA: less no results
- ✓ Less mosaicism: less error rate
- ✓ No impact of embryo biopsy
- ✓ Less embryos to test: lower cost
- ✓ eSET possible
- ✓ Frozen ET: better endometrial environment

CONS
- ✓ Not all embryos reach blasctocyst
- ✓ Requires experience

Schoolcraft et al. Fertil Steril 2010

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Cleavage-stage or Blastocyst biopsy?

Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial

Richard T. Scott Jr., M.D.,[a,b] Kathleen M. Upham, B.S.,[a] Eric J. Forman, M.D.,[b] Tian Zhao, M.S.,[a] and Nathan R. Treff, Ph.D.[a,b,c]

[a] Reproductive Medicine Associates of New Jersey, Morristown; [b] Division of Reproductive Endocrinology, Department of Obstetrics, Gynecology, and Reproductive Sciences, Robert Wood Johnson Medical School, Rutgers University, New Brunswick; and [c] Department of Genetics, Rutgers–State University of New Jersey, Piscataway, New Jersey

Fertility and Sterility® VOL. 100 NO. 3 / SEPTEMBER 2013

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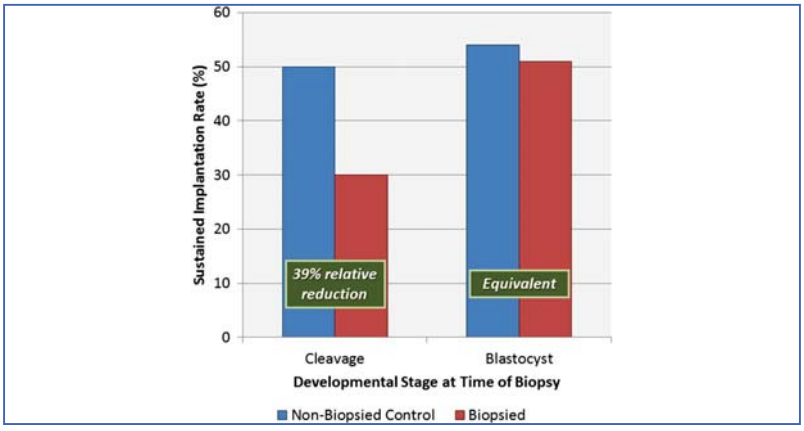
D3 versus D5-6



Scott et al. Fertil Steril 2013

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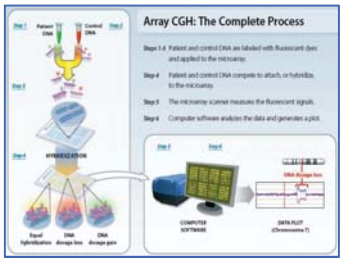
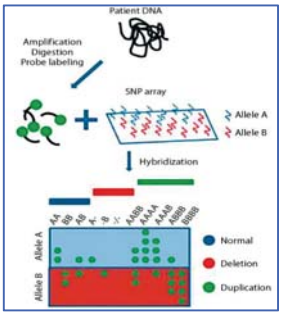
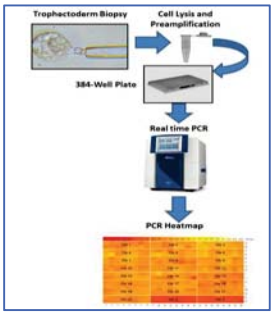
Genetic Analysis in PGS

- ✓ FISH: old technology
- ✓ Comprehensive Chromosome Screening (CCS): new technology
  - aCGH
  - SNP microarray
  - qPCR







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Advantages of CCS

- ✓ <u>Complete 24-chromosome analysis</u>
- ✓ No pre-IVF validation required by parental DNA
- ✓ Automated analysis, < 24 hours (aCGH, qPCR)
- ✓ ICSI not required
- ✓ Fresh ET still possible (if D3 or D5 biopsy+aCGH/qPCR)

Handyside A. Fertil Steril 2013

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Studies for PGS with CCS technology

- ✓ MOSTLY: Prospective & Retrospective Observational studies
- ✓ Randomized controlled trials (RCTs)
  - ONLY 3 RCTs
  - 1 with aCGH, 2 with qPCR
  - 2 from same group !

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PGS-aCGH on D5-6: 1st application

Clinical application of comprehensive chromosomal screening at the blastocyst stage

William B. Schoolcraft, M.D.,[a] Elpida Fragouli, Ph.D.,[b,c] John Stevens, M.S.,[a] Santiago Munne, Ph.D.,[d] Mandy G. Katz-Jaffe, Ph.D.,[b] and Dagan Wells, Ph.D., F.R.C.Path.[b,c]

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TABLE 2

Comparison of patient characteristics and treatment cycle outcome for patients with aneuploidy screening and a set of contemporary cycles from the same clinic.

	Contemporary comparison group (n = 113)	Comprehensive chromosome screening group (n = 45)
Average maternal age (y)	37.1	37.7
Average no. of previous failed IVF treatments	1.24	2.42
Day 3 FSH (IU)	7.6	7.3
Average no. of oocytes retrieved per cycle	19.4	18.6
Average no. of blastocysts transferred per cycle	2.7 (299 transfrred in 113 cycles)	2.0 (90 transferred in 45 cycles)
Biochemical pregnancy (positive pregnancy test) per cycle[a]	84.0% (95/113)	82.2% (37/45)[a]
Implantation rate (proportion of transferred embryos producing a fetal sac)	46.5% (139/299)	72.2% (65/90)[b]
Implantation rate (proportion of transferred embryos producing a fetus with heartbeat)	44.8% (134/299)	68.9% (62/90)[c]

[a] Only one patient had no euploid embryos available for transfer. The pregnancy rate per cycle with an embryo transfer was 84.1% (37/44).
[b] $P<.0001$ (chi-squared test with Yates correction).
[c] $P<.0001$ (chi-squared test with Yates correction).

Schoolcraft. Clinical application of CGH on blastocysts. Fertil Steril 2010.

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Summary of RCTs on New PGS

- ✓ **Yang et al 2012: aCGH on D5-6 + fresh ET D6 (eSET)**
- ✓ **Forman et al 2013: qPCR on D5-6 + Fresh-Frozen ET (eSET vs DET)**
- ✓ **Scott et al 2013: qPCR on D5-6 + Fresh ET D6 (DET vs DET)**

Study Group	N	Age	Blastocysts
Yang et al. 2012	55	31.2	8.3
Forman et al. 2013	89	35.1	5.8
Scott et al. 2013	72	32.2	7.1

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PGS: eSET+aCGH and Fresh D6 ET

Yang et al. Molecular Cytogenetics 2012, 5:24
http://www.molecularcytogenetics.org/content/5/1/24

MOLECULAR CYTOGENETICS

METHODOLOGY Open Access

Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study

Zhihong Yang[1], Jiaen Liu[2], Gary S Collins[3], Shala A Salem[1], Xiaohong Liu[2], Sarah S Lyle[1], Alison C Peck[1], E Scott Sills[1*] and Rifaat D Salem[1]

Table 3 Comparison of laboratory findings and clinical outcome among IVF patients undergoing SET with embryo assessment by aCGH + morphology (Group A) and blastocyst morphology alone (Group B)

	A	B	*p*
Fresh blastocyst transfer *according to morphology assessment:*	55 (100)	48 (100)	
Grade 5/6	31 (56.4)	28 (58.3)	
Grade 4	21 (38.2)	19 (39.6)	0.677[a]
Grade 3	3 (5.4)	1 (2.1)	
Clinical pregnancy	39 (70.9)	22 (45.8)	0.017[a]
Ongoing pregnancy (≥20wks GA)	38 (69.1)	20 (41.7)	0.009[a]
Missed abortion	1 (2.6)	2 (9.1)	0.597[b]

Notes: All data reported as *n* (%). SET = single embryo transfer; aCGH = array comparative genomic hybridization; GA = gestational age [a] by Chi-squared test [b] by Fisher's exact test.

1st IVF , ≤ 35 years old, Normal Karyotype

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In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial

Eric J. Forman, M.D.,[a,b] Kathleen H. Hong, M.D.,[a,b] Kathleen M. Ferry, B.Sc.,[a] Xin Tao, M.Sc.,[a] Deanne Taylor, Ph.D.,[a] Brynn Levy, Ph.D.,[a,c] Nathan R. Treff, Ph.D.,[a,b] and Richard T. Scott Jr., M.D.[a,b]

[a] Reproductive Medicine Associates of New Jersey, Department of Reproductive Endocrinology, Basking Ridge, New Jersey; [b] UMDNJ-Robert Wood Johnson Medical School, Department of Obstetrics, Gynecology & Reproductive Sciences, New Brunswick, New Jersey; and [c] Department of Pathology, Columbia University, College of Physicians and Surgeons, New York, New York

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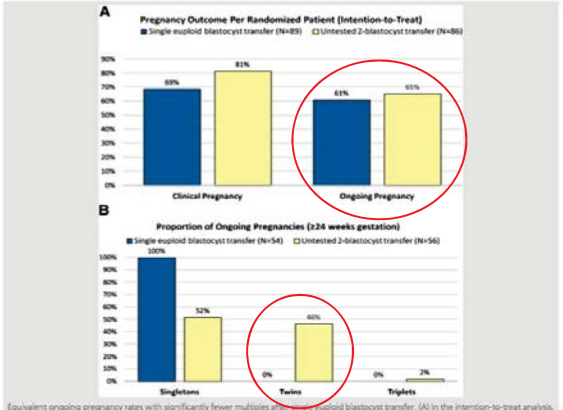
FIGURE 2



Equivalent ongoing pregnancy rates with significantly fewer multiples after single euploid blastocyst transfer. (A) In the intention-to-treat analysis, the ongoing pregnancy rate (≥ 24 weeks gestation) after single euploid blastocyst transfer was not inferior to the rate after transferring two untested blastocysts. (B) In the single euploid blastocyst transfer group, all ongoing pregnancies were singletons. After accounting for second trimester losses and vanishing twins, multiples accounted for 48% of the ongoing pregnancies after untested two-blastocyst transfer.

Forman. Blastocyst Euploid Selective Transfer (BEST) Trial. Fertil Steril 2013

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Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial

Richard T. Scott Jr., M.D.,[a,b] Kathleen M. Upham, B.S.,[a] Eric J. Forman, M.D.,[a] Kathleen H. Hong, M.D.,[a] Katherine L. Scott, M.S.,[a,b] Deanna Taylor, Ph.D.,[a,b] Xin Tao, M.S.,[a] and Nathan R. Treff, Ph.D.[a,b]

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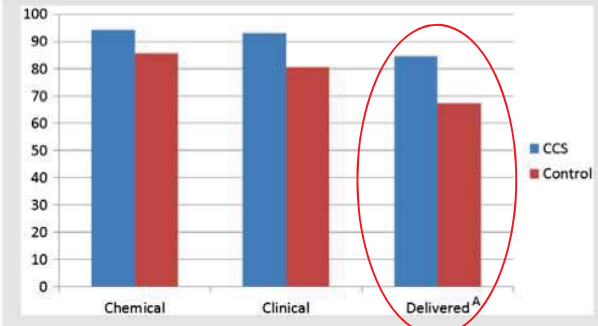

Outcome per treatment cycle: Delivery rates are statistically significantly increased in treatment cycles in which embryos undergo comprehensive chromosome screening (*P*=.03). The initial chemical and clinical pregnancy rates were not different.

Scott. RCT showing CCS improves delivery rates. Fertil Steril 2013.

24

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Reproductive BioMedicine Online (2015) 30, 281–289

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ELSEVIER

ARTICLE

Impact of blastocyst biopsy and comprehensive chromosome screening technology on preimplantation genetic screening: a systematic review of randomized controlled trials

CrossMark

Elias M Dahdouh [a,b,c,*], Jacques Balayla [c], Juan Antonio García-Velasco [d]

Université de Montréal

Human Reproduction, Vol.30, No.2 pp. 473–483, 2015
Advanced Access publication on November 28, 2014 doi:10.1093/humrep/deu303

human reproduction

REVIEW *Reproductive genetics*

The clinical effectiveness of preimplantation genetic diagnosis for aneuploidy in all 24 chromosomes (PGD-A): systematic review

Evelyn Lee[1,*], Peter Illingworth[2], Leeanda Wilton[3], and Georgina Mary Chambers[1]

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Human Reproduction, Vol.0, No.0 pp. 1–2, 2015

human reproduction

LETTER TO THE EDITOR

Preimplantation genetic screening using comprehensive chromosome screening: evidence and remaining challenges

Elias M. Dahdouh[1,2,3,*], Jacques Balayla[3] and Juan Antonio García-Velasco[4]

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SOGC TECHNICAL UPDATE

No. 323, May 2015 (Replaces No. 232, August 2009)

Technical Update: Preimplantation Genetic Diagnosis and Screening

This technical update has been prepared by the Genetics Committee and approved by the Executive and Board of the Society of Obstetricians and Gynaecologists of Canada.

PRINCIPAL AUTHORS

Elias M. Dahdouh, MD, Montreal QC

Jacques Balayla, MD, Montreal QC

François Audibert, MD, Montreal QC

GENETICS COMMITTEE

J Obstet Gynaecol Can 2015;37(5):451–463

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Recommendations

8. Preimplantation genetic screening using fluorescence in situ hybridization technology on day-3 embryo biopsy is associated with decreased live birth rates and therefore should not be performed with in vitro fertilization. (I-E).
9. Preimplantation genetic screening using comprehensive chromosome screening technology on blastocyst biopsy increases implantation rates and improves embryo selection in IVF cycles in patients with a good prognosis. (I-B).

Dahdouh et al. SOGC Guidelines PGD-PGS. J Obstet Gynaecol Can 2015

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ORIGINAL ARTICLE: GENETICS

Comprehensive chromosome screening improves embryo selection: a meta-analysis

Elias M. Dahdouh, M.D., M.Sc.,[a,b,c] Jacques Balayla, M.D.,[c] and Juan Antonio García-Velasco, M.D., Ph.D.[d]

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Fertility and Sterility® VOL. 104 NO. 6 / DECEMBER 2015

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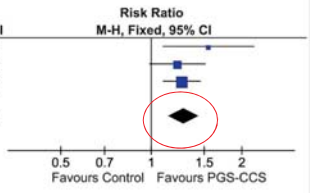
FIGURE 2

Clinical implantation rate

Study or Subgroup	PGS-CCS Events	PGS-CCS Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Yang et al. 2012	39	55	22	48	13.3%	1.55 [1.09, 2.20]
Forman et al. 2013	55	87	89	172	33.9%	1.22 [0.98, 1.52]
Scott et al. 2013	107	134	103	163	52.7%	1.26 [1.09, 1.46]
Total (95% CI)		**276**		**383**	**100.0%**	**1.29 [1.15, 1.45]**
Total events	201		214			

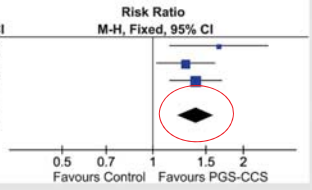
Heterogeneity: Chi² = 1.34, df = 2 (P = 0.51); I² = 0%
Test for overall effect: Z = 4.27 (P < 0.0001)



Sustained implantation rate (> 20 weeks gestation)

Study or Subgroup	PGS-CCS Events	PGS-CCS Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Yang et al. 2012	38	55	20	48	14.5%	1.66 [1.14, 2.42]
Forman et al. 2013	54	87	83	172	37.8%	1.29 [1.03, 1.61]
Scott et al. 2013	89	134	78	163	47.7%	1.39 [1.14, 1.70]
Total (95% CI)		**276**		**383**	**100.0%**	**1.39 [1.21, 1.60]**
Total events	181		181			

Heterogeneity: Chi² = 1.29, df = 2 (P = 0.53); I² = 0%
Test for overall effect: Z = 4.61 (P < 0.00001)



Meta-analysis of RCTs on PGS-CCS vs. routine care.

Dahdouh. CCS and embryo selection. Fertil Steril 2015.

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FIGURE 3

Clinical implantation rate

Study or Subgroup	PGS-CCS Events	PGS-CCS Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Sher et al. 2009	36	94	43	311	8.8%	2.77 [1.90, 4.04]
Schoolcraft et al. 2010	62	90	134	299	27.5%	1.54 [1.27, 1.85]
Fishel et al. 2011	31	112	15	187	5.0%	3.45 [1.95, 6.10]
Forman et al. 2012	86	140	101	182	38.9%	1.11 [0.92, 1.33]
Keltz et al. 2013	30	57	253	1321	9.3%	2.75 [2.10, 3.60]
Greco et al. 2014	28	41	9	41	4.0%	3.11 [1.68, 5.75]
Lee et al. 2015	28	55	16	63	6.6%	2.00 [1.22, 3.29]
Total (95% CI)		**589**		**2404**	**100.0%**	**1.78 [1.60, 1.99]**
Total events	301		571			

Heterogeneity: Chi² = 51.50, df = 6 (P < 0.00001); I² = 88%
Test for overall effect: Z = 10.31 (P < 0.00001)

Risk Ratio M-H, Fixed, 95% CI

0.2 0.5 1 2 5
Favours Control Favours PGS-CCS

Sustained implantation rate (> 20 weeks gestation)

Study or Subgroup	PGS-CCS Events	PGS-CCS Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Sher et al. 2009	34	94	39	311	14.9%	2.88 [1.94, 4.29]
Forman et al. 2012	77	140	76	182	54.6%	1.32 [1.05, 1.65]
Lee et al. 2015	25	55	12	63	9.2%	2.39 [1.33, 4.29]
Feichtinger et al. 2015	29	110	60	403	21.2%	1.77 [1.20, 2.62]
Total (95% CI)		**399**		**959**	**100.0%**	**1.75 [1.48, 2.07]**
Total events	165		187			

Heterogeneity: Chi² = 13.10, df = 3 (P = 0.004); I² = 77%
Test for overall effect: Z = 6.48 (P < 0.00001)

Risk Ratio M-H, Fixed, 95% CI

0.2 0.5 1 2 5
Favours Control Favours PGS-CCS

Meta-analysis of observational studies on PGS-CCS vs. routine care.

Dahdouh. CCS and embryo selection. Fertil Steril 2015.

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Prerequisites for PGS-CCS

- ✓ **Experience in extended embryo culture (%blastulation)**
- ✓ **Experience in Blastocyst biopsy (or D3 biopsy)**
- ✓ **Validated and tested CCS platform**
- ✓ **Effective cryopreservation program (Frozen ET)**

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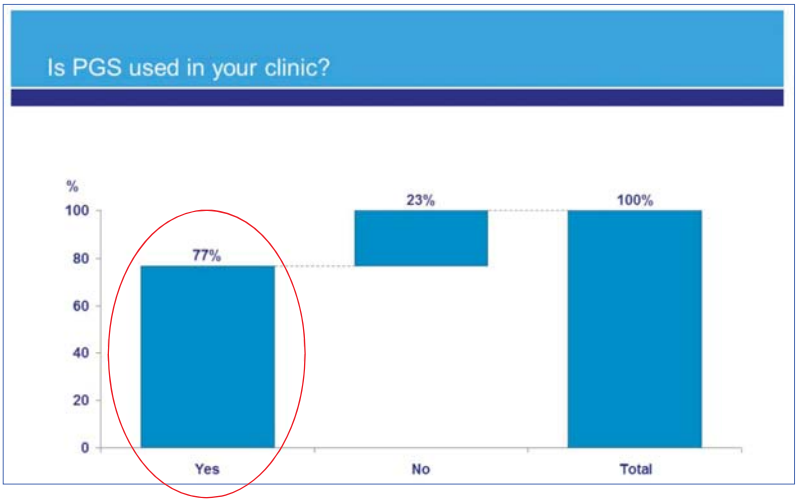
Is PGS used in your clinic?



IVF Worldwide. PGS survey 2016

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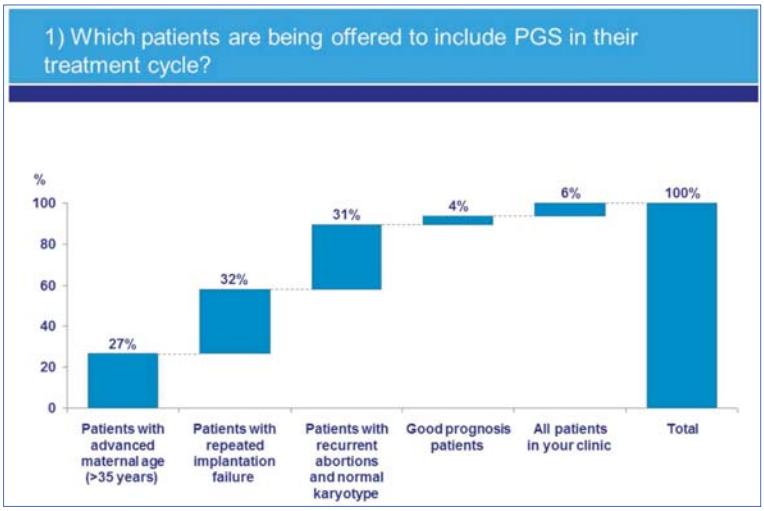
1) Which patients are being offered to include PGS in their treatment cycle?



IVF Worldwide. PGS survey 2016

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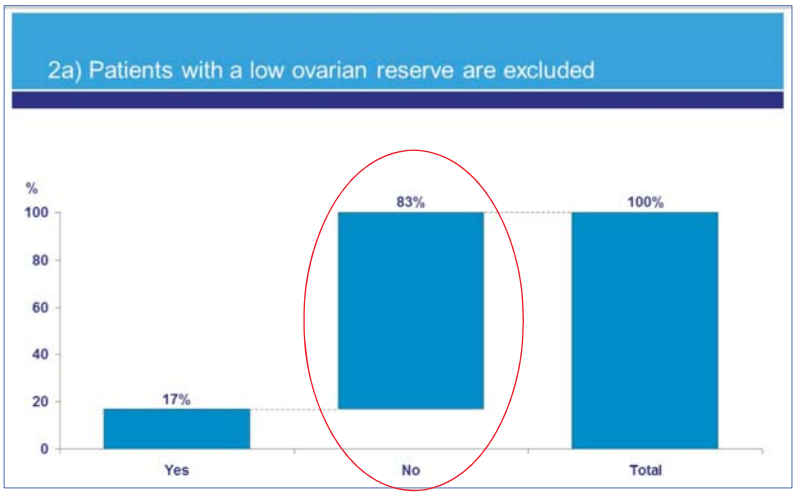

IVF Worldwide. PGS survey 2016

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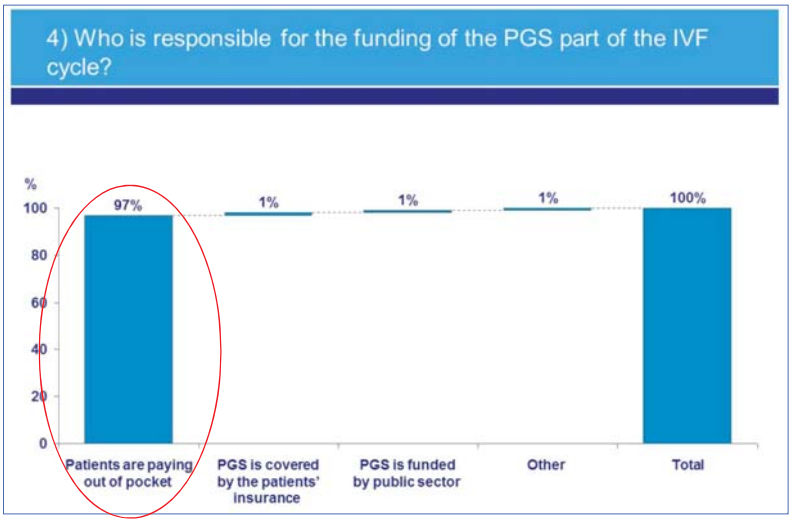

IVF Worldwide. PGS survey 2016

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TABLE 1

Characteristics of included randomized and observational studies.

Study	Design	Indication	Embryo biopsy	Genetic platform	Main outcomes
Randomized studies					
Yang et al., 2012 (24)	Pilot RCT	Good-prognosis patients, 1st IVF cycle	Blastocyst	aCGH	Clinical PR, ongoing PR (>20 wk)
Forman et al. 2013 (22)	Noninferiority trial (RCT)	Normal ovarian reserve, ≤1 previous IVF failure	Blastocyst	qPCR	Ongoing PR (>24 wk), multiple PR
Scott et al., 2013 (75)	RCT	Normal ovarian reserve, ≤1 previous IVF failure	Blastocyst	qPCR	Sustained IR, delivery rate
Observational studies					
Sher et al., 2009 (58)	PCS	AMA + RIF + RPL	Cleavage	mCGH	IR, Live birth rate
Schoolcraft et al., 2010 (55)	PCS	Previous IVF failure	Blastocyst	aCGH	IR
Fishel et al., 2011 (79)	PCS	RIF	Polar Body	aCGH	IR
Forman et al., 2012 (23)	RCS	1st SET cycle	Blastocyst	qPCR	Ongoing PR (>12 wk)
Keltz et al., 2013 (76)	RCC	AMA + RIF + RPL	Cleavage	aCGH	IR
Greco et al., 2014 (43)	PCS	RIF	Blastocyst	aCGH	IR
Lee et al., 2015 (77)	RCS	AMA	Blastocyst	aCGH	IR, Live birth rate
Feichtinger et al., 2015 (78)	RCS	RIF + AMA	Polar Body	aCGH	Live birth rate

Note: aCGH = array comparative genomic hybridization; AMA = advanced maternal age; IR = implantation rate; mCGH = metaphase comparative genomic hybridization; qPCR = quantitative polymerase chain reaction; PCS = prospective cohort study; PR = pregnancy rate; RCC = retrospective case-control; RCS = retrospective cohort study; RCT = randomized controlled trial; RIF = repeated implantation failure; RPL = recurrent pregnancy loss; SET = single-embryo transfer.

Dahdouh. CCS and embryo selection. Fertil Steril 2015.

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PGS-CCS: the debate still Continues !

- ✓ Embryo banking in poor prognosis: No Proven benefit !
- ✓ Extrapolation to other patient categories remains unclear:
  - Low responders: AMH<1.1 or AFC<8 (Scott et al. NCT01977144)
  - D3 biopsy with CCS (Rubio et al. 2014 NCT01571076)
  - PGS-CCS for RIF or RPL…
  - D3 vs. D5 biopsy with CCS…
- ✓ Cost effectiveness: cumulative live births after PGS-CCS vs. controls ?

Dahdouh et al. Fertil Steril 2015
Dahdouh et al. RBMOnline 2015

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CORRESPONDENCE

Healthy Babies after Intrauterine Transfer of Mosaic Aneuploid Blastocysts

N Engl J Med 2015; 373:2089-2090 | November 19, 2015 | DOI: 10.1056/NEJMc1500421

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Take Home Messages
PGS: Is it delivering on its promise?

- ✓ To increase LBR with FISH on D3?
  NO (I-E)
- ✓ To increase IR and improve embryo selection with CCS?
  YES, for good prognosis patients (I-B)
- ✓ To increase LBR with CCS?
  PROBABLY YES, for good prognosis patients (I-C)
- ✓ To increase LBR for RIF & RPL?
  PENDING, YES (II-B)
- ✓ To increase LBR in poor responders?
  PROBABLY NOT (III-C)

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Bibliography

- Dahdouh EM, Balayla J, García-Velasco JA. Comprehensive chromosome screening improves embryo selection: a meta-analysis. Fertil Steril. 2015 December; 104(6): 1503-12.
- Dahdouh EM, Balayla J, García-Velasco JA. Preimplantation genetic screening using comprehensive chromosome screening: evidence and remaining challenges. Hum Reprod. 2015 June; 30(6):1515-6.
- Dahdouh EM, Balayla J, Audibert F; SOGC Genetics Committee. Technical Update: Preimplantation Genetic Diagnosis and Screening. J Obst Gynaecol Ca. 2015 May; 37(5): 451-463
- Dahdouh EM, Balayla J, García-Velasco JA. Impact of blastocyst biopsy and comprehensive chromosome screening technology on preimplantation genetic screening: a systematic review of randomized controlled trials. Reprod BioMed Online. 2015 March; 30(3):281-9.
- Forman EJ, Hong KH, Ferry KM, Tao X, Taylor D, Levy B, Treff NR, Scott RT Jr. In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial. Fertil Steril 2013;100:100-107.
- Lee E, Illingworth P, Wilton L, Chambers GM. The clinical effectiveness of preimplantation genetic diagnosis for aneuploidy in all 24 chromosomes (PGD-A): systematic review. Hum Reprod 2015;30:473–483.
- Rubio C, Bellver J, Rodrigo L, Bosch E, Mercader A, Vidal C, De los Santos MJ, Giles J, Labarta E, Domingo J et al. Preimplantation genetic screening using fluorescence in situ hybridization in patients with repetitive implantation failure and advanced maternal age: two randomized trials. Fertil Steril 2013;99:1400–1407.
- Scott RT Jr, Upham KM, Forman EJ, Hong KH, Scott KL, Taylor D, Tao X, Treff NR. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. Fertil Steril 2013a;100:697–703.
- Scott RT Jr, Upham KM, Forman EJ, Zhao T, Treff NR. Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. Fertil Steril 2013b;100:624–630.
- Yang Z, Liu J, Collins GS, Salem SA, Liu X, Lyle SS, Peck AC, Sills ES, Salem RD. Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study. Mol Cytogenet 2012;5:24.

IMSI vs ICSI
Between Hope and Hype?

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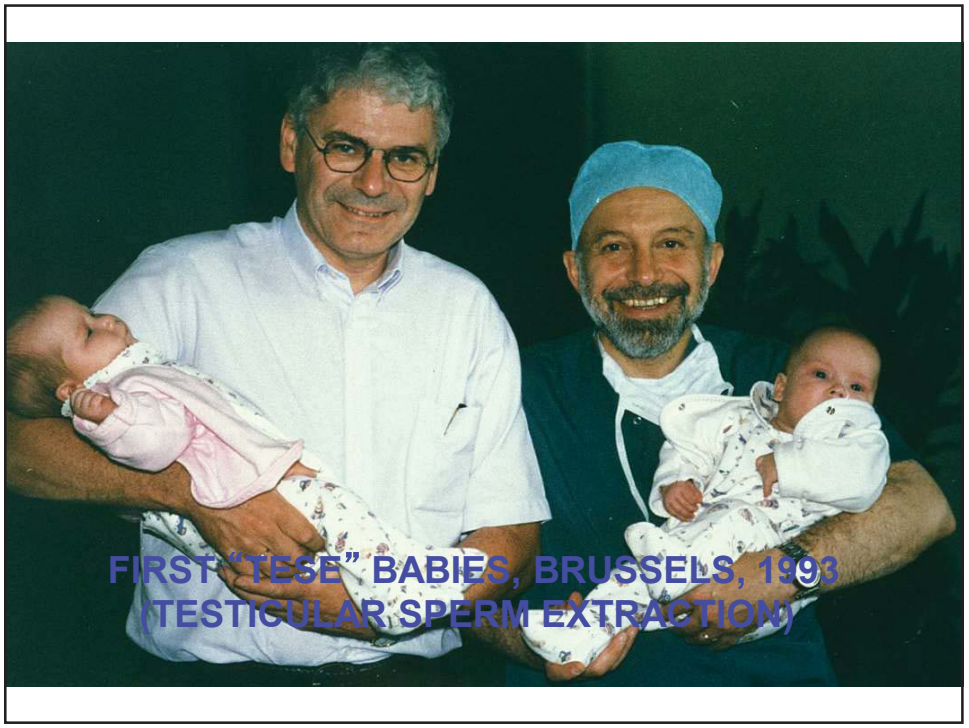
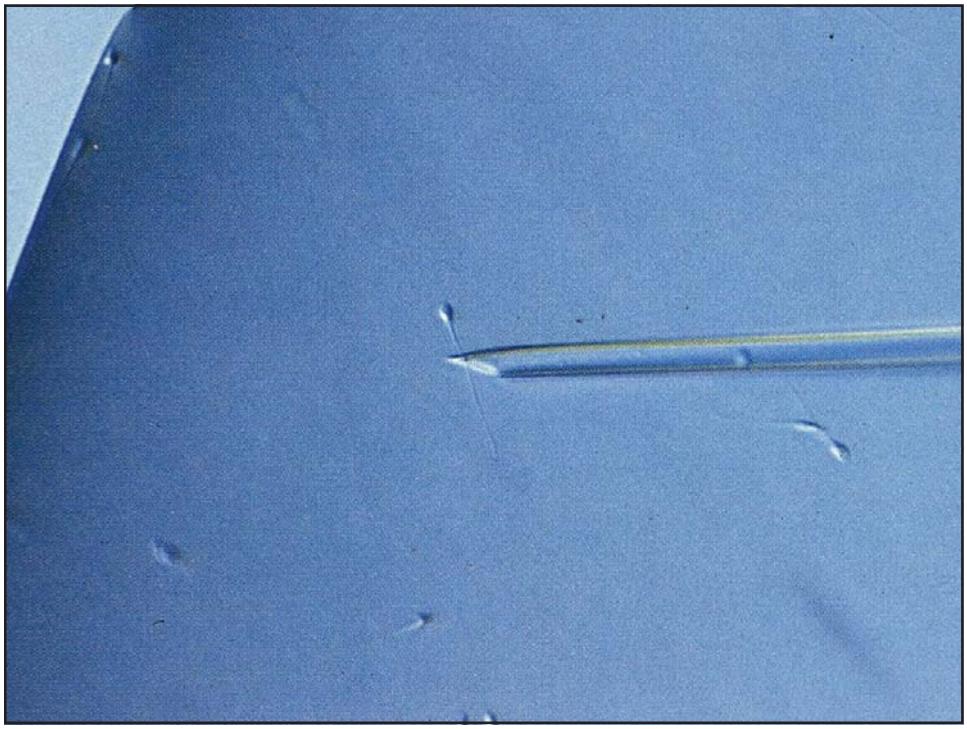
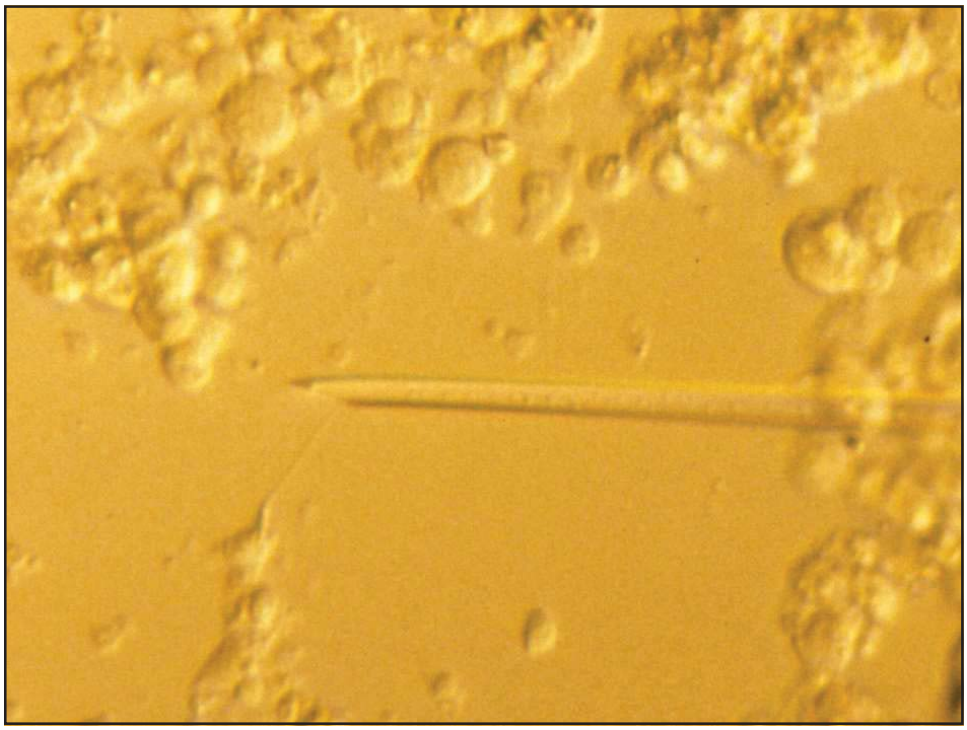
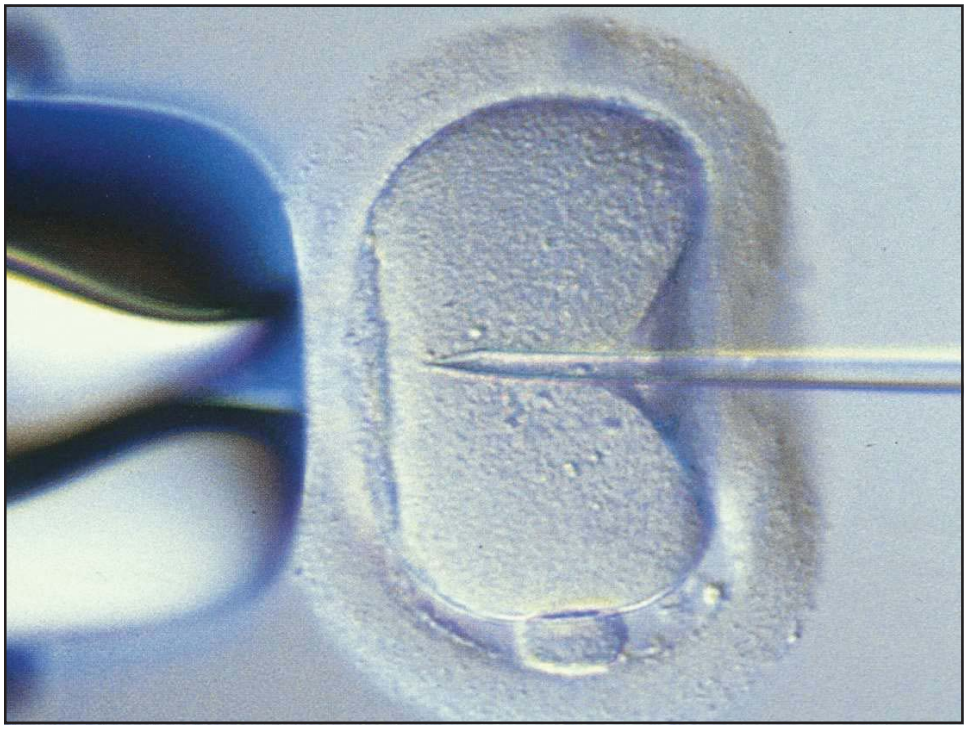
NO CONFLICT OF INTEREST


TREATING MALE
INFERTILITY
With ICSI or With IMSI


FIRST "TESE" BABIES, BRUSSELS, 1993
(TESTICULAR SPERM EXTRACTION)







ICSI Cycle Results with Varying Degrees of Male Factor Infertility

ICSI Pregnancy Rates for Obstructive Azoospermia (Testis Vs. Epididymis)

Age	MESA OA Fresh & Frozen		TESE OA Fresh & Frozen		Overall	
≤35	206/377	55%	44/99	44%	250/476	52%
36-40	47/109	43%	11/33	33%	58/142	41%
>40	12/29	41%	1/6	16%	13/35	37%
Overall	253/486	52%	55/132	42%	308/618	50%

ICSI Live Birth Rates for Obstructive Azoospermia (Testis Vs. Epididymis)

Age	MESA OA Fresh & Frozen		TESE OA Fresh & Frozen		Overall	
≤35	159/377	42%	33/99	33%	192/476	40%
36-40	27/109	25%	5/33	15%	32/142	22%
>40	4/29	14%	0/6	0%	4/35	11%
Overall	190/486	39%	38/132	29%	228/618	37%

ICSI Pregnancy Rates for Non-Obstructive Azoospermia (Testis Vs. Epididymis)

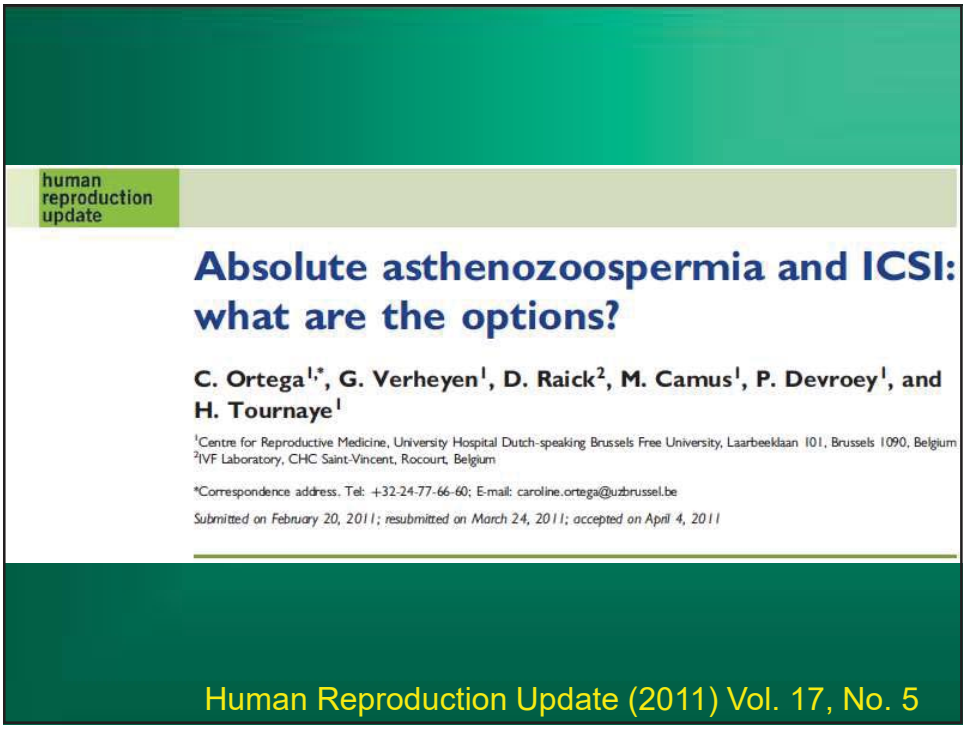
Age	MESA OA Fresh & Frozen		TESE OA Fresh & Frozen		TESE NOA Fresh & Frozen		Overall	
≤35	206/377	55%	44/99	44%	90/230	39%	340/706	48%
36-40	47/109	43%	11/33	33%	30/70	43%	88/212	41%
>40	12/29	41%	1/6	16%	2/16	12%	15/51	29%
Overall	253/486	52%	55/132	42%	122/316	39%	430/934	46%

ICSI Live Birth Rates for Non-Obstructive Azoospermia (Testis Vs. Epididymis

Age	MESA OA Fresh & Frozen		TESE OA Fresh & Frozen		TESE NOA Fresh & Frozen		Overall	
≤35	159/377	42%	33/99	33%	52/230	23%	244/706	35%
36-40	27/109	25%	5/33	15%	20/70	29%	52/212	24%
>40	4/29	14%	0/6	0%	0/16	0%	4/51	7%
Overall	190/486	39%	38/132	29%	72/316	23%	300/934	32%

Pregnancy Rates for Ejaculated Sperm in 2,186 Consecutive ICSI Cycles

Age	<2 Million Sperm		2-5 Million Sperm		6-20 Million Sperm		>20 Million Sperm		Overall	
≤35	222/473	47%	102/184	55%	137/262	52%	402/747	54%	863/1666	52%
36-40	59/139	42%	23/53	43%	39/98	40%	88/230	38%	209/520	40%
>40	6/46	13%	4/16	25%	4/19	21%	9/45	20%	23/126	18%
Overall	287/658	44%	129/253	51%	180/379	47%	499/1022	49%	1095/2312	47%



Human Reproduction Update (2011) Vol. 17, No. 5

Sperm Parameters and ICSI (1995): Only No Motility Had A Negative Effect

- Only the injection of a totally immotile spermatozoon has an overall negative impact on fertilization and pregnancy rates (Liu et al., 1995).
- Of the three basic sperm parameters (total sperm count, sperm motility and morphology) : in 996 cycles 'only one condition had a negative influence on the result of ICSI: where a completely immotile (presumably dead) spermatozoon was injected into the oocyte' (Nagy et al., 1995a).

Human Reproduction Update (2011) Vol. 17, No. 5

Absolute asthenozoospermia and ICSI: What are the options?

- Necrozoospermia is a rare condition reported in only 0.2-0.5% of infertile males and may have its origin either in the epididymis or in the testis (Ahmadi and Ng, 1999).
- But viable spermatozoa may be retrieved by testicular sperm extraction (TESE) (Devreoy et al.,1994; Tournaye et al., 1996).
- Therefore, it is recommended to perform ICSI in combination with TESE in patients with proven necrozoospermia (Tournaye et al., 1996)

Human Reproduction Update (2011) Vol. 17, No. 5

Special applications of intracytoplasmic sperm injection: the influence of sperm count, motility, morphology, source and sperm antibody on the outcome of ICSI

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[1]To whom correspondence should be addressed

Human Reproduction (1998) Vol. 13 No. 1

Special applications of intracytoplasmic sperm injection:
The influence of sperm count, motility, morphology, source and sperm antibody on the outcome of ICSI

- The results showed that neither the type nor the extent of sperm impairment had an important influence on the outcome of ICSI when ejaculated spermatozoa were used.

- Only two very rare conditions had a strongly negative influence on the result of ICSI, i.e. where immotile (presumably dead) spermatozoa or where round-headed spermatozoa were injected into the oocyte.

Human Reproduction (1998) Vol. 13 No. 1

IMSI vs ICSI

SPERM SELECTION BY MORPHOLOGY?

Influence of individual sperm morphology on fertilization, embryo morphology, and pregnancy outcome of intracytoplasmic sperm injection

Anick De Vos, Ph.D., Hilde Van De Velde, Ph.D., Hubert Joris, M.T., Greta Verheyen, Ph.D., Paul Devroey, M.D., Ph.D., and André Van Steirteghem, M.D., Ph.D.

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Fertility and Sterility (2003) Vol. 79, No. 1

Influence of individual sperm morphology and sperm origin on oocyte fertilization and embryo quality after ICSI.

	Individual sperm morphology at microinjection					
	Normal			Abnormal		
Variable	Ejaculated	Nonejaculated	Total	Ejaculate	Nonejaculated	Total
No. of oocytes injected	4,406	465	4,871	418	397	815
Fertilization rate (%)[a]	72.5±25.1	65.7±30.6	71.7±25.9	64.4±38.0	54.7±32.5	60.7±36.2
Embryo quality[b]	73.6±29.8	73.8±34.2	73.7±30.4	72.5±35.9	72.1±35.2	72.3±35.5

Note: Values are mean (±SD) percentages of two-pronuclei oocytes per injected oocyte for fertilization rate and percentages of type A and B embryos (see text) per two-pronuclei oocyte for embryo quality.

[A] The two origin groups differed significantly (P<.001), and the difference between the two morphology groups approached significance (P=.058). No interaction was observed between origin and morphology (P=.532).

[B] No significant difference by origin or morphology was observed

Fertility and Sterility (2003) Vol. 79, No. 1

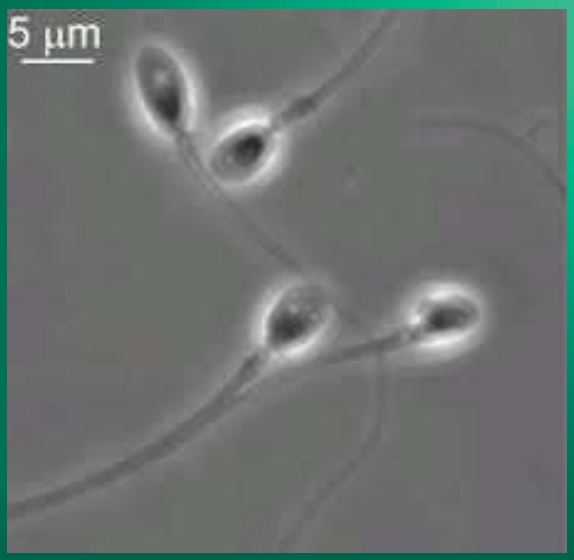
Influence of Individual Sperm

•Embryo cleavage quality did not differ between groups.

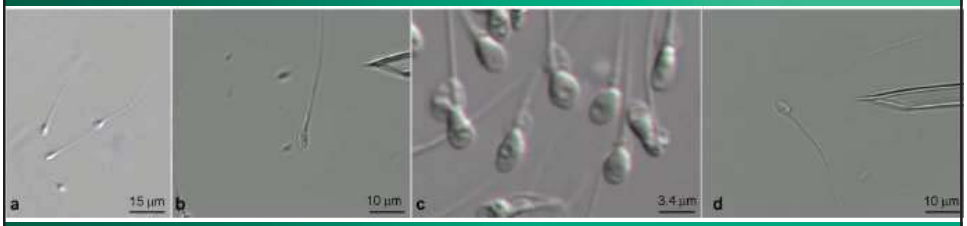
•Higher pregnancy and implantation rates were obtained with normal sperm morphology (36.7% and 18.7%) than with abnormal sperm morphology (20.2% and 9.6%)

The implantation rate was lower when only the injection an abnormal spermatozoon was possible.

Fertility and Sterility (2003) Vol. 79, No. 1



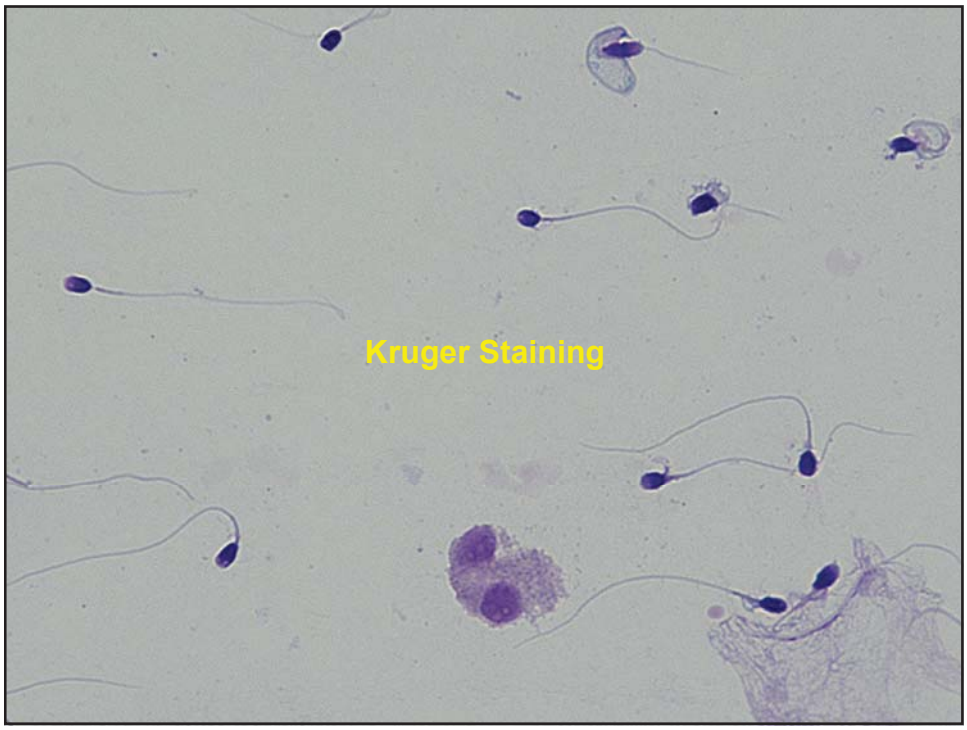
Spermatozoa observed

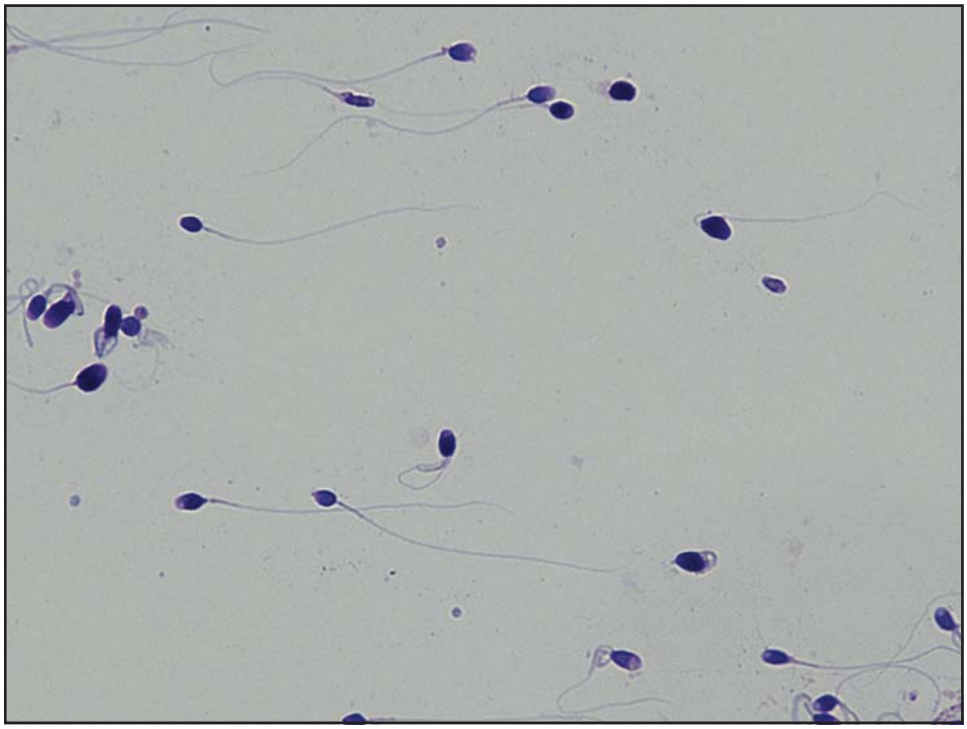


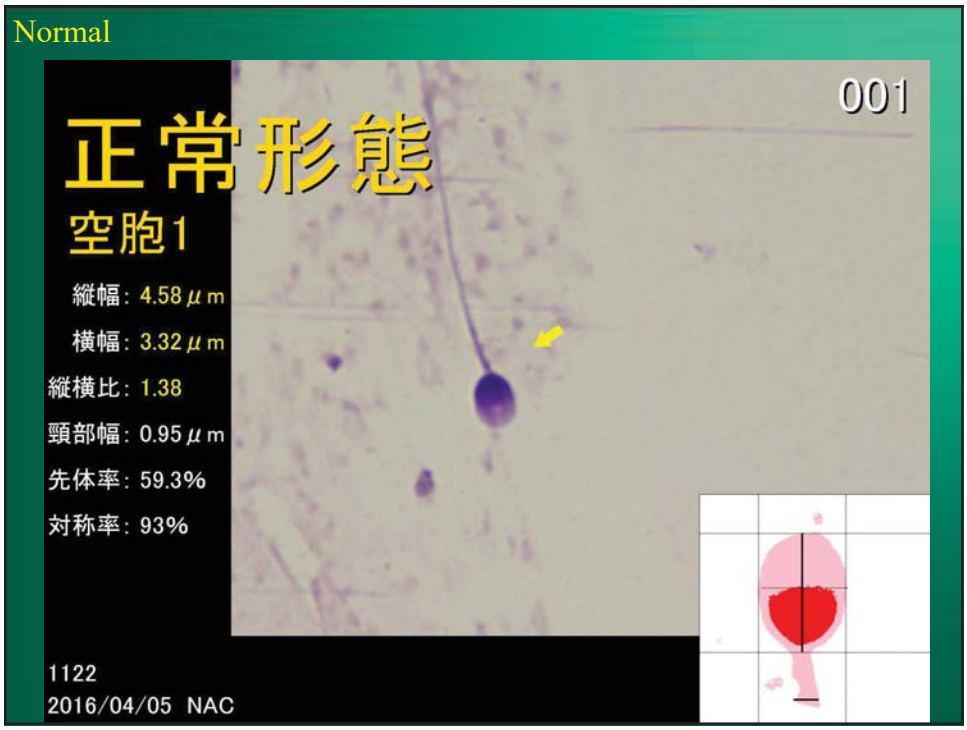
(a) Low magnification
(b-d) High magnification
(c) The shape and presence of vacuoles can be clearly observed
(d) A vacuole-free spermatozoon

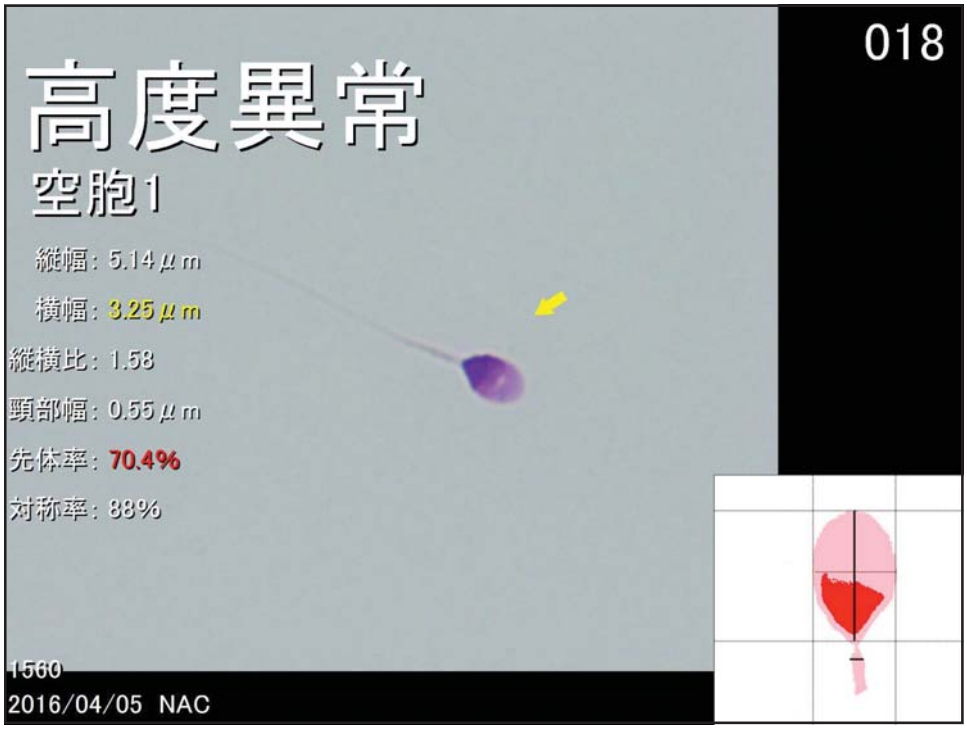
Asian Journal of Andrology (2013) Vol. 15 No. 1

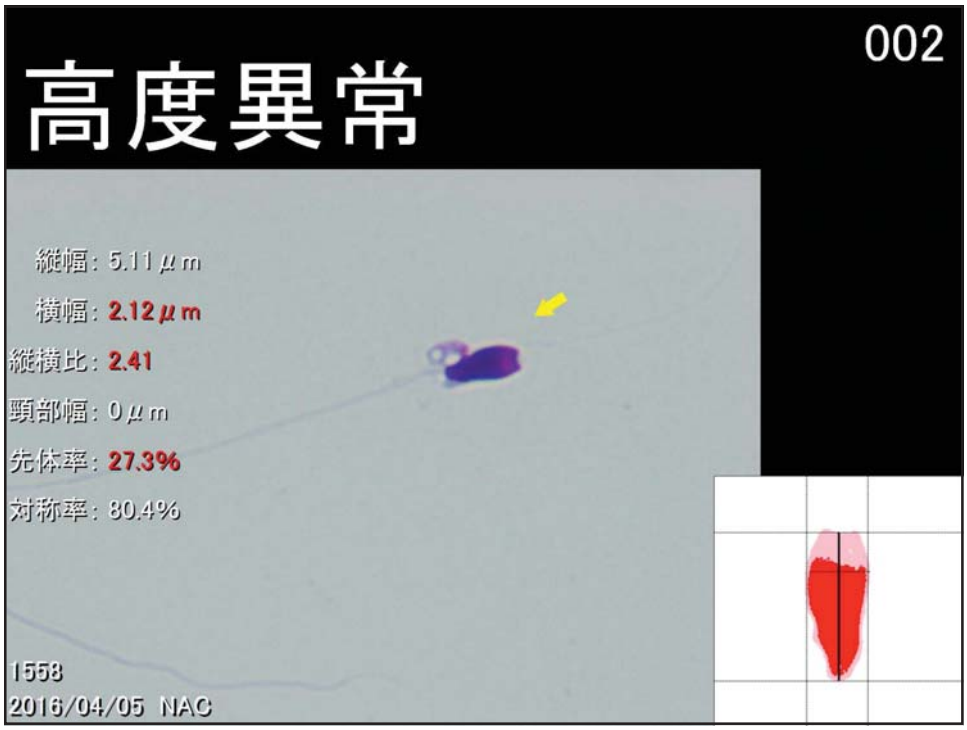
Sperm Morphology A Heterogeneous Diversity

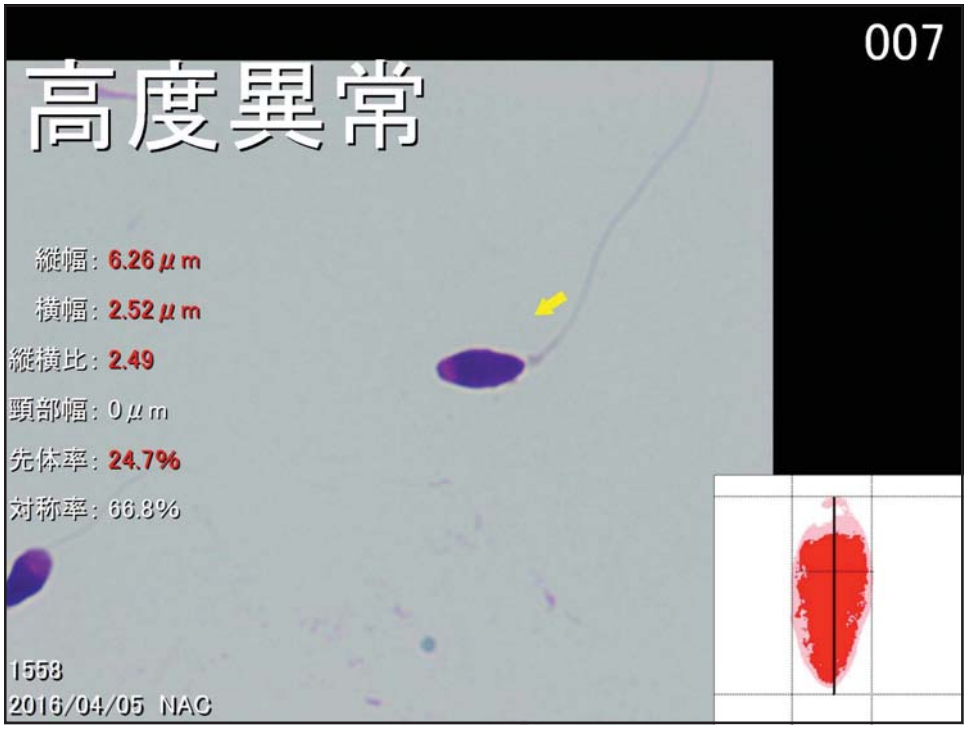

Kruger Staining

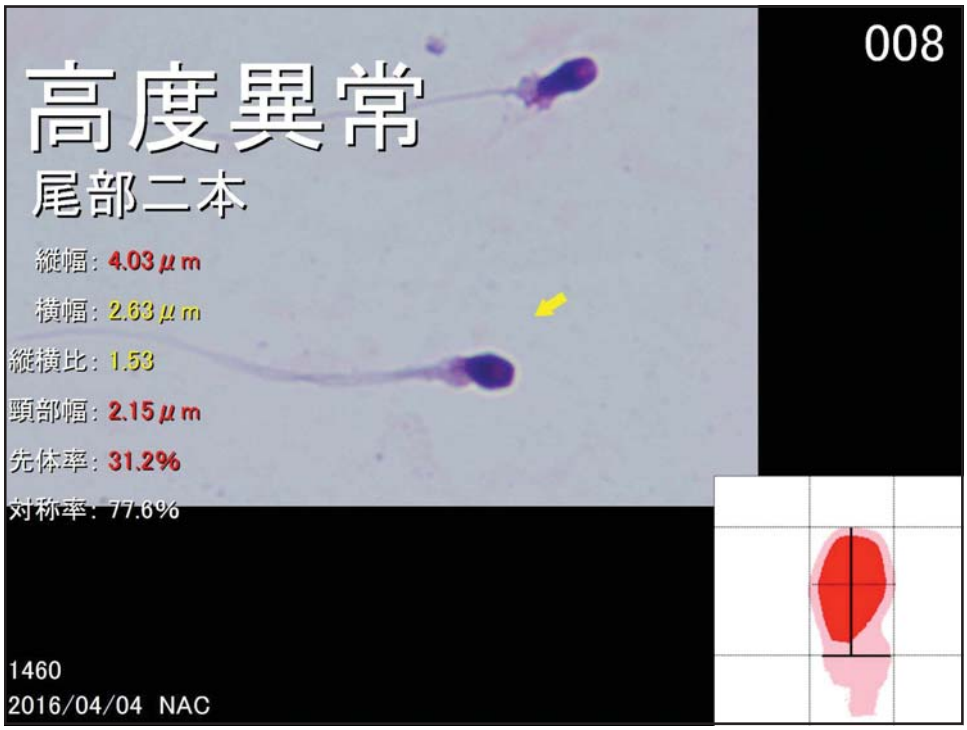


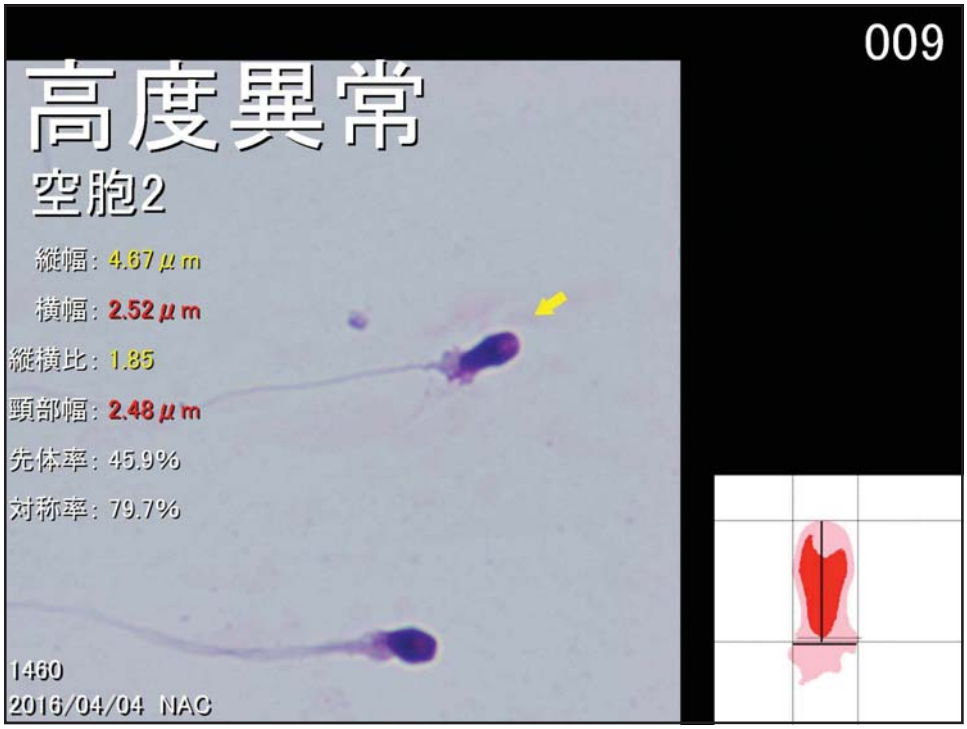

Normal
001
正常形態
空胞1
縦幅: 4.58 μm
横幅: 3.32 μm
縦横比: 1.38
頸部幅: 0.95 μm
先体率: 59.3%
対称率: 93%
1122
2016/04/05 NAC

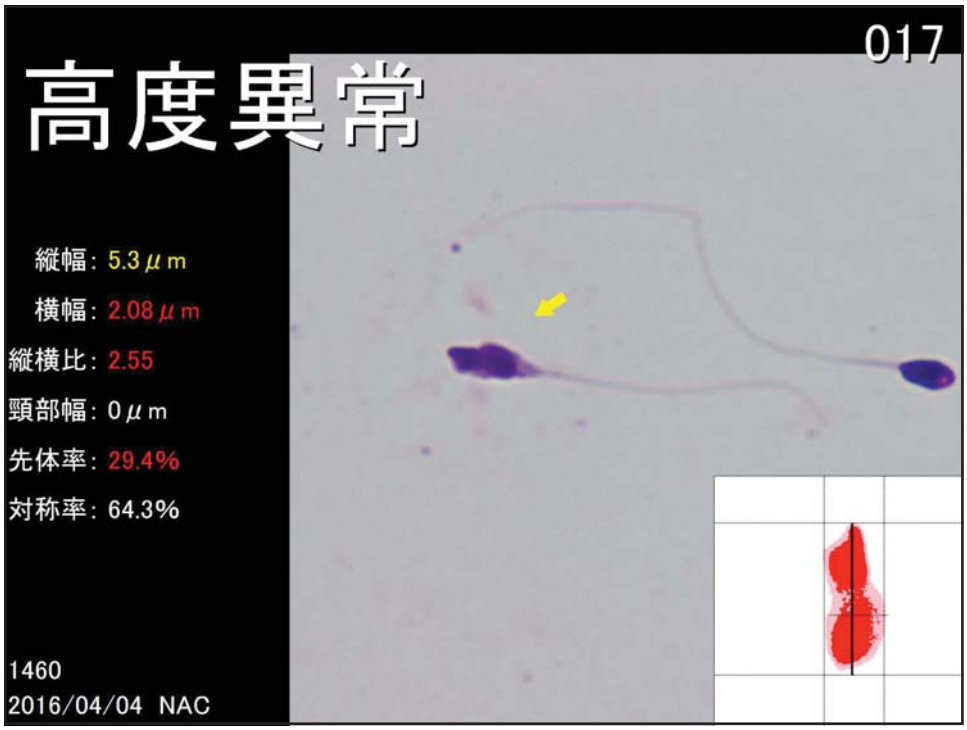

018
高度異常
空胞1
縦幅：5.14μm
横幅：3.25μm
縦横比：1.58
頸部幅：0.55μm
先体率：70.4%
対称率：88%
1560
2016/04/05 NAC

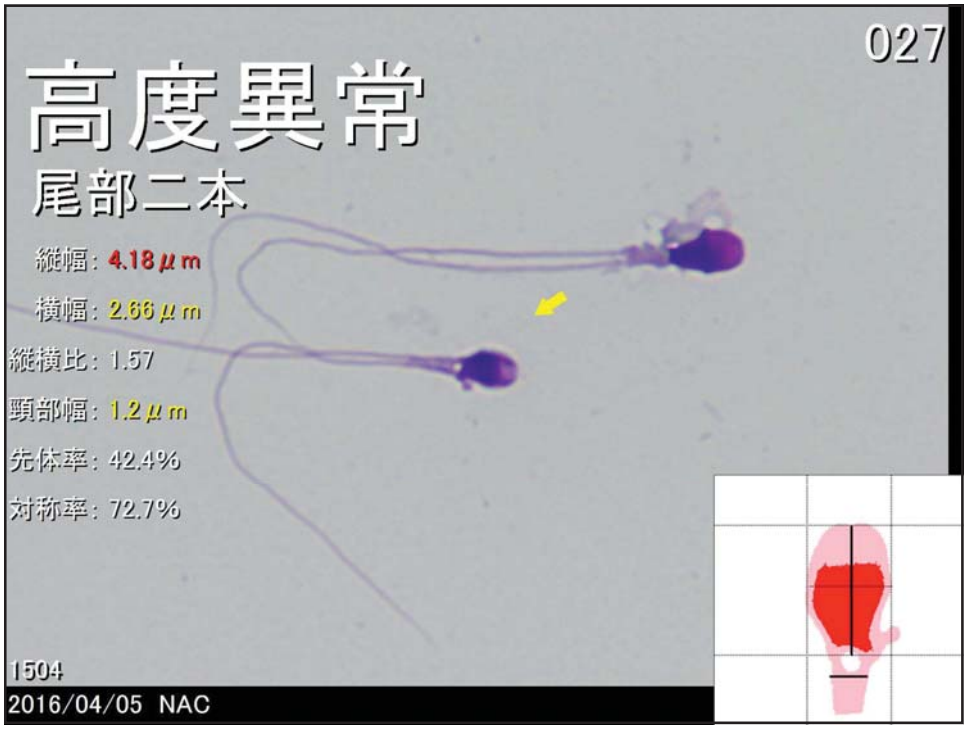

002
高度異常
縦幅：5.11μm
横幅：2.12μm
縦横比：2.41
頸部幅：0μm
先体率：27.3%
対称率：80.4%
1558
2016/04/05 NAC

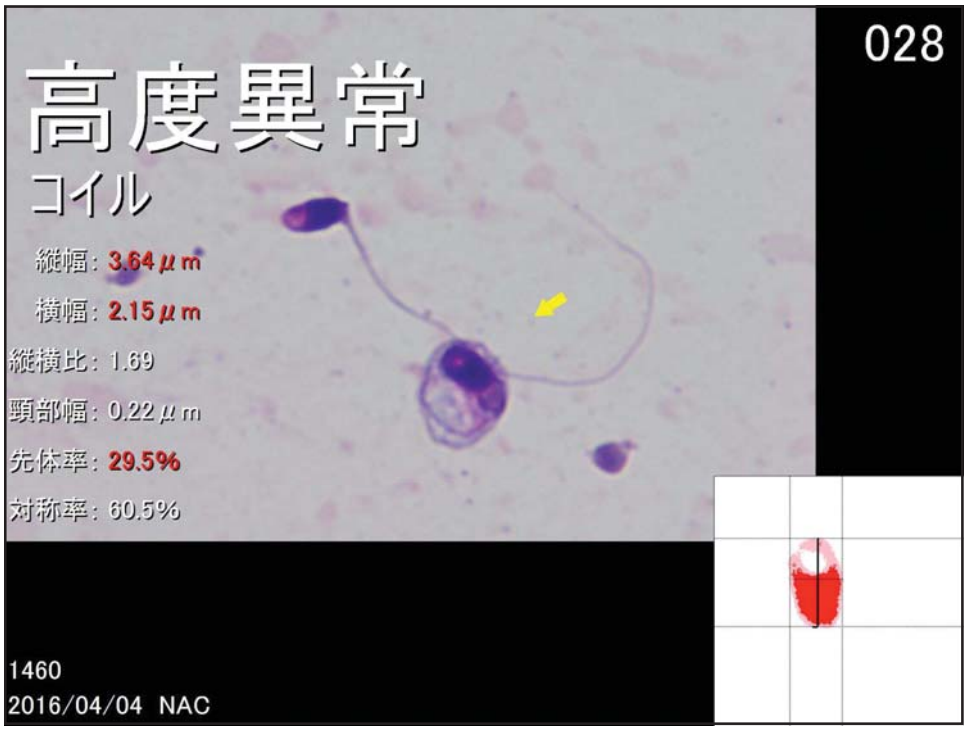

007
高度異常
縦幅: 6.26 μm
横幅: 2.52 μm
縦横比: 2.49
頸部幅: 0 μm
先体率: 24.7%
対称率: 66.8%
1558
2016/04/05 NAC

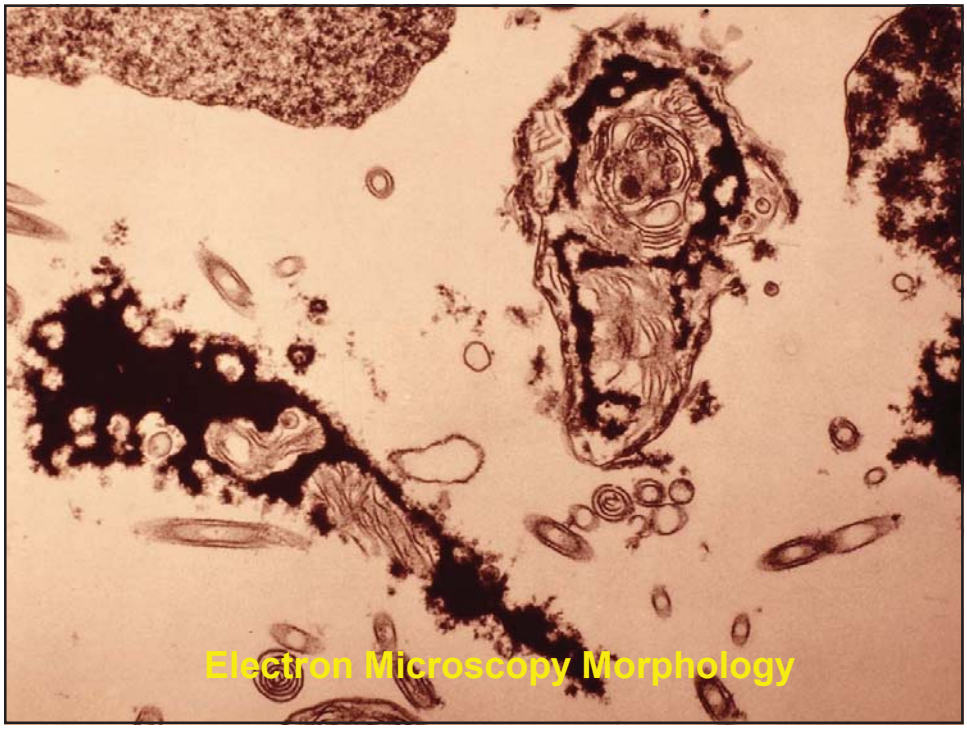

008
高度異常
尾部二本
縦幅: 4.03 μm
横幅: 2.63 μm
縦横比: 1.53
頸部幅: 2.15 μm
先体率: 31.2%
対称率: 77.6%
1460
2016/04/04 NAC

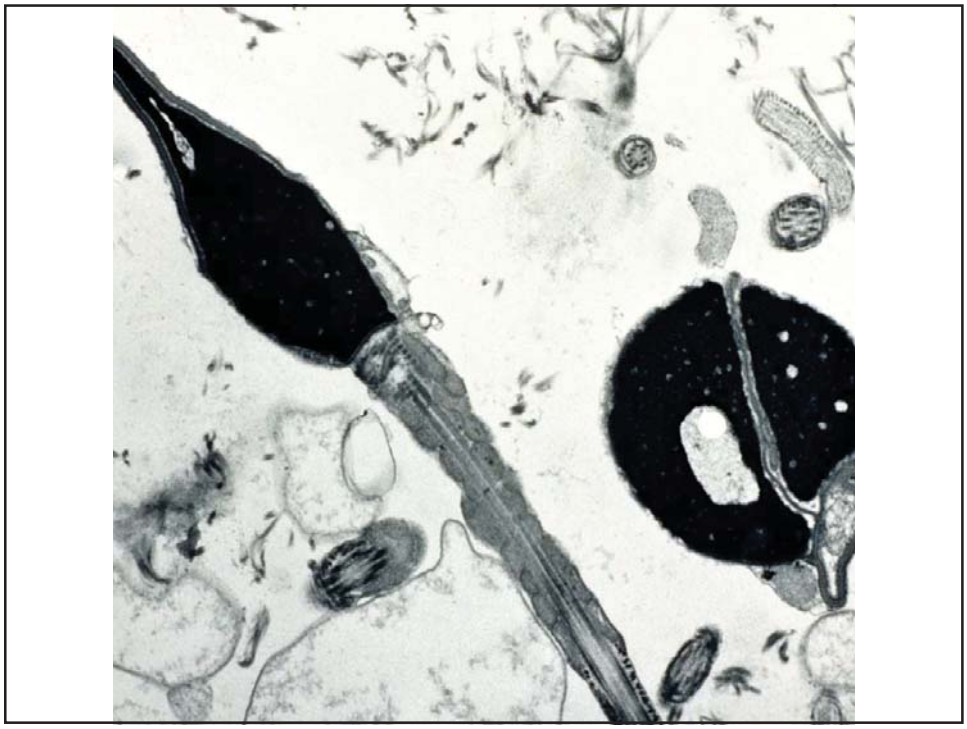

009
高度異常
空胞2
縦幅: 4.67 μm
横幅: 2.52 μm
縦横比: 1.85
頸部幅: 2.48 μm
先体率: 45.9%
対称率: 79.7%
1460
2016/04/04 NAC

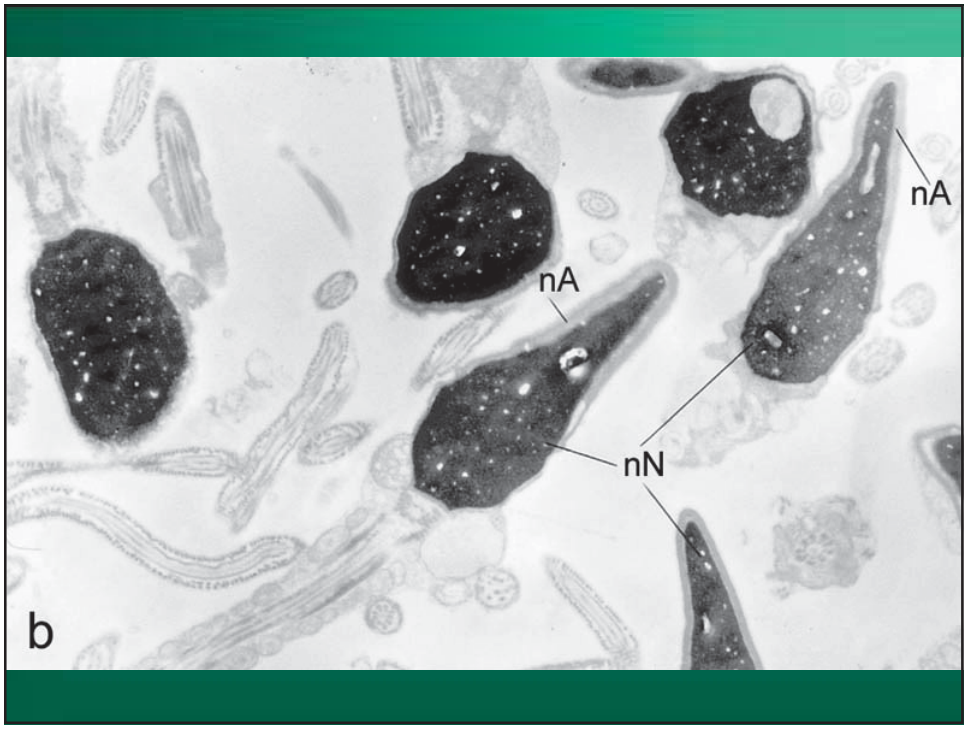

017
高度異常
縦幅: 5.3 μm
横幅: 2.08 μm
縦横比: 2.55
頸部幅: 0 μm
先体率: 29.4%
対称率: 64.3%
1460
2016/04/04 NAC

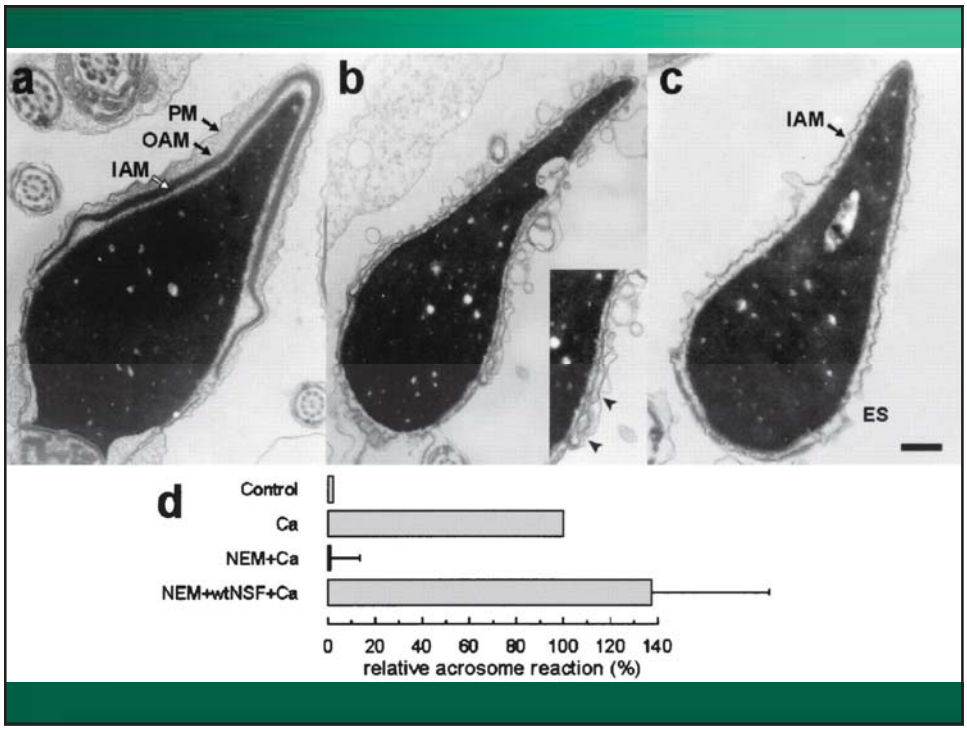

027
高度異常
尾部二本
縦幅：4.18μm
横幅：2.66μm
縦横比：1.57
頸部幅：1.2μm
先体率：42.4%
対称率：72.7%
1504
2016/04/05 NAC


028
高度異常
コイル
縦幅：3.64μm
横幅：2.15μm
縦横比：1.69
頸部幅：0.22μm
先体率：29.5%
対称率：60.5%
1460
2016/04/04 NAC

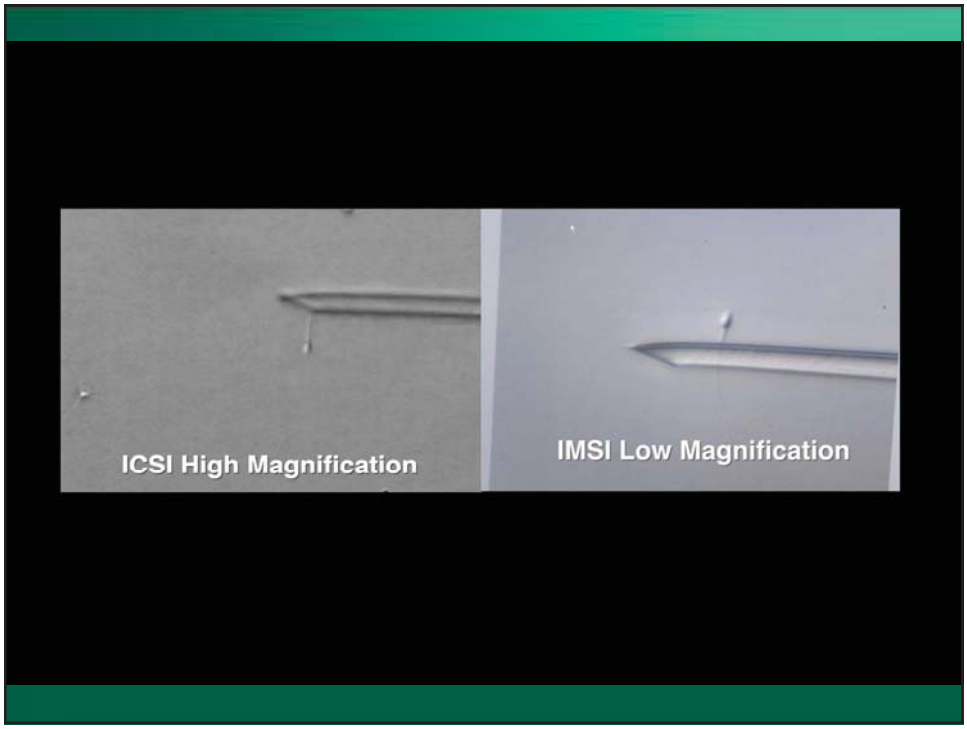

Electron Microscopy Morphology




nA
nA
nN
b


a
PM
OAM
IAM
b
c
IAM
ES
d
Control
Ca
NEM+Ca
NEM+wtNSF+Ca
0 20 40 60 80 100 120 140
relative acrosome reaction (%)


ICSI High Magnification
IMSI High Magnification
ICSI Low Magnification
IMSI Low Magnification



Intracytoplasmic injection of morphologically selected spermatozoa (IMSI) improves outcome after assisted reproduction by deselecting physiologically poor quality spermatozoa

Martin Wilding · Gianfranco Coppola · Loredana di Matteo · Antonio Palagiano · Enrico Fusco · Brian Dale

J Assist Reprod Genet (2011) Vol. 28 No. 1

Review of the IMSI Literature

	Nu of cycles		Fertilization rate (%)			Implantation rate (%)	
	IMSI	ICSI	IMSI	ICSI	P	IMSI	ICSI
Bartoov, 2003	50	50	64.5±17.5	65.5±21.5	NS	27.9±26.4	9.5±15.3
Berkovitz, 2006	80	80	67.4±20.8	69.1±22.6	NS	31.3±36.3	9.4±17.4
Antinori, 2008	227	219	94.8	94.5	NS	17.3	11.3
Knez, 2011	20	37	51.2	52.7	NS	17.1	6.8
Setti, 2011	250	250	68	73	=0.013	23.8	25.4
Oliveira, 2011	100	100	65.4±23.5	62±26.5	NS	13.6	9.8
Balaban, 2011	87	81	81.6±10.65	80.87±15	NS	28.9	19.5
Marci, 2013	51	281	77.3	80	NS	16.8	16.7

Asian Journal of Andrology (2013) Vol. 15 No. 1

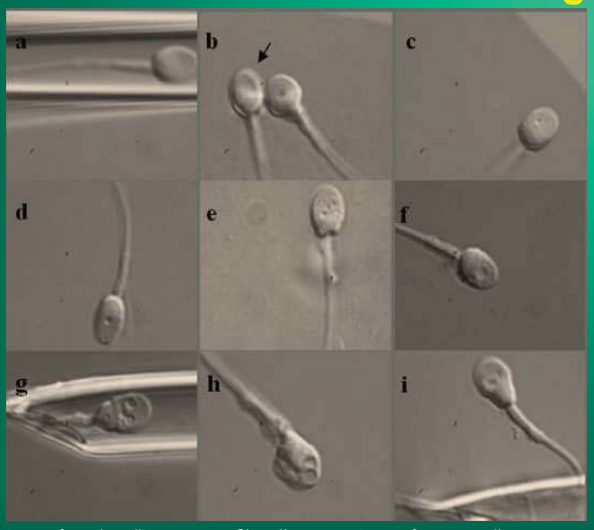
RESEARCH Open Access

The IMSI procedure improves poor embryo development in the same infertile couples with poor semen quality: A comparative prospective randomized study

Katja Knez*, Branko Zorn, Tomaz Tomazevic, Eda Vrtacnik-Bokal and Irma Virant-Klun

Reproductive Biology and Endocrinology (2011) Vol. 9 No.1

Classification of spermatozoa selected at 6,000 x magnification into 3 different categories



- Class I – spermatozoa of good quality Class II – spermatozoa of worse quality
- Class III – spermatozoa of poor quality
- Legend: a,b,c – spermatozoa of Class I; d,e,f – spermatozoa of class II; g,h,I – spermatozoa of Class III

Reproductive Biology and Endocrinology (2011) Vol. 9 No.1

human reproduction

ORIGINAL ARTICLE *Embryology*

Does intracytoplasmic morphologically selected sperm injection improve embryo development? A randomized sibling-oocyte study

A. De Vos*, H. Van de Velde, G. Bocken, G. Eylenbosch, N. Franceus, G. Meersdom, S. Tistaert, A. Vankelecom, H. Tournaye, and G. Verheyen

Centre for Reproductive Medicine, Universitair Ziekenhuis Brussel, Laarbeeklaan 101, B-1090 Brussels, Belgium

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Submitted on September 27, 2012; resubmitted on October 28, 2012; accepted on November 26, 2012

Human Reproduction (2013) Vol. 28 No.3

Does intracytoplasmic morphologically selected sperm injection improve embryo development? A randomized sibling-oocyte study

- All couples were enrolled for ICSI because of oligo-astheno-teratozoospermia
- The present cohort of single embryo transfers in a comparable patient population does not support an improved clinical outcome with IMSI compared with ICSI

Human Reproduction (2013) Vol. 28 No.3

Fertilization and embryo development IMSI VS ICSI

	IMSI (n = 1557)	ICSI (n = 1548)	P-value, paired t-test
Fertilization (% per injected MII oocyte) Embryo quality	79.1±1.2	77.3±1.3	0.220
Day 2 (% top quality embryos/2-PN)	35.0±1.8	38.5±2.0	0.047
Day 3 (% top quality embryos/2-PN)	37.0±1.9	38.5±1.9	0.362
Day 5 (% top quality blastocysts/2-PN)	9.8±1.3	11.4±1.6	0.428
Day 2 (% total blastocyst formation/2-PN)	39.9±2.3	43.4±2.6	0.247

Human Reproduction (2013) Vol. 28 No.3

Clinical Outcomes IMSI VS ICSI

	IMSI	ICSI
Mean female age	30.7±3.5	30.9±3.3
Mean number of embryos replaced	1.2±0.4	1.2±0.4
Number of positive hCG (% per ET)	55(44.0)[a]	68(48.9)[a]
Clinical pregnancy [b] (% per ET)	43(34.4)[a]	51(36.7)[a]
Implantation rate per embryo transferred (%)	30.3	32.2

Human Reproduction (2013) Vol. 28 No.3

Does intracytoplasmic morphologically selected sperm injection improve embryo development? A randomized sibling-oocyte study

- The present sibling-oocyte study compares conventional ICSI with a sperm selection method using higher magnification (IMSI). No difference neither in oocyte fertilization rate, nor in embryo quality was observed.
- The clinical pregnancy rate and the implantation rate per embryo transferred was similar for IMSI-only and ICSI-only transfers.
- The present data do not support any benefit of IMSI in a non-selected population as tested here, with fresh ejaculated sperm containing ≥ 1 million/ml.

Human Reproduction (2013) Vol. 28 No.3

Does intracytoplasmic morphologically selected sperm injection improve embryo development? A randomized sibling-oocyte study

- Those without vacuoles on the one hand and those with large vacuoles on the other hand are very rare in patients (respectively, 2.6 and 4.6%).
- Prevalence of small vacuoles found in normal shaped spermatozoa was extremely high (92.8% in patients, comparable with 95.8% in fertile donors.
- These should be considered as a common feature in normal human sperm and not associated with pathology or DNA damage

Human Reproduction (2013) Vol. 28 No.3

Does intracytoplasmic morphologically selected sperm injection improve embryo development? A randomized sibling-oocyte study

- While Vanderwalmen et al. (2008) found that blastocyst formation is severely affected by the presence of large vacuoles and/ or abnormal head shapes, the present study only shows that blastocyst formation was not jeopardized when using either Grade III or IV spermatozoa
- Beyond blastocyst formation the implantation rate per embryo transferred was not affected
- No significant differences were observed between conventional ICSI and IMSI

Human Reproduction (2013) Vol. 28 No.3

Does intracytoplasmic morphologically selected sperm injection improve embryo development? A randomized sibling-oocyte study

- The proportion of spermatozoa with vacuoles within semen samples hardly compromised the selection of suitable spermatozoa for oocyte injection
- The use of so-called ‘second-best’ spermatozoa had no major implications on fertilization and blastocyst formation.
- IMSI and conventional ICSI were comparable in terms of oocyte fertilization rate and embryo development up to the blastocyst stage.
- Clinical outcome was similar for IMSI-only and ICSI- only transfers

Human Reproduction (2013) Vol. 28 No.3


J Assist Reprod Genet (2016) 33:349–355
DOI 10.1007/s10815-015-0645-5

GAMETE BIOLOGY

Intracytoplasmic morphologically selected sperm injection (IMSI) does not improve outcome in patients with two successive IVF-ICSI failures

N. Gatimel[1,2] · J. Parinaud[1,2] · R. D. Leandri[1,2]

J Assist Reprod Genet (2016) Vol.33 No.1

Intracytoplasmic morphologically selected sperm injection (IMSI) does not improve outcome in patients with two successive IVF-ICSI failures

- Retrospective comparative study between IMSI and conventional ICSI during a third ART attempt.
- Two hundred sixteen couples with two previous ICSI failures were studied between February 2010 and June 2014.
- IMSI did not significantly improve the clinical outcomes compared with ICSI, either for implantation (12 vs 10%), clinical pregnancy (23 vs 21%), or live birth rates (20 vs 19%)

J Assist Reprod Genet (2016) Vol.33 No.1

Results of ICSI and IMSI cycles after two previous ICSI failures

	ICSI group	IMSI group	Statistical comparison
Number of cycles	127	89	
Ovarian stimulation protocol			
Long agonist	31 (24%)	22(25%)	NS
Antagonist	96 (76%)	67(75%)	
Total injected FSH units	2085±1021	2010±833	NS
No. follicles ≥15mm (at last US monitoring)	7.3±2.5	7.5±2.9	NS
No. metaphase II oocytes	6.9±3.1	8.1±3.6	P<0.01
Fertilization rate (%)	61±26	54±24	P<0.05
No. embryos obtained	4.3±2.6	4.5±2.8	NS
% of good morphology embryos (score 3 and 4, Giorgetti classification)	32±30	36±35	NS
No. embryo transfers	119(94%)	86 (97%)	NS
No. transferred embryos	2.3±0.8	2.3±0.8	NS
Clinical pregnancy rate per oocyte retrieval	28% (35/119)	27% (24/89)	NS
Implantation rate	10% (28/270)	12% (23/194)	NS
Ongoing pregnancy rate	21% (25/119)	23% (20/86)	NS
Delivery rate per embryo transfer	19% (23/119)	20% (17/86)	NS
Cycles with frozen embryo (% per transfer)	19% (23/119)	22% (19/86)	NS
Number of frozen embryos per freezing	2.3±1.0	1.9±0.7	NS

J Assist Reprod Genet (2016) Vol.33 No.1

Characteristics of studies assessing the results of IMSI after several ICSI failures

Authors	Study Design	Study Population	Number of previous ICSI failures	Implantation rate (%)
Bartoov et al.	Retrospective study	62 couples with altered semen analysis, at least two ICSI failures; comparison with 50 couples paired according to number of previous ICSI failures	4.1	ICSI 27.9 ICSI 9.5 P<0.01
Berkovitz et al.	Retrospective study	80 couples with at least 2 ICSI failure 3.9	3.9	IMSI 31.3 ICSI 9.4 P<0.05
Antinori et al.	RCT	OAT 139 couples (62 ICSI, 77 IMSI)	≥2 (in subgroup C)	
Knez et al.	RCT	57 couples (37 ICSI, 20 IMSI) male infertility with altered sperm parameters and arrested embryos after prolonged 5-day embryo culture in previous ICSI cycles	Not specified	IMSI 17.1 ICSI 6.8 NS (low number of couples)
El Khattabi et al.	Prospective non-randomized observational study	220 couples (90 IMSI, 130 ICSI)	2 or more previous ICSI failures	IMSI 16.7 ICSI 16.1 NS
Klement et al.	Propective non-randomized observational study	449 couples male infertility factor (127 IMSI, 322 ICSI)	One ICSI failure	

J Assist Reprod Genet (2016) Vol.33 No.1

Intracytoplasmic morphologically selected sperm injection (IMSI) does not improve outcome in patients with two successive IVF-ICSI failures

- IMSI does not improve the morphology of early embryos.
- The way in which conventional ICSI is performed: accuracy of sperm selection and particularly the magnification used: X200 or X400 (some abnormalities that are not visible at X200 might be detected at magnification X400
- Benefit of IMSI was enhanced in the case of severe morphological alterations
- IMSI does not improve clinical outcomes in couples with two previous ICSI failures

J Assist Reprod Genet (2016) Vol.33 No.1

Twelve years of MSOME and IMSI: a review

Amanda Souza Setti [a,b], Daniela Paes de Almeida Ferreira Braga [a,b], Assumpto Iaconelli Jr [a,b], Tsutomu Aoki [c], Edson Borges Jr [a,b,*]

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[*] Corresponding author. *E-mail address:* edson@fertility.com.br (E Borges).

Amanda S. Setti obtained her BSc degree in 2005 at the University of Santo Amaro (UNISA) and specialized in human assisted reproduction in 2007 at the Sapientiae Institute. At present, she is a scientific researcher of Fertility – Centro de Fertilização Assistida and Instituto Sapientiae and a Master's degree student at Faculdade de Ciências Médicas da Santa Casa de São Paulo in São Paulo, Brazil.

Reproductive BioMedicine Online (2013) Vol.27 No. 1

Twelve years of MSOME and IMSI: a review

- Determine the proportion of spermatozoa, otherwise selected for ICSI, that had morphological abnormalities.
- The results showed that 64.8% of the analyzed spermatozoa were deselected after digital analysis.
- Reasons for rejection of spermatozoa included poor morphology, the presence of multiple vacuoles, the presence of vacuoles that occupied >4% of the nuclear area and poor morphology of the mid-piece.
- High magnification reveals morphological features not visible using the conventional ICSI procedure

Reproductive BioMedicine Online (2013) Vol.27 No. 1

IMSI drawbacks

- Sperm selection under high magnification is performed using a glass-bottomed dish that is appropriate for Nomarski microscopy.
- On the other hand, the ICSI procedure is performed with a plastic-bottomed dish that works with Hoffman modulation contrast.
- Therefore it is important to emphasize that switching between the two systems requires additional time, delaying the injection procedure.

Reproductive BioMedicine Online (2013) Vol.27 No. 1

Twelve years of MSOME and IMSI: a review

- Ai et al. (2010) investigates whether IMSI with testicular spermatozoa improves the clinical outcome in patients with azoospermia. A total of 66 azoospermic patients were provided with conventional ICSI and 39 with IMSI.
- The results showed no difference between groups regarding pregnancy rates

Reproductive BioMedicine Online (2013) Vol.27 No. 1

Twelve years of MSOME and IMSI: a review

- The results are controversial. These conflicting results might have occurred due to differences in inclusion criteria, stimulation protocols, seminal and oocyte qualities and many other confounding variables.
- SJS, “The conflict could be possibly that technicians are more observant of sperm morphology with IMSI, but if they are super observant with ICSI, results could be the same with 400X as with 600X to 1500X”.

Reproductive BioMedicine Online (2013) Vol.27 No. 1

Is intracytoplasmic morphologically selected sperm injection (IMSI) beneficial in the first ART cycle? A multicentric randomized controlled trial

[1,2]R. D. Leandri, [1,2]A. Gachet, [3]J. Pfeffer, [4]C. Celebi, [5]N. Rives, [6]F. Carre-Pigeon, [7]O. Kulski, [8]V. Mitchell and [1,2]J. Parinaud

[1]Médecine de la Reproduction, CHU de Toulouse, Toulouse, France, [2]EA 3694, Groupe de Recherche en Fertilité Humaine, Université Paul Sabatier, Toulouse, France, [3]Laboratoire ZTP, Clinique de la DHUYS, Bagnolet, France, [4]Laboratoire de Biologie de la Reproduction, CMCO Schiltigheim, CHRU de Lille, Schiltigheim, France, [5]Laboratoire de Biologie de la Reproduction-CECOS, EA 4308 « Gamétogenèse et Qualité du Gamète », CHU Hôpitaux de Rouen, Rouen, France, [6]Laboratoire de Biologie de la Reproduction-CECOS, CHU de Reims, Reims, France, [7]Laboratoire de Biologie de la Reproduction, Hôpital des 4 Villes, Sèvres, France, and [8]EA4308-Institut de la Biologie de la Reproduction, CHRU de Lille, Lille, France

Andrology (2013) Vol. 1 No. 1

Is intracytoplasmic morphologically selected sperm injection (IMSI) beneficial in the first ART cycle? A multicentric randomized controlled trial

- IMSI did not provide any significant improvements in the clinical outcomes compared with ICSI neither for implantation (24% vs. 23%), nor clinical pregnancy (31% vs 33%) nor live birth rates 27% vs. 30%).
- Moreover the results of IMSI were similar to the ICSI ones whatever the degree of sperm DNA fragmentation, nuclear immaturity and sperm morphology.
- These results show that IMSI instead of ICSI has no advantage in the first ART attempts. However, this does not rule out IMSI completely and more randomized trials must be performed

Andrology (2013) Vol. 1 No. 1

Comparison of implantation rates between IMSI and ICSI according to sperm characteristics

	Total	ICSI	IMSI	Statistical Comparison
Implantation rate according DFI (%)				
<10%	24	29	18	NS
10-22%	23	21	25	NS
>23%	29	30	28	NS
Implantation rate according aniline blue staining (%)				
<10%	22	26	15	NS
10-23%	24	21	27	NS
>23%	28	30	25	NS
Implantation rate according the percentage of morphologically normal spermatozoa (%)				
<1%	17	11	23	NS
1-7%	30	30	32	NS
>7%	27	30	25	NS
Implantation rate (%) according the number of motile spermatozoa recovered after preparation (10^6)				
<0.13	30	32	26	NS
0.13-0.7	16	13	20	NS
>0.7	35	40	29	NS

Andrology (2013) Vol. 1 No. 1

Pregnancy outcomes in women with repeated implantation fialures after intracytoplasmic morphologically selected sperm injection (IMSI)

- **Results:** No statistically significant differences between the two groups were observed with regard to rates of fertilisation, implantation and pregnancy/ cycle.

Reproductive Biology and Endocrinology (2011) Vol. 9 No.1

General study population; comparison between morphologically selected sperm injection (IMSI) and conventional intracytoplasmic sperm injection (ICSI) groups

	Total		
	IMSI	ICSI	p
Fertilisation rate (%)	65.4±23.5	62±26.5	0.34
Implantation rate (%)	13.6	9.8	0.21
Pregnancy/cycle (%)	26	19	0.73

Reproductive Biology and Endocrinology (2011) Vol. 9 No.1

Cochrane Library

Cochrane Database of Systematic Reviews

Regular (ICSI) versus ultra-high magnification (IMSI) sperm selection for assisted reproduction (Review)

Teixeira DM, Barbosa MAP, Ferriani RA, Navarro PA, Raine-Fenning N, Nastri CO, Martins WP

Cochrane Library (2013) Vol. 7 No. 1

Regular (ICSI) versus ultra-high magnification (IMSI) sperm selection for assisted reproduction (Review)

- We concluded that the current evidence does not support using IMSI: there is no evidence of benefit for live birth and miscarriage, we are very uncertain of the beneficial effect of IMSI in clinical pregnancy, and there is no evidence of the effect of this intervention on congenital abnormalities.
- More studies to improve the evidence quality are necessary before recommending IMSI in clinical practice.

Cochrane Library (2013) Vol. 7 No. 1

RESEARCH Open Access

Clinical outcome after IMSI procedure in an unselected infertile population: a pilot study

Roberto Marci[1,5*], Fabien Murisier[2,3,4], Giuseppe Lo Monte[1], Ilaria Soave[1], Alain Chanson[2], Françoise Urner[2,4] and Marc Germond[2,4]

Reproductive Health (2013) Vol. 10 No. 16

Clinical outcome after IMSI procedure in an unselected infertile population: a pilot study

- **Methods:** Three hundred and thirty-two couples were analyzed: 281 couples underwent conventional ICSI procedure and 51 underwent IMSI technique.
- **Conclusions:** Our preliminary results show that the IMSI technique does not significantly improve IVF outcomes in an unselected infertile population.

Reproductive Health (2013) Vol. 10 No.

Main characteristics of the patient and clinical-laboratory outcomes in IMSI and ICSI groups

	ICSI		IMSI	P-value
	Count/medium	d.s	Count/medium	
Nº of cycles	281		51	
Women age at pickup	34,98	3,19	35,65	
Pregnancy rate (%)	30,96		33,33	0,74
Live birth rate (%)	11,39		13,72	0,23
Ongoing pregnancy rate (%)	7,47		5,88	0,69

Reproductive Health (2013) Vol. 10 No.

FUTURE OF TESE: Stem Cells

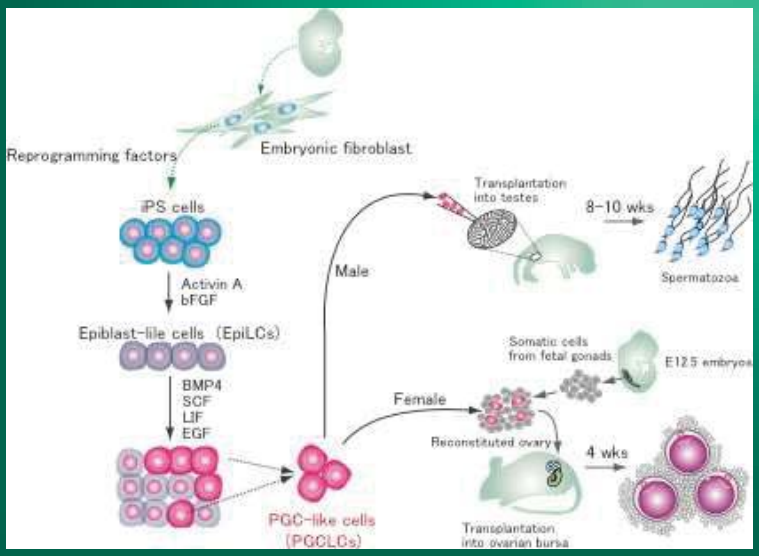
- Retrieve testis tissue prepubertal male cancer patients.
- Culture spermatogonial stem cells in multiple passages to eliminate cancer cells.
- Transfer pure stem cells back to testis.

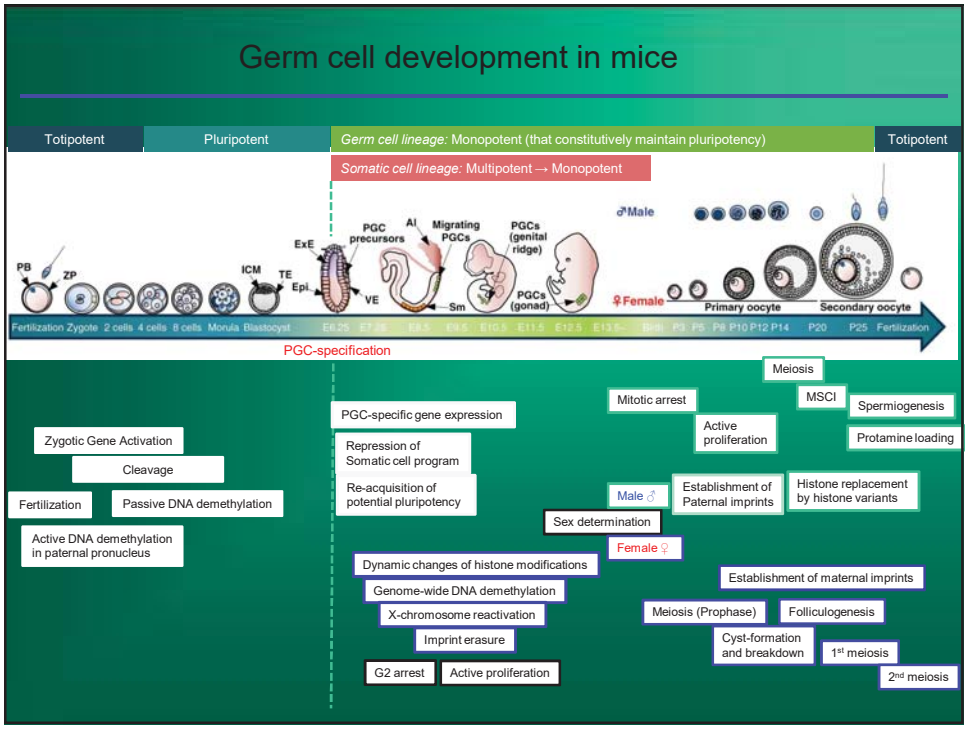
FUTURE OF TESE: Stem Cells

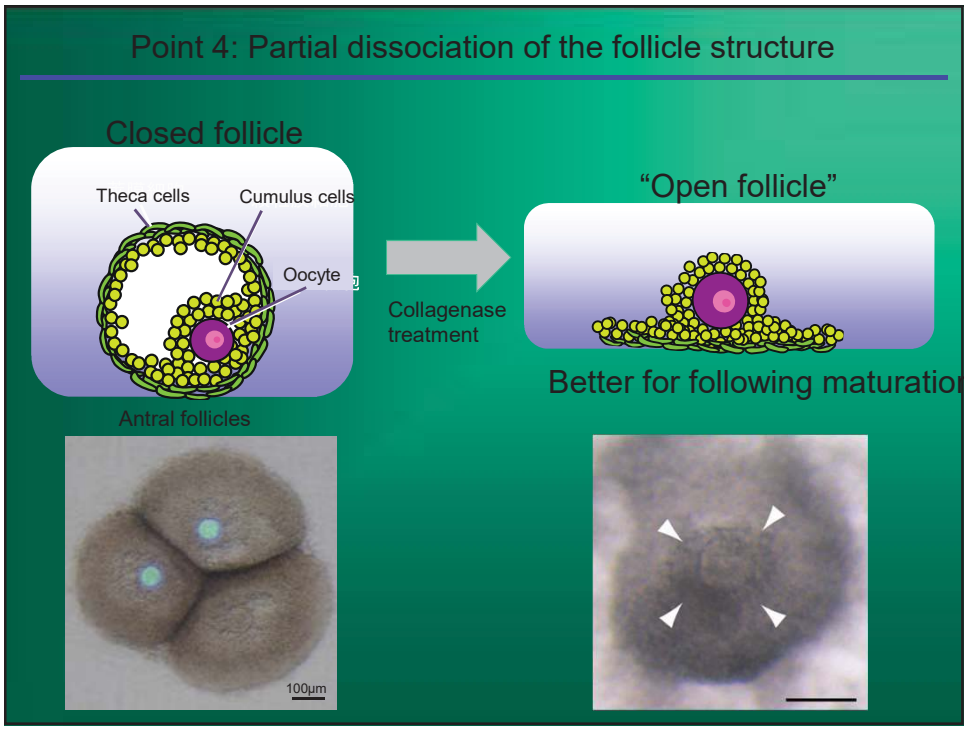
- For severe oligospermic males, retrieve testis tissue and culture spermatogonial stem cells to exponentially increase number.
- Then transfer back to testis via rete testis to increase sperm count.

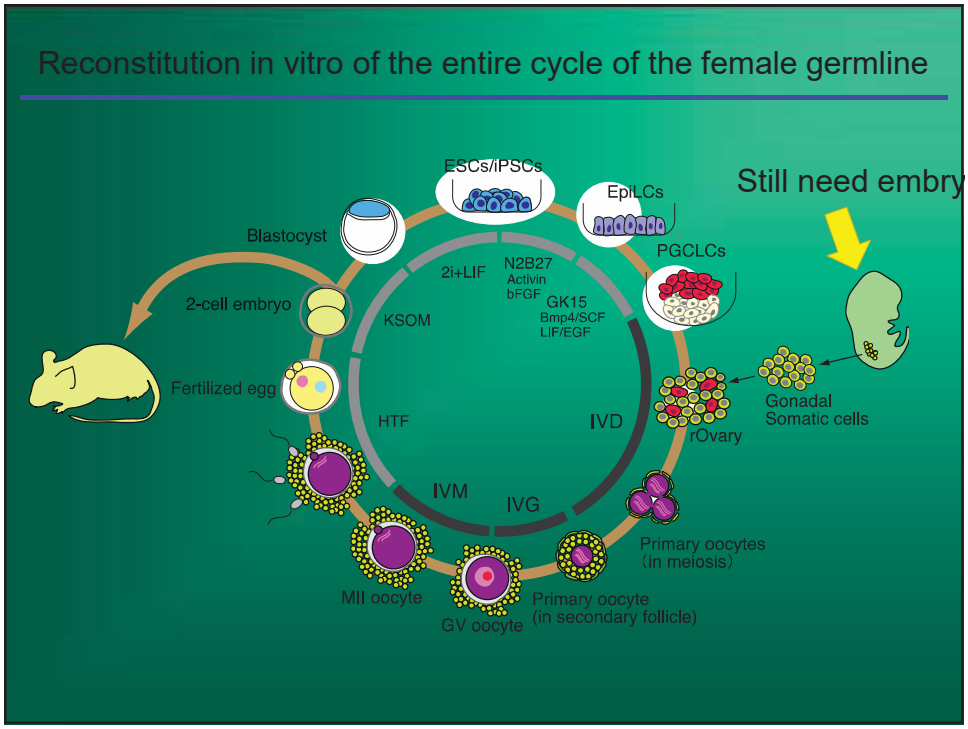
SPERM AND EGGS FROM SKIN CELLS

Derivation of artificial gametes from iPS cells in mouse




Germ cell development in mice
Totipotent
Pluripotent
Germ cell lineage: Monopotent (that constitutively maintain pluripotency)
Totipotent
Somatic cell lineage: Multipotent → Monopotent
PB
ZP
ICM
TE
Epi
ExE
VE
PGC precursors
Al
Migrating PGCs
Sm
PGCs (genital ridge)
PGCs (gonad)
♂Male
♀Female
Primary oocyte
Secondary oocyte
Fertilization Zygote 2 cells 4 cells 8 cells Morula Blastocyst
P20
P25 Fertilization
PGC-specification
Meiosis
MSCI
Spermiogenesis
Mitotic arrest
Active proliferation
Protamine loading
PGC-specific gene expression
Repression of Somatic cell program
Re-acquisition of potential pluripotency
Zygotic Gene Activation
Cleavage
Fertilization
Passive DNA demethylation
Active DNA demethylation in paternal pronucleus
Male ♂
Establishment of Paternal imprints
Histone replacement by histone variants
Sex determination
Female ♀
Dynamic changes of histone modifications
Genome-wide DNA demethylation
X-chromosome reactivation
Imprint erasure
G2 arrest
Active proliferation
Establishment of maternal imprints
Meiosis (Prophase)
Folliculogenesis
Cyst-formation and breakdown
1st meiosis
2nd meiosis


Point 4: Partial dissociation of the follicle structure
Closed follicle
Theca cells
Cumulus cells
Oocyte
Antral follicles
Collagenase treatment
"Open follicle"
Better for following maturatio
100μm


Reconstitution in vitro of the entire cycle of the female germline
ESCs/iPSCs
EpiLCs
Still need embry
Blastocyst
PGCLCs
2i+LIF
N2B27
Activin
bFGF
GK15
Bmp4/SCF
LIF/EGF
2-cell embryo
KSOM
Fertilized egg
HTF
IVD
Gonadal
Somatic cells
rOvary
IVM
IVG
Primary oocytes
(in meiosis)
MII oocyte
Primary oocyte
(in secondary follicle)
GV oocyte

ADHERENCE COMPOUNDS IN EMBRYO TRANSFER MEDIA

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St. Jude Children's Research Center
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Fertility Associates of Memphis

St. Jude Children's Research Hospital

FERTILITY ASSOCIATES of MEMPHIS
William H. Kutteh, MD, Ph.D Raymond W. Ke, MD

Vanderbilt Medical Center

DISCLOSURES
Adherence Compounds in Embryo Transfer Media

- No conflict of Interest

LEARNING OBJECTIVES
Adherence Compounds in Reproduction

At the end of this presentation, the participant should be able to:

- Define the adherence compounds
- Discuss the recent studies in this area
- Understand the Cochran review studies
- Be able to counsel patients about adherence compounds

Adherence compounds in embryo transfer media

Background
- Early modifications of transfer media
- The transition towards HSA
- Macromolecules in media

Hyaluronic acid and reproduction

Hyaluronic acid in human IVF
- Implantation
- Pregnancy rates
- Adverse events
- Live birth

Summary

Background

- Modifications in embryo transfer media to improve implantation have been made since early days of human IVF
- Patient serum as a source of protein was commonly used in early days of human IVF
- Hypothesis that high protein levels have a beneficial effect on implantation

Feichtinger W, Kemeter P, Menezo Y. J In Vitro Fert Embryo Transf. 1986 Apr;3(2):87-92

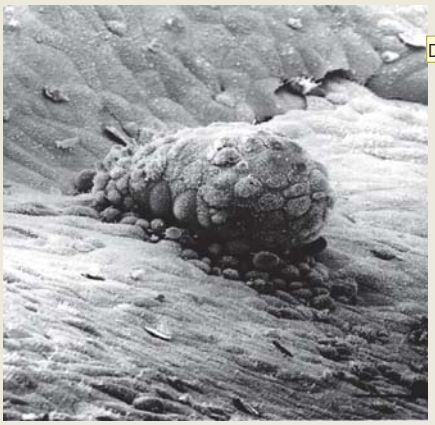
Background

- Some comparative studies found better results with human cord blood
- Human serum albumin (HSA) is used but raises concerns of infectious disease transmission.
- Synthetic serum substitute (SSS) has been used to avoid infectious disease risk

Fertil Steril. 1991 Jul;56(1):98-101.. Khan I, Staessen C, Devroey P, Van Steirteghem AC.
Fertil Steril. 1995 Dec;64(6):1162-6. Hargreaves CA, Rahman F, Cowan D, et.al
Hum Reprod. 1997 Oct;12(10):2263-6.. Laverge H, De Sutter P, Desmet R,. et al.

Background- Adherence Compounds

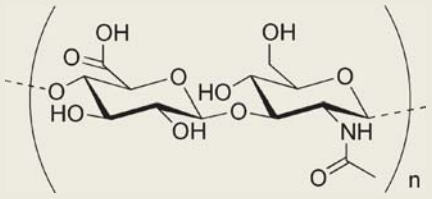
- Role for macromolecules in embryo transfer
- Use of different molecules for improvement of embryo transfer media:
  - fibrin sealant
  - Increased viscosity



Fertil Steril. 1989 Oct;52(4):680-2. Menezo Y[1], Arnal F, Humeau C, et al.
Hum Reprod. 1992 Jul;7(6):890-3.. Feichtinger W[1], Strohmer H, Radner KM, et al.

Structure of Hyaluronic Acid

- Chemical structure first determined by Weissman and Meyer in 1954
- Anionic, non-sulfated glycosaminoglycan
- Found in almost all vertebrate organs, widely distributed throughout
  - Connective tissue
  - Epithelial tissue
  - Neural tissues



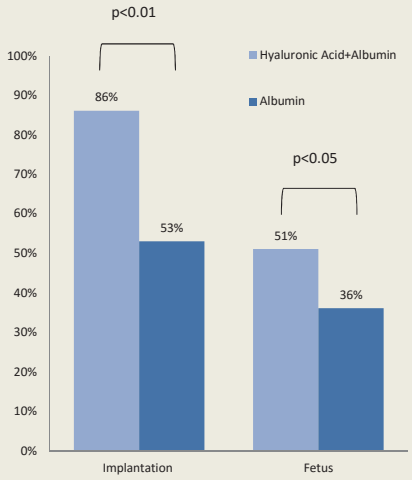
Weissman B, Meyer K The structure of hyalobiuronic acid and of hyaluronic acid from ombilical cord. J AM Chem Soc . 1954;27: 1753–1757.

Hyaluronic acid and reproduction

- Sperm function
  - Huszar et al. 2007; Worrilow et al. 2013
- Follicle development
  - Babayan et al., 2008
- Effects on pre-implantation development
  - Gardner et al. 1999; Stojkovic et al. 2002; Palasz et al. 2006
- Effects during cryopreservation
  - Stojkovic et al. 2002; Lane et al. 2003; Palasz et al. 2008
- Effects on implantation

Hyaluronan and mouse implantation

- Effect of different macromolecules on mouse embryo development
  - BSA
  - PVA
  - Dextran
  - Hyaluronan
- Effect of concentration on embryo development
- Assessment of embryo viability
- Assessment of implantation and fetal development



Gardner DK, Rodriegez-Matinez H, Lane M. Human Reproduction. 1999, 14(10):2575-80

Hyaluronan for embryo transfer

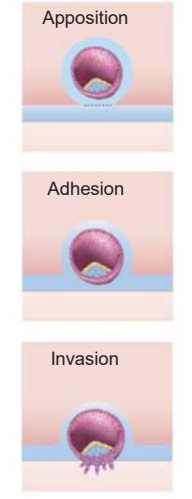
- Hyaluronic acid is the major glycosaminoglycan present in follicular, oviductal and uterine fluids

  -Lee and Ax, 1984; Suchanek et al., 1994; Rodriguez-Martinez et al.,1998

- Levels of the glycosaminoglycan Hyaluronic acid have been shown to increase significantly in the uterus at the time of implantation:
  - In mouse (Zorn et al., 1995)
  - In humans (Salamonsen et al., 2001) uterus
- Inclusion of Hyaluronic acid in embryo transfer medium has a significant beneficial effect in the mouse model (Gardner et al., 1999).

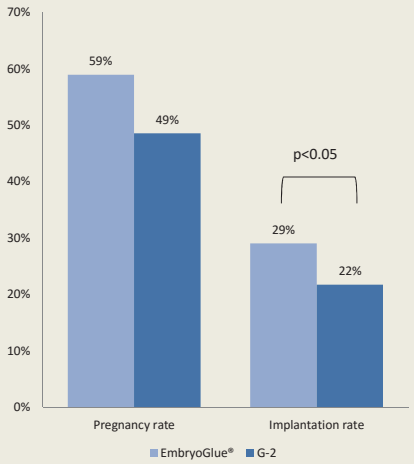
How does hyaluronic acid promote implantation?

- Improve cell-cell and cell-matrix adhesion (Turley and Moore, 1984)
- Degradation products of hyaluronan improve implantation (West *et al*., 1985)
- Improved physical diffusion with uterine secretion (Eytan *et al*., 2004)
- Receptor mediated biological function (Campbell *et al*., 1995)



Effect of HA on Human Implantation

- Randomized controlled trial presented at ASRM 2002
- Day 3 transfer
- Embryo transfer in G-2 or in EmbryoGlue
- Control and study group similar for
  - Age
  - FSH levels
  - Ratio of ICSI
  - Number of 2 PN
  - Number of embryos transfered



Schoolcraft, et al. Increased hyaluronan concentration in the embryo transfer medium results in a significant increase in human embryo implantation rate. Fertil Steril 2002; 76:55

Cochran Review 2010: Hyaluronic acid improves Implantation

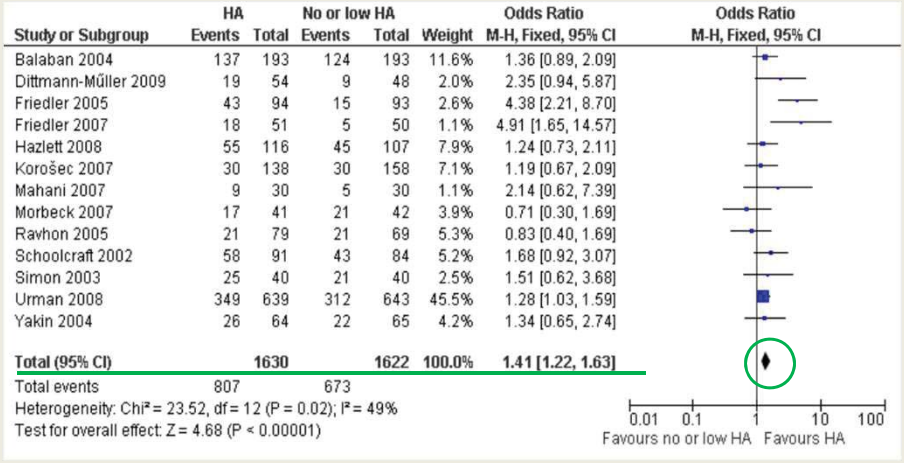
- In 2010, The Cochrane collaboration released a report:

  "Adherence compounds in embryo transfer media for assisted reproductive technologies"
- Systematic review on adherence compounds in IVF
- Included 15 randomized controlled studies involving hyaluronan
- Higher pregnancy rate (13 RCT's)
  - >3200 patients
  - Clinical pregnancy rate **50% vs 41%**

THE COCHRANE COLLABORATION

Bontekoe et al., 2010 Cochrane Database Syst Rev, Jul7;(7):CD007421

HA Improves Human Implantation



Study or Subgroup	HA Events	HA Total	No or low HA Events	No or low HA Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Balaban 2004	137	193	124	193	11.6%	1.36 [0.89, 2.09]
Dittmann-Müller 2009	19	54	9	48	2.0%	2.35 [0.94, 5.87]
Friedler 2005	43	94	15	93	2.6%	4.38 [2.21, 8.70]
Friedler 2007	18	51	5	50	1.1%	4.91 [1.65, 14.57]
Hazlett 2008	55	116	45	107	7.9%	1.24 [0.73, 2.11]
Korošec 2007	30	138	30	158	7.1%	1.19 [0.67, 2.09]
Mahani 2007	9	30	5	30	1.1%	2.14 [0.62, 7.39]
Morbeck 2007	17	41	21	42	3.9%	0.71 [0.30, 1.69]
Ravhon 2005	21	79	21	69	5.3%	0.83 [0.40, 1.69]
Schoolcraft 2002	58	91	43	84	5.2%	1.68 [0.92, 3.07]
Simon 2003	25	40	21	40	2.5%	1.51 [0.62, 3.68]
Urman 2008	349	639	312	643	45.5%	1.28 [1.03, 1.59]
Yakin 2004	26	64	22	65	4.2%	1.34 [0.65, 2.74]
Total (95% CI)		**1630**		**1622**	**100.0%**	**1.41 [1.22, 1.63]**
Total events	807		673			

Heterogeneity: Chi² = 23.52, df = 12 (P = 0.02); I² = 49%
Test for overall effect: Z = 4.68 (P < 0.00001)

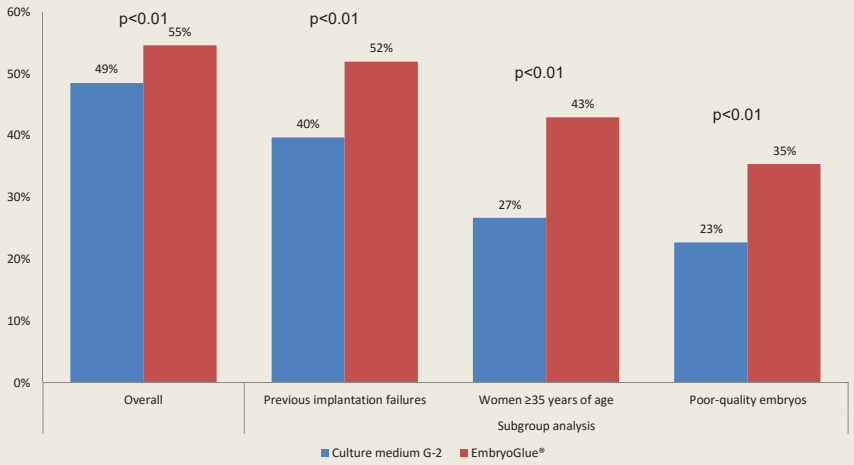
Bontekoe et al., 2010 Cochrane Database Syst Rev, Jul7;(7):CD007421

HA Benefits IR and CPR

- Purpose of study:
  - Assess impact of hyaluronic acid on implantation and clinical pregnancy rate
  - Includes cleavage-stage (825) and blastocyst (457) transfers
- Study design:
  - N=1,282 consecutive fresh embryo transfer cycles randomly allocated into two groups
  - Trial group: EmbryoGlue® 639 women
  - Control group: G-2 643 women

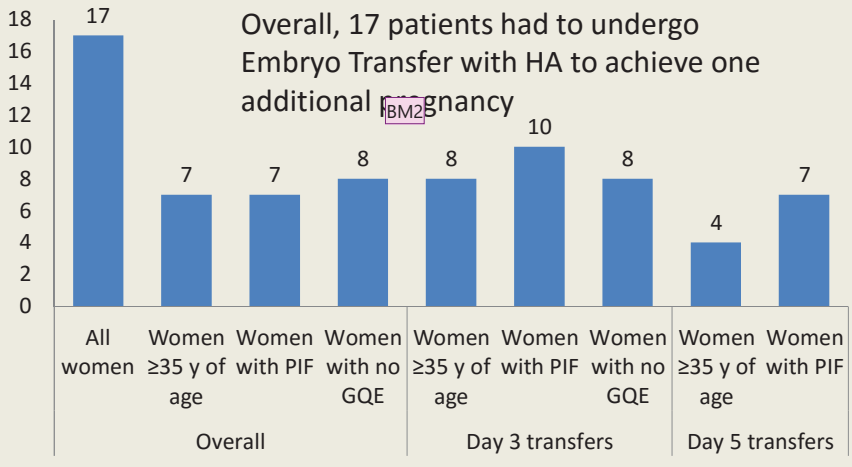
Urman et al. Fertility and Sterility 90 (3). 2008

Hyaluronic Acid Improves clinical pregnancy rate (per embryo transfer)



Urman et al. Fertility and Sterility 90 (3). 2008

Number needed to treat on clinical pregnancy rates



Overall, 17 patients had to undergo Embryo Transfer with HA to achieve one additional pregnancy

Urman et al. Fertility and Sterility 90 (3). 2008

Urman: HA Benefits IR and CPR

- Summary of Urman study:
  - Improved implantation rate
  - Improved clinical pregnancy rate
  - Improved day 3 and day 5 transfers
- Benefits seen in women with:
  - Age > 35 years
  - Prior implantation failure
  - No good quality embryos

Urman et al. Fertility and Sterility 90 (3). 2008

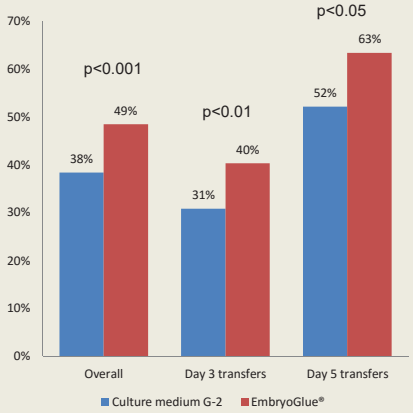
Live birth

- Follow-up study from Urman et al. 2008
- Presented at ESHRE 2011, Stockholm
- Study design:
  - 1282 fresh cycles
  - Double blinded (clinician and patient)
  - Stimulation protocol, oocyte retrieval and embryo transfer procedure described in Urman et al. 2008

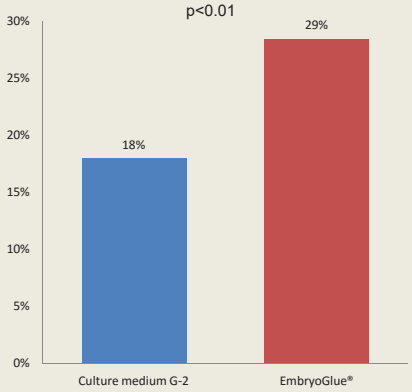
B Balaban et al., Hum Reprod 2011; 26: i24

Live birth rate : HA vs control

Delivery rate per embryo transfer



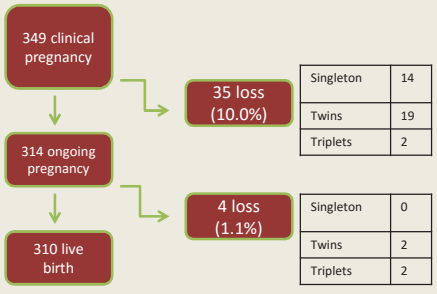
Children born per embryo transfer



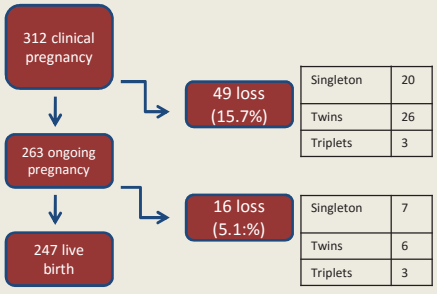
B Balaban et al., Hum Reprod 2011; 26: i24

Adverse events with HA: Biochemical Loss and Miscarriage

HA Enhriched Tranfer Medium



Control without HA



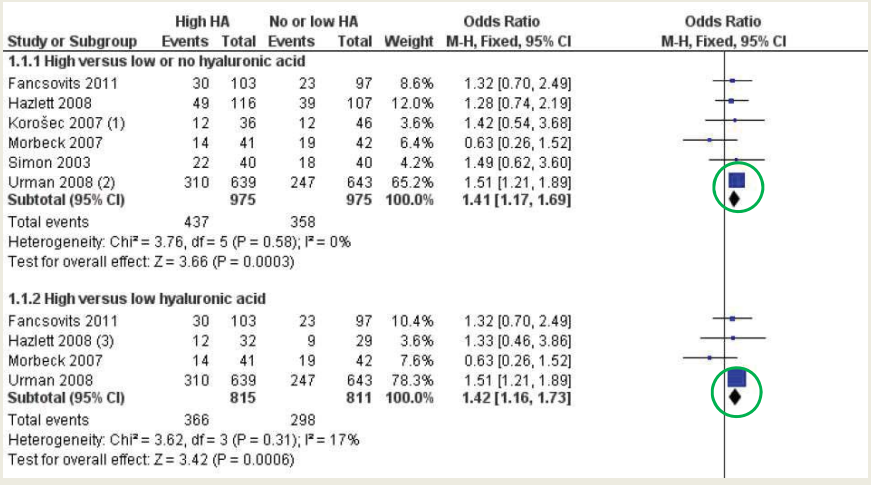
B Balaban et al., Hum Reprod 2011; 26: i24

Cochran Review Update 2014:
Hyaluronic acid improves Pregnancy and Live Birth Rate

- Functional levels of HA (0.5 mg/ml)
  - 14 studies reporting clinical pregnancy (n=3452)
  - 6 studies reporting live births (n=1950)
- Increased pregnancy rate OR 1.41 (1.17-1.69)
- Increased live birth rate OR 1.39 (1.21-1.60)
- Increased multiple pregnancy OR 1.86 (1.49-2.31)
- ”Moderate quality evidence”

Bontekoe et al., Cochrane Database Syst Rev. 2014 Feb 25;2:CD007421

Hyaluronic Acid Improves Live birth



Study or Subgroup	High HA Events	Total	No or low HA Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
1.1.1 High versus low or no hyaluronic acid						
Fancsovits 2011	30	103	23	97	8.6%	1.32 [0.70, 2.49]
Hazlett 2008	49	116	39	107	12.0%	1.28 [0.74, 2.19]
Korošec 2007 (1)	12	36	12	46	3.6%	1.42 [0.54, 3.68]
Morbeck 2007	14	41	19	42	6.4%	0.63 [0.26, 1.52]
Simon 2003	22	40	18	40	4.2%	1.49 [0.62, 3.60]
Urman 2008 (2)	310	639	247	643	65.2%	1.51 [1.21, 1.89]
Subtotal (95% CI)		**975**		**975**	**100.0%**	**1.41 [1.17, 1.69]**
Total events	437		358			
Heterogeneity: Chi² = 3.76, df = 5 (P = 0.58); I² = 0%						
Test for overall effect: Z = 3.66 (P = 0.0003)						
1.1.2 High versus low hyaluronic acid						
Fancsovits 2011	30	103	23	97	10.4%	1.32 [0.70, 2.49]
Hazlett 2008 (3)	12	32	9	29	3.6%	1.33 [0.46, 3.86]
Morbeck 2007	14	41	19	42	7.6%	0.63 [0.26, 1.52]
Urman 2008	310	639	247	643	78.3%	1.51 [1.21, 1.89]
Subtotal (95% CI)		**815**		**811**	**100.0%**	**1.42 [1.16, 1.73]**
Total events	366		298			
Heterogeneity: Chi² = 3.62, df = 3 (P = 0.31); I² = 17%						
Test for overall effect: Z = 3.42 (P = 0.0006)						

Bontekoe et al., Cochrane Database Syst Rev. 2014 Feb 25;2:CD007421

HA-Enriched ET Medium on IVF Outcome: A prospective Randomized Controlled Trial

- Prospective randomized study
- Looked at poor prognosis patients:
  - > 40 years old
  - 2 or more failed IVF cycles
  - 3 or fewer oocytes
  - Only poor quality embryos
- 581 women randomized
  - 290 HA in media
  - 291 control media

Fancovitz, P et al. Arch Gynaecol Obstet. 2015;291:1173-1179.

HA-Enriched ET Medium on IVF Outcome: A prospective Randomized Controlled Trial

	HA group		Control group		*P* value
No. of ET cycles	290		291		
Pregnancy (+hCG)	141	48.6 %	126	43.3 %	0.198
Biochemical pregnancy	18	6.2 %	12	4.1 %	0.257
Clinical pregnancy	123	42.4 %	114	39.2 %	0.427
Multiple pregnancy	28	22.8 %	37	32.5 %	0.095
Implantation	151/647	23.3 %	155/669	23.2 %	0.942
Delivery (/ET)	90	31.0 %	85	29.2 %	0.808
Abortion (/ET)	33	11.4 %	29	10.0 %	0.581
No. of newborns	111/151	73.5 %	110/155	71.0 %	0.620
Birth weight (g)[a]	3,018	±598	2,724	±698	0.001

Fancovitz, P et al. Arch Gynaecol Obstet. 2015;291:1173-1179.

No difference in clinical outcome of IVF-ET in poor prognosis patients with HA-Enriched ET Medium

	HA group		Control group		*P* value
Clinical pregnancy rate					
Female age ≥40 years	12/59	20.3 %	13/56	23.2 %	0.709
≥2 previous IVF failure	16/47	34.0 %	20/53	37.7 %	0.701
≤3 oocytes collected	17/60	28.3 %	6/34	17.6 %	0.247
Only poor quality embryos transferred	35/133	26.3 %	34/125	27.2 %	0.873
Implantation rate					
Female age ≥40 years	13/174	7.5 %	16/179	8.9 %	0.616
≥2 previous IVF failure	13/117	11.1 %	28/118	23.7 %	0.073
≤3 oocytes collected	19/121	15.7 %	6/63	9.5 %	0.246
Only poor quality embryos transferred	40/280	14.3 %	46/275	16.7 %	0.427
Delivery rate					
Female age ≥40 years	6/59	10.2 %	6/56	10.7 %	0.924
≥2 previous IVF failure	7/47	14.9 %	12/53	22.6 %	0.324
≤3 oocytes collected	14/60	23.3 %	6/34	17.6 %	0.517
Only poor quality embryos transferred	28/133	21.1 %	22/125	17.6 %	0.483

Fancovitz, P et al. Arch Gynaecol Obstet. 2015;291:1173-1179.

Fancovitz: HA- ET Medium on IVF Outcome:

- HA addition showed no change in:
  - -biochemical loss or miscarriage
  - -implantation or pregnancy rates
  - -clinical pregnancy or live birth
- No benefit of HA in patients:
  - -> 40 years old
  - -2 or more failed IVF cycles
  - -3 or fewer oocytes
  - -Only poor quality embryos
- Birth weigh significantly higher with HA

Fancovitz, P et al. Arch Gynaecol Obstet. 2015;291:1173-1179.

Adhesion Compounds Summary

- Supplements are important for transfer media
- Protein additives are accepted world wide as an important supplement
- Hyaluronic acid appears to improve implantation, clinical pregnancy, and live birth rate in some studies (moderate quality evidence)
- Further research need to determine appropriate patient populations

References Adherence Compounds (1)

- Feichtinger W, Kemeter P, Menezo Y.The use of synthetic culture medium and patient serum for human in vitro fertilization and embryo replacement. J In Vrtro Fert Embryo Transf. 1986;3:87-92.
- Khan I, Staessen C, Devroey P, Van Steirteghem AC. Human serum albumin versus serum: a comparative study on embryo transfer medium. Fertil Steril. 1991 Jul;56(1):98-101.
- Hargreaves CA, Rahman F, Cowan D, et al.Serum is more effective than albumin in promoting human embryo development and implantation. Fertil Steril. 1995 Dec;64(6):1162-6.
- Laverge H, De Sutter P, Desmet R,. Prospective randomized study comparing human serum albumin with fetal cord serum as protein supplement in culture medium for in-vitro fertilization. Hum Reprod. 1997 Oct;12(10):2263-6 Menezo Y[1], Arnal F, Humeau C, Ducret L, Nicollet B.Increased viscosity in transfer medium does not improve the pregnancy rates after embryo replacement. Fertil Steril. 1989 Oct;52(4):680-2.
- Feichtinger W[1], Strohmer H, Radner KM, Goldin M.The use of fibrin sealant for embryo transfer: development and clinical studies. Hum Reprod. 1992 Jul;7(6):890-3.
- Weissman B, Meyer K (1954) The structure of hyalobiuronic acid and of hyaluronic acid from ombilical cord. J AM Chem Soc 27: 1753–1757.
- Schoolcraft, et al. Increased hyaluronan concentration in the embryo transfer medium results in a significant increase in human embryo implantation rate. Fertil Steril 2002; 76:55

References: Adherence Compounds (2)

- B. Balaban, A. Isiklar, K. Yakin, H. Fursoy, B. Urman, Increased implantation rates in patients with recurrent implantation failures following the use a new transfer medium enriched with hyaluronan. *Human reproduction* 19, i7 (2004).
- M. Ludwig, B. Nitz, [Optimisation of possible success in an IVF program]. *Zentralbl Gynakol* 126, 368 (Dec, 2004).
- Yakin. K., Balaban. B., Isiklar. H., Bozdag. B., U. B., Improved clinical outcome in frozen-thawed embryo transfers with the use of hyaluronan-enriched transfer medium. *Human reproduction* 19, i89 (2004).
- Friedler. S. *et al.*, Efficacy of hyaluronan-enriched embryo transfer medium in patients with repeated IVF-ET failures. *Human Reproducution* 20, i159 (2005).
- Valojerdi MR *et al.*, Efficacy of a human embryo transfer medium: a prospective, randomized clinical trial study. *J Assist Reprod Genet.* may, 23, 207 (2006).
- S. Friedler *et al.*, A randomized clinical trial comparing recombinant hyaluronan/recombinant albumin versus human tubal fluid for cleavage stage embryo transfer in patients with multiple IVF-embryo transfer failure. *Human reproduction* 22, 2444 (Sep, 2007).
- S. Korosec, I. Virant-Klun, T. Tomazevic, N. H. Zech, et al. Single fresh and frozen-thawed blastocyst transfer using hyaluronan-rich transfer medium. *Reproductive biomedicine online* 15, 701 (Dec, 2007).
- K. E. Loutradi *et al.*, Evaluation of a transfer medium containing high concentration of hyaluronan in human in vitro fertilization. *Fertil Steril* 87, 48 (Jan, 2007).

References: Adherence Compounds (3)

- C. W. Mi, W. C. Venier, S. N. Kokjohn, W. P. Hummel, L. M. Kettel, Comparison of two human embryo transfer media in blastocyst frozen embryo transfer cycles. *Fertility and Sterility* 88, S318 (2007).
- M. Svobodova, J. Brezinova, I. Oborna, J. Dostal, M. Krskova, [EmbryoGlue the transfer medium with hyaluronan in the IVF+ET program]. *Ceska Gynekol* 72, 15 (Jan, 2007).
- W. D. Hazlett, L. R. Meyer, T. E. Nasta, P. A. Mangan, V. C. Karande, Impact of EmbryoGlue as the embryo transfer medium. *Fertil Steril* 90, 214 (Jul, 2008).
- K. E. Loutradi, T. B. Tarlatzi, E. M. Kolibianakis, B. C. Tarlatzis, Does hyaluronan improve embryo implantation? *Current opinion in obstetrics & gynecology* 20, 305 (Jun, 2008).
- B. Urman, K. Yakin, B. Ata, A. Isiklar, B. Balaban, Effect of hyaluronan-enriched transfer medium on implantation and pregnancy rates after day 3 and day 5 embryo transfers: a prospective randomized study. *Fertil Steril* 90, 604 (Sep, 2008).
- S. Bontekoe, M. J. Heineman, N. Johnson,, Adherence compounds in embryo transfer media for assisted reproductive technologies. *The Cochrane database of systematic reviews* 2, CD007421 (2014).
- S. Bontekoe, D. Blake, M. J. Heineman, E. C. Williams, N. Johnson, Adherence compounds in embryo transfer media for assisted reproductive technologies. *The Cochrane database of systematic reviews*, CD007421 (2010).
- B. Balaban, K. Yakin, B. Ata, A. Isiklar, B. Urman, Effect of hyaluronan-enriched transfer medium on take home baby rate after day 3 and day 5 embryo transfers: a prospective randomized study. *Human reproduction* 26, i24 (2011).

Gene profiling in endometrium: Does personalized embryo transfer correct for implantation failure?

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Disclosure

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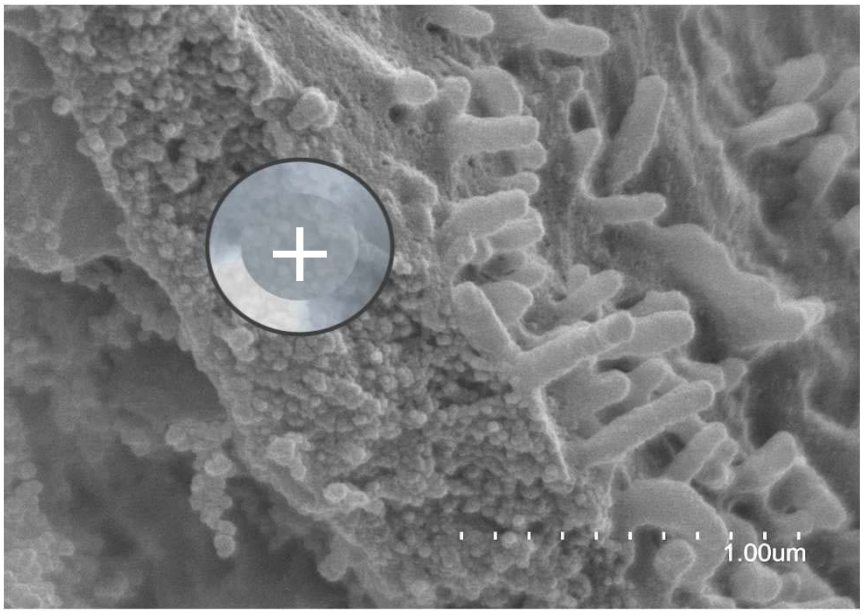
ERA patent inventor

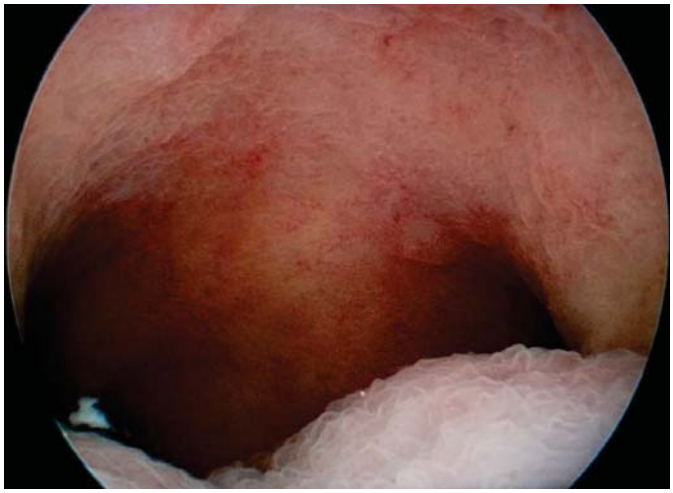
Learning objectives

- ✓ To discuss the concept of personalized medicine applied to human endometrial receptivity.
- ✓ To learn about the molecular diagnosis of endometrial receptivity using ERA, and its surrogate therapeutic option personalized ET (pET).
- ✓ To discuss non-invasive diagnostic methods of ER “in progress” by means of secreted molecules or single cell analysis.

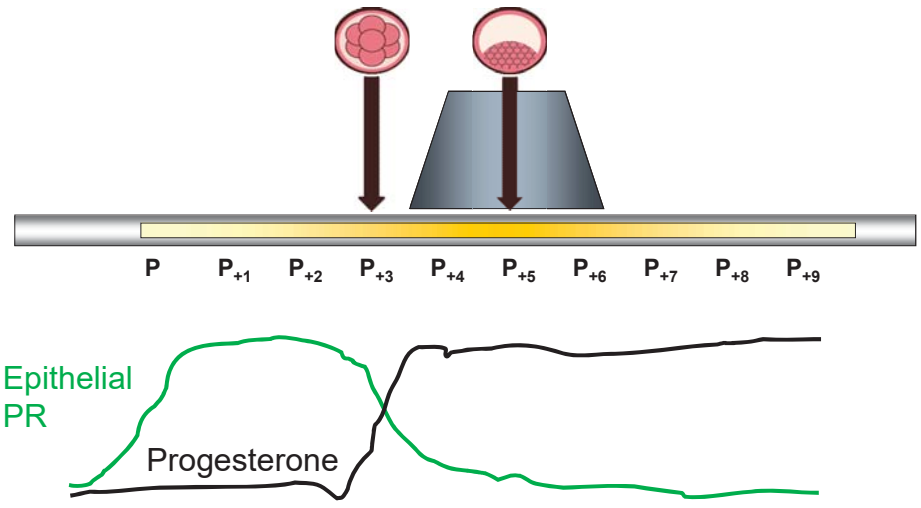
The sticky embryo



The uterine view



Window of endometrial receptivity (WOI)

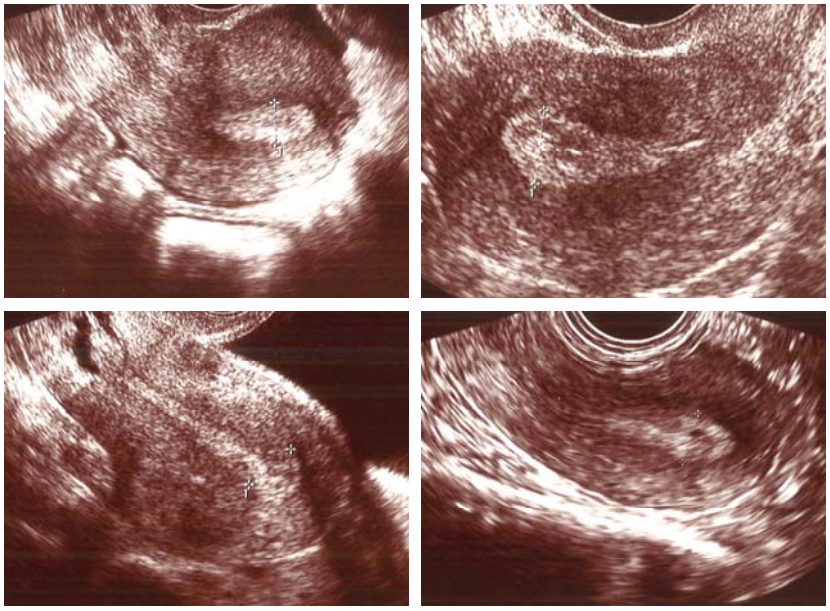


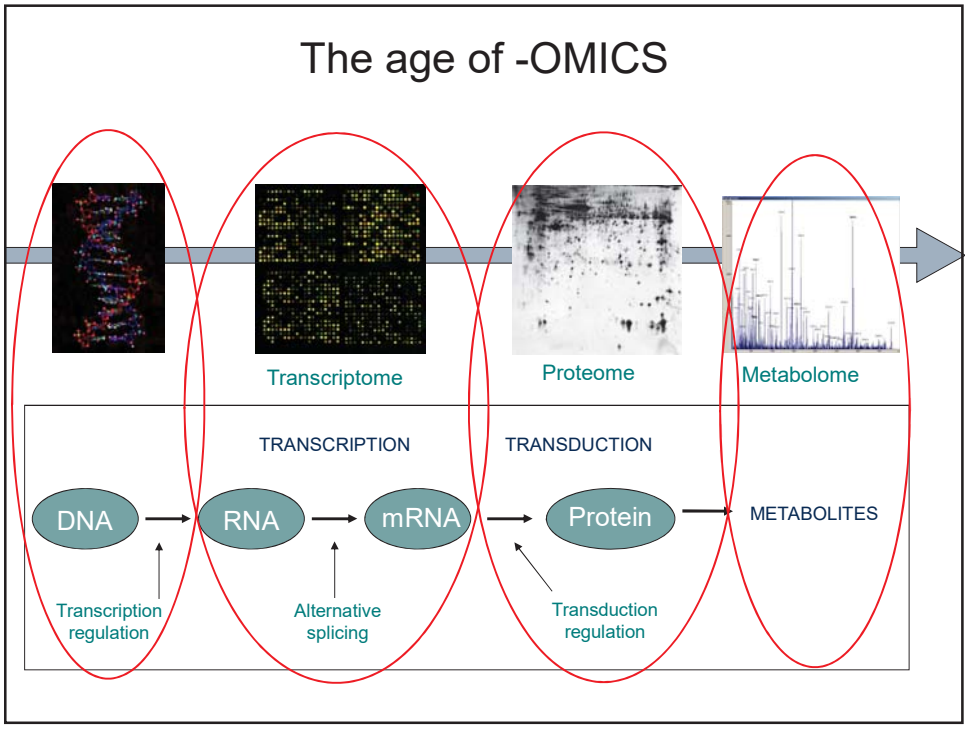
Dating the endometrial biopsy[1]

- ✓ Randomized studies
  - Interobserver and cycle-to-cycle (60%) variations[2]
  - Endometrial dating is not related to fertility status[3]

Histological dating is not a valid method for the diagnosis of luteal phase deficiency neither guidance throughout clinical management in infertility

1. Noyes, et al. 1950
2. Murray, et al. 2004
3. Coutifaris, et al. 2004





Summary of studies performed in human endometrium using microarray analysis. All the gene information from these studies is available at http://www.endometrialdatabase.com.

Process studied	Microarray	Company	Number of gene targets	Study
Decidualization	Clontech	Atlas array Stanford University	588	Popovici *et al.* (2000)
Decidualization	Incyte human GEM-V	Incyte Genomics	6918	Brar *et al.* (2001)
Endometrial cancer	Hu6800	Affymetrix	6000	Mutter *et al.* (2001)
WOI	HG-U95A	Affymetrix	12686	Carson *et al.* (2002)
Endometriosis	Human gene genefilter GF211	Research Genetics	4133	Eyster *et al.* (2002)
WOI	HG-U95A	Affymetrix	12686	Kao *et al.* (2002)
Endometriosis	Atlas human cDNA expression Array	Clontech	597	Lebovic *et al.* (2002)
Endometriosis	Home-made	University of Tokio	23040	Arimoto *et al.* (2003)
WOI	HG-U95A-E	Affymetrix	>60000	Borthwick *et al.* (2003)
RU486	Home-made	University of Cambridge	~1000	Catalano *et al.* (2003)
WOI	Human cytokine expression array	R&D Systems	375	Domínguez *et al.* (2003)
Endometriosis	HG-U95A	Affymetrix	12686	Kao *et al.* (2003)
Endometrial cancer	Oncochip	Centro Nacional de Investigaciones Oncológicas	6386	Moreno-Bueno *et al.* (2003a)
Progesterone effect	Human Chip 1K set 1	Takara Shuzo	1066	Okada *et al.* (2003)
WOI	HG-U95A	Affymetrix	12686	Riesewijk *et al.* (2003)
Endometrial cancer	Home-made	National Cancer Institute	9984	Risinger *et al.* (2003)
Decidualization	HG-U95A	Affymetrix	12686	Tierney *et al.* (2003)
Endometrial cancer	GEMarray clones	Incyte Genomics	18098	Cao *et al.* (2004)
Endometrial cancer	HG-U133A	Affymetrix	>22000	Ferguson *et al.* (2004)
Endometriosis	Atlas human 1.2 cDNA expression array	Clontech	1172	Matsuzaki *et al.* (2004)
Stimulated cycles	HG-U95Av2	Affymetrix	12686	Mirkin *et al.* (2004)
Menstrual cycle	[illegible]	[illegible]	[illegible]	[illegible]
Endometrial cancer	[illegible]	[illegible]	[illegible]	[illegible]
In vitro models	[illegible]	[illegible]	[illegible]	[illegible]
Endometrial cancer	[illegible]	[illegible]	[illegible]	[illegible]
Stimulated cycles	[illegible]	[illegible]	[illegible]	[illegible]
In vitro models	HG-U133A 2.0	Affymetrix	14500	Rekker *et al.* (2005)
Endometriosis	Atlas human 1.2 cDNA expression array	Clontech	1176	Matsuzaki *et al.* (2005)
Endometrial cancer	HG-U133A – U133B	Affymetrix	>22000	Maxwell *et al.* (2005)
In vitro models	Home-made	[illegible]	[illegible]	[illegible]
RU486	Home-made	[illegible]	[illegible]	[illegible]
Stimulated cycles	HG-U133A	Affymetrix	>22000	Talbi *et al.* (2006)
Menstrual cycle	HG-U133 Plus 2.0	Affymetrix	> 47000	Talbi *et al.* (2006)
In vitro models	Home-made	University of Cambridge	>15000	White *et al.* (2005)
Proliferative phase	HG-Arraytyon cDNA expression array	BD Biosciences Clontech	588	Yanokihara *et al.* (2005)
Endometrial cancer	[illegible]	[illegible]	[illegible]	[illegible]
Endometriosis	[illegible]	[illegible]	[illegible]	[illegible]
RU486	[illegible]	[illegible]	[illegible]	[illegible]
In vitro models	[illegible]	[illegible]	[illegible]	[illegible]
Endometrial cancer	[illegible]	[illegible]	[illegible]	[illegible]
In vitro models	GeneChip U133 Plus 2.0	Affymetrix	7400	Matsumura *et al.* (2006)
Endometriosis	[illegible]	Affymetrix	> 47000	Sarraj *et al.* (2006)
Endometrial cancer	[illegible]	Incyte Genomics	9600	Wang *et al.* (2006)
Endometriosis	CodeLink Whole Human Genome Bioarrays	GE Amersham Biosciences	45674	Eyster *et al.* (2007)
WOI	Home-made	University of Cambridge	>16000	Feroze-Zaidi *et al.* (2007)
In vitro models	HG-U133 Plus 2.0	Affymetrix	> 47000	Gielen *et al.* (2007)
Endometriosis	Atlas human 1.2 Array	Clontech	1176	Mettler *et al.* (2007)
Endometriosis	miRNA Bioarray	Ambion	287	Pan *et al.* (2007)
Endometrial cancer	35K Human oligo microarray	Norwegian Microarray Consortium	24650	Paulsson *et al.* (2007)
Endometriosis	HG-U133A 2.0	Affymetrix	14500	Van Langendonckt *et al.* (2007)
Endometrial cancer	HG-U133 Plus 2.0	Affymetrix	> 47000	Wong *et al.* (2007)
Pregnancy	HG-U133A 2.0	Affymetrix	14500	Berenger *et al.* (2008)
Menstrual cycle	Sentrix Human Illumina 6V1 Expression Bead chip	Illumina	> 47000	Cloke *et al.* (2008)
Pregnancy	HG-U133 Plus 2.0	Affymetrix	> 47000	Evans *et al.* (2008)
Signaling	HG-U133 Plus 2.0	Affymetrix	> 47000	Gielen *et al.* (2008)
Endometrial cancer	Cancer profiling array	Clontech	241	Hanna *et al.* (2008)
Stimulated cycles	HG-U133A	Affymetrix	>22000	Horcajadas *et al.* (2008)
Decidualization	HG-U133 Plus 2.0	Affymetrix	> 47000	Horne *et al.* (2008)
Endometriosis	HG-U95Av2	Affymetrix	12686	Hull *et al.* (2008)
Stimulated cycles	HG-U133A	Affymetrix	>22000	Liu *et al.* (2008)
Menstrual cycle	HG-U133 Plus 2.0	Affymetrix	> 47000	Liu *et al.* (2008)
Menstrual cycle	HG-U133 Plus 2.0	Affymetrix	> 47000	Macklon *et al.* (2008)
Pregnancy	HG-U133 Plus 2.0	Affymetrix	> 47000	Savaris *et al.* (2008)
Endometriosis	Home-made	University of Cambridge	22000	Sherwin *et al.* (2008)
WOI	Home-made	National Cancer Institute	9128	Tapia *et al.* (2008)
Stimulated cycles	HG-U133 Plus 2.0	Affymetrix	> 47000	Zhou *et al.* (2008)
Menstrual cycle	HG-U133A	Affymetrix	> 22000	Ball *et al.* (2009)
Paracrine effects	Home-made	University of Cambridge	>15000	Germeyer *et al.* (2005)
WOI	HG-U133 Plus 2.0	Affymetrix	> 47000	Haouzi *et al.* (2009)
Stimulated cycles	HG-U133 Plus 2.0	Affymetrix	> 47000	Van Vaerenbergh *et al.* (2009)
Adenomyosis	HG-U133 Plus 2.0	Affymetrix	> 47000	Chen *et al.* (2010)
WOI	Whole Human Genome Oligo Microarray	Agilent	>40000	Labarta *et al.* (2011)

All the gene information from these studies are available at http://www.endometrialdatabase.com.

Garrido-Gómez T, Domínguez F, Ruiz-Alonso M, **Simón C**. The Analysis of Endometrial receptivity. In: Textbook of Assisted Reproductive Techniques. UK; Informa Healthcare; 2012: 366-79.

Endometrial receptivity array (ERA)

GENETICS

A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature

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[a] Fundación IVI-Instituto Universitario IVI, University of Valencia, Valencia; [b] Instituto de Investigación, Sanitaria del Hospital Clinico de Valencia, Valencia University, Valencia; [c] iGenomix, Valencia; [d] Department of Experimental Biology, University of Jaén, Jaén; and [e] Centro de Investigación Príncipe Felipe, Valencia, Spain

Objective: To create a genomic tool composed of a customized microarray and a bioinformatic predictor for endometrial dating and to detect pathologies of endometrial origin. To define the transcriptomic signature of human endometrial receptivity.
Design: Two cohorts of endometrial samples along the menstrual cycle were used: one to select the genes to be included in the customized microarray (endometrial receptivity array [ERA]), and the other to be analyzed by ERA to train the predictor for endometrial dating and to define the transcriptomic signature. A third cohort including pathological endometrial samples was used to train the predictor for pathological classification.
Setting: Healthy oocyte donors and patients.
Patient(s): Healthy fertile women (88) and women with implantation failure (5) or hydrosalpinx (2).
Intervention(s): Human endometrial biopsies.
Main Outcome Measure(s): The gene expression of endometrial biopsies.
Result(s): The ERA included 238 selected genes. The transcriptomic signature was defined by 134 genes. The predictor showed a specificity of 0.8857 and sensitivity of 0.99758 for endometrial dating, and a specificity of 0.1571 and a sensitivity of 0.995 for the pathological classification.
Conclusion(s): This diagnostic tool can be used clinically in reproductive medicine and gynecology. The transcriptomic signature is a potential endometrial receptivity biomarkers cluster. (Fertil Steril® 2011;95:50–60. ©2011 by American Society for Reproductive Medicine.)

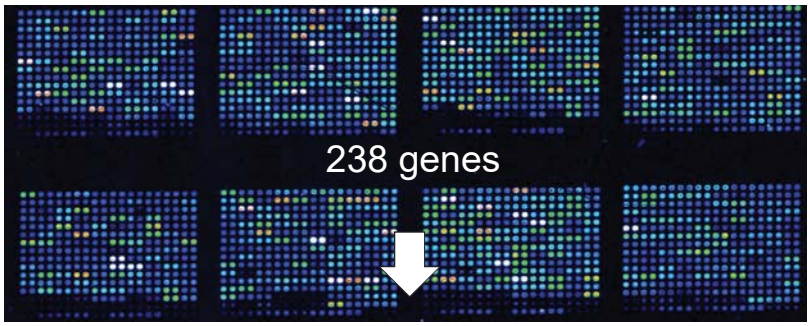
Key Words: Endometrial receptivity, endometrial dating, microarray, transcriptomic signature, predictor, diagnostic tool

Fertility and Sterility® Vol. 95, No. 1, January 2011 0015-0282/$36.00

doi:10.1016/j.fertnstert.2010.04.063

Gene symbol	Gene name	Fold change	No. of probes
GPX3[a,b,c]	Glutathione peroxidase 3 (plasma)	35.49	2
PAEP[b,c]	Progestagen-associated endometrial protein (placental protein 14, pregnancy-associated endometrial alpha-2-globulin, alpha uterine protein) (PAEP), transcript variant 2	31.43	1
COMP[c]	Cartilage oligomeric matrix protein	30.95	2
SLC1A1[c]	Solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1	17.57	3
LIF[b]	Leukemia inhibitory factor (cholinergic differentiation factor)	15.03	3
TCN1[c]	Transcobalamin I (vitamin B12-binding protein, R binder family)	14.76	1
CXCL14[c]	Chemokine (C-X-C motif) ligand 14	14.02	2
C4BPA[c]	Complement component 4 binding protein, alpha	13.14	2
TSPAN8[c]	Tetraspanin 8	12.90	2
LAMB3[c]	Laminin, beta 3, transcript variant 2	11.32	1
MAOA[c]	Monoamine oxidase A, nuclear gene encoding mitochondrial protein	9.39	2
SOD2[c]	Superoxide dismutase 2, mitochondrial, nuclear gene encoding mitochondrial protein, transcript variant 2	9.06	2
GADD45A[c]	Growth arrest and DNA damage inducible, alpha	8.25	1
MUC16	Mucin 16, cell surface associated	8.01	8
THBD[c]	Thrombomodulin	7.84	3
NNMT[c]	Nicotinamide *N*-methyltransferase	7.74	2
DPP4[b,c]	Dipeptidylpeptidase 4 (CD26, adenosine deaminase complexing protein 2)	7.72	3
SCGB2A2[c]	Secretoglobin, family 2A, member 2	7.43	2
S100P[c]	S100 calcium-binding protein P	6.95	1
SNX10[c]	Sorting nexin 10	6.56	2
CP[c]	Ceruloplasmin (ferroxidase)	6.34	2
G0S2	Putative lymphocyte G0/G1 switch gene	6.20	2
C4.4A[c]	GPI-anchored metastasis-associated protein homologue	6.03	1
ANG[c]	Angiogenin, ribonuclease, RNase A family, 5	5.98	2
ABCC3[c]	ATP-binding cassette, subfamily C (CFTR/MRP), member 3	5.98	1
XCL1	Chemokine (C motif) ligand 1	5.80	3
ADRA2A	Adrenergic, alpha-2A, receptor	5.78	2
EFNA1	Ephrin-A1, transcript variant 1	5.77	3
KLRC1	Killer cell lectin-like receptor subfamily C, member 1, transcript variant 2	5.75	2
TAGLN[b]	Transgelin	5.71	3
SLC15A1	Solute carrier family 15 (oligopeptide transporter), member 1	5.59	2

Endometrial receptivity array (ERA)
Endometrial receptivity analysis (ERA-NGS)

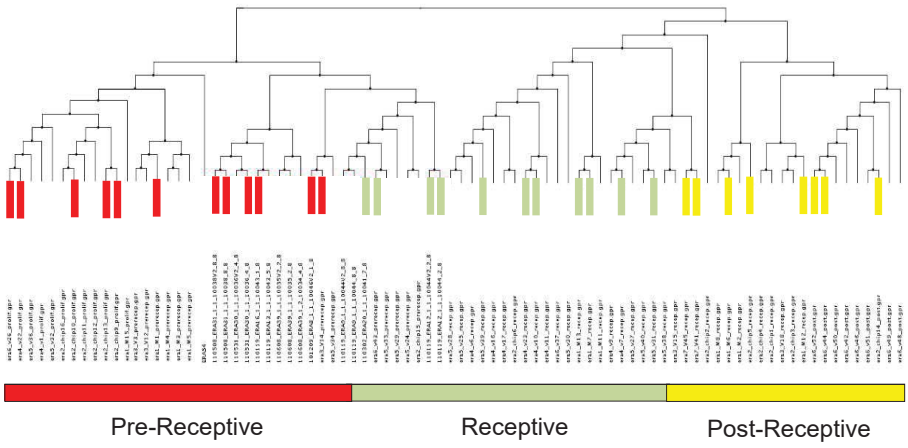


Bioinformatic analysis of data

Classification and prediction from gene expression

Patented in 2009: PCT/ES 2009/000386

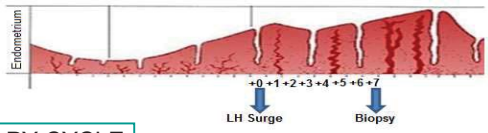
Predictor classifies the molecular receptivity status of the endometrium



Endometrial receptivity array (ERA)
Timing of the biopsy

NATURAL CYCLE

Endometrial biopsy must be taken on the 7th day after the LH surge (LH+7) (urine or serum preferible).

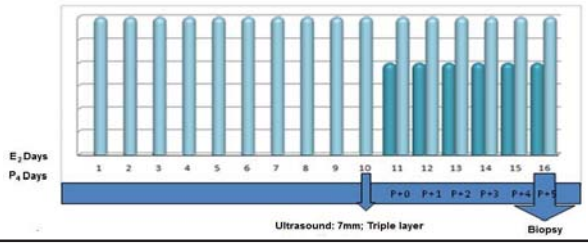


HORMONE REPLACEMENT THERAPY CYCLE

Endometrial biopsy must be taken on day P+5, after proper E_2 priming

E_2 : 6 mg/day

P_4 : 800 mg/day

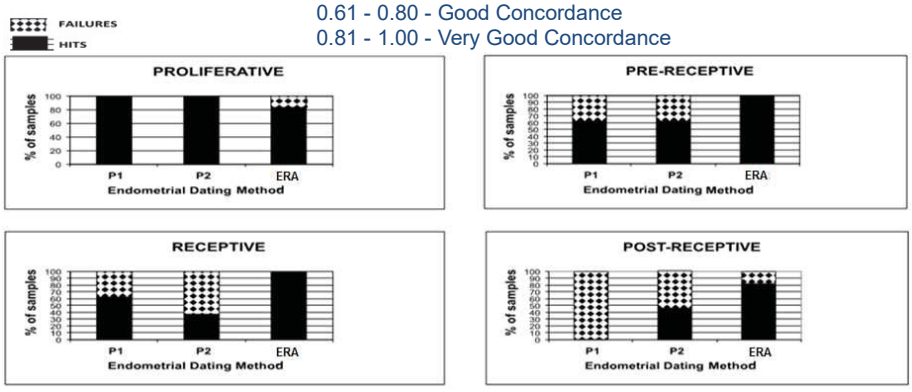


Endometrial receptivity array (ERA) – Accuracy

In a blinded study ERA classifies better than Noyes criteria

	Pathologist 1 (P1)	Pathologist 2 (P2)	P1 vs P2	ERA
Kappa value	**0.618** (0.446-0.791)	**0.685** (0.545-0.824)	**0.622** (0.435-0.839)	**0.922** (0.815-1.000)

0.61 - 0.80 - Good Concordance
0.81 - 1.00 - Very Good Concordance



Díaz-Gimeno, et al. 2013

Endometrial receptivity array (ERA) – Consistency

ERA TEST ANALYZED IN THE SAME PATIENT, same day, 3-years apart

Code	Date First Biopsy	Date Second Biopsy	Months between	First Biopsy Results	Second Biopsy Results
CON1	09/2009	02/2012	29	Receptive	Receptive (0.908)
CON2	09/2009	03/2012	30	Receptive	Receptive (0.908)
CON3	05/2009	04/2012	35	Receptive	Receptive (0.908)
CON4	05/2009	05/2012	36	Proliferative	Non Receptive (0.864)
CON5	01/2009	05/2012	40	Proliferative	Non Receptive (0.864)
CON6	07/2009	05/2012	35	Receptive	Receptive (0.908)

Díaz-Gimeno, et al. 2013

The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure

Maria Ruiz-Alonso, M.Sc.,[b] David Blesa, Ph.D.,[a,b] Patricia Díaz-Gimeno, Ph.D.,[a,c] Eva Gómez, M.Sc.,[a] Manuel Fernández-Sánchez, M.D.,[d] Francisco Carranza, M.D.,[d] Joan Carrera, M.D.,[e] Felip Vilella, Ph.D.,[a] Antonio Pellicer, M.D., Ph.D.,[a,b] and Carlos Simón, M.D., Ph.D.[a,b]

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http://dx.doi.org/10.1016/j.fertnstert.2013.05.004

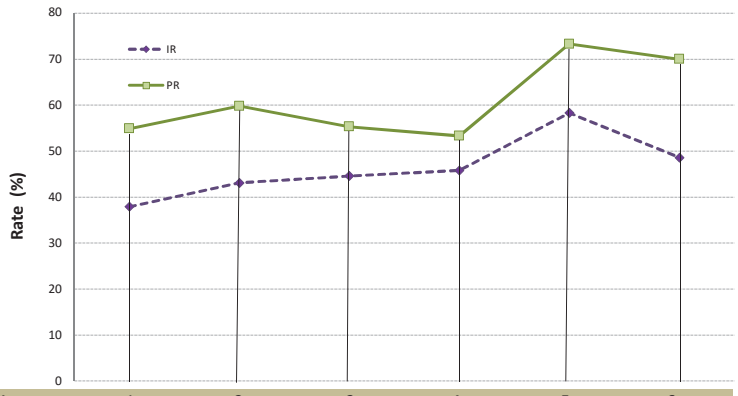
TABLE 1

	RIF	Control
No. of patients	85	25
Age (y)	38.4 ± 4.7	39.9 ± 5.1
No. of Receptive ERA/total analyzed	63/85 (74.1)	22/25 (88.0)
No. of previous failed cycles	4.8 ± 2.1	0.5 ± 0.5
Total Patients with pET after Receptive ERA	29	11
Implantation rate after 1st pET	19/56 (33.9)	11/20 (55.0)
Pregnancy rate after 1st pET	15/29 (51.7)	9/11 (81.8)
Biochemical pregnancies	3/15 (20.0)	2/9 (22.2)
Clinical abortions	0/15 (0.0)	0/9 (0.0)
No. of previous failed cycles	5.0 ± 1.8	0.3 ± 0.6
Ovum donation patients/total	11/22 (50.0)	2/3 (66.6)
IVF/ICSI patients/total	11/22 (50.0)	1/3 (33.3)

Values are presented as mean ± SD or n (%).

Ruiz-Alonso, et al. 2013

pET outcome after receptive ERA in patients with RIF (n=310)



Months after ERA test	1	2	3	4	5	6
Number of patients	91	87	47	30	15	40
Implantation Rate (%)	37.9	43.1	44.6	45.8	58.3	48.6
Pregnancy Rate (%)	54.9	59.8	55.3	53.3	73.3	70.0

Ruiz-Alonso, et al. 2013

A Clinical outcome of non receptive RIF and control patients that underwent pET

	Non Receptive
No. of patients	25
No. of previous failed cycle RIF Patients	5.0±1.8
No. of previous failed cycle Control Patients	0.3±0.6
ERA Prediction	
Pre-receptive	21/25 (84.0)
Post-receptive	4/25 (16.0)
2nd ERA at the specified day (P+4;P+6;P+7;LH+9) [a]	18
Months between 1st and 2nd ERA	2.6±2.8
2nd ERA Receptive at the specified day	15
Patients with pET[b] after 2nd RECEPTIVE ERA	8
Months between 2nd RECEPTIVE ERA and pET	1.8±0.7
Implantation rate using pET	5/13 (38.5)
Pregnancy rate using pET	4/8 (50.0)
Biochemical pregnancies (%)	0/4 (0.0)
Clinical abortions (%)	0/4 (0.0)

Ruiz-Alonso, et al. 2013

ERA clinical applicability

A case report and pilot study comparing routine embryo transfer versus pET (Ruiz-Alonso et al. 2014 Hum Reprod 2014 Apr 15).

human reproduction CASE REPORT *Infertility*

What a difference two days make: "personalized" embryo transfer (pET) paradigm: A case report and pilot study

M. Ruiz-Alonso[1], N. Galindo[2], A. Pellicer[3], and C. Simón[1,3,4,*]

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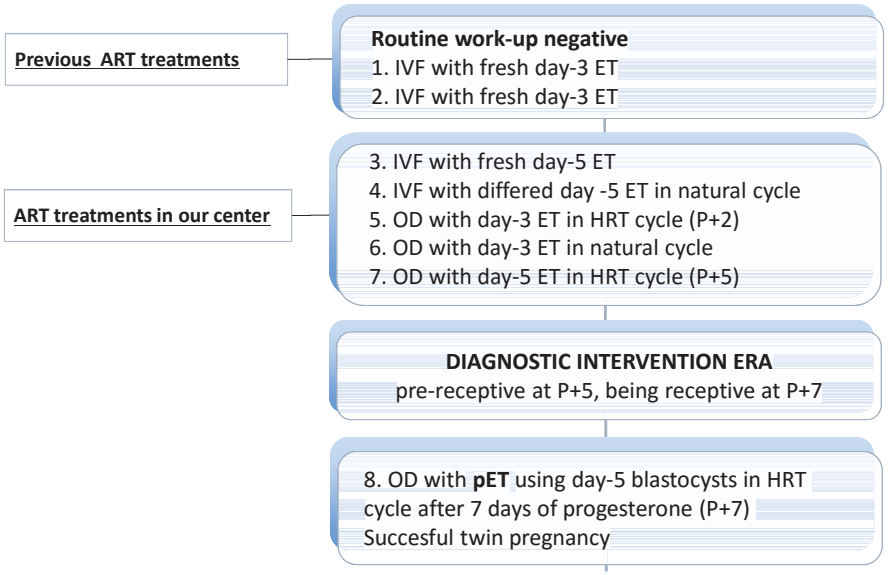
ABSTRACT: Embryo implantation requires that the blastocyst will attach during the receptive stage of the endometrium, known as window of implantation (WOI). Historically, it has been assumed that the WOI is always constant in all women. However, molecular analyses of endometrial receptivity demonstrates a personalized WOI (pWOI) that is displaced in one out of four patients suffering from recurrent implantation failure (RIF) of endometrial origin and illustrates the utility of a personalized endometrial diagnostic approach. Here, we report a clinical case of successful personalized embryo transfer (pET) after four IVF and three oocyte donation failed attempts in which different embryo transfer strategies were attempted. This case report is complemented by a pilot study of 17 patients undergoing oocyte donation and who suffered failed implantations with routine embryo transfer (ET) but were then treated with pET after the personalized diagnosis of their WOI.

Key words: personalized embryo transfer / window of implantation / recurrent implantation failure / assisted reproduction

CASE REPORT



Ruiz-Alonso, et al. 2014

CLINICAL OUTCOME		
		ET
	Number of patients	17
	Source of oocytes	Ovum donation
	Age	40.7 ± 4.7 (32-49)
First attempt	Number of embryos transferred	1.8 ± 0.4
	Implantation rate	**12.9%** (4/31)
	Pregnancy rate	**23.5%** (4/17)
	Ongoing pregnancy rate	**0%** (0/4)
	Clinical abortion	**100%** (4/4)
	Biochemical pregnancy	**0.0%** (0/4)
Cumulative	Total attempts	2.1 ± 1.3
	Number of embryos transferred	1.8 ± 0.4
	Implantation rate	**10.8%** (7/65)
	Pregnancy rate	**19.4%** (7/36)
	Ongoing pregnancy rate	**0%** (0/7)
	Clinical abortion	**71.4%** (5/7)
	Biochemical pregnancy	**28.6%** (2/7)

Ruiz-Alonso, et al. 2014

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Cumulative	Clinical abortion	**71.4%** (5/7)
Cumulative	Biochemical pregnancy	**28.6%** (2/7)

ENDOMETRIAL RECEPTIVITY DIAGNOSIS USING ERA	
	ET
Receptive	**0%** (0/17)
Pre-receptive	**94%** (16/17)
WOI delayed 1 day	**19%** (3/16)
WOI delayed 2 days	**81%** (13/16)
Post-receptive	**6%** (1/17)
WOI advanced 1 day	**100%** (1/1)

	CLINICAL OUTCOME		
		ET	**pET**
	Number of patients	17	
	Source of oocytes	Ovum donation	
	Age	40.7 ± 4.7 (32-49)	
First attempt	Number of embryos transferred	1.8 ± 0.4	1.7 ± 0.5
First attempt	Implantation rate	**12.9%** (4/31)	**34.5%** (10/29)
First attempt	Pregnancy rate	**23.5%** (4/17)	**52.9%** (9/17)
First attempt	Ongoing pregnancy rate	**0%** (0/4)	**66.7%** (6/9)
First attempt	Clinical abortion	**100%** (4/4)	**0%** (0/9)
First attempt	Biochemical pregnancy	**0.0%** (0/4)	**33.3%** (3/9)
Cumulative	Total attempts	2.1 ± 1.3	1.2 ± 0.4
Cumulative	Number of embryos transferred	1.8 ± 0.4	1.8 ± 0.4
Cumulative	Implantation rate	**10.8%** (7/65)	**40.0%** (14/35)
Cumulative	Pregnancy rate	**19.4%** (7/36)	**60.0%** (12/20)
Cumulative	Ongoing pregnancy rate	**0%** (0/7)	**75.0%** (9/12)
Cumulative	Clinical abortion	**71.4%** (5/7)	**0%** (0/12)
Cumulative	Biochemical pregnancy	**28.6%** (2/7)	**25.0%** (3/12)

ENDOMETRIAL RECEPTIVITY DIAGNOSIS USING ERA	
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Cumulative	Total attempts	2.1 ± 1.3	1.2 ± 0.4
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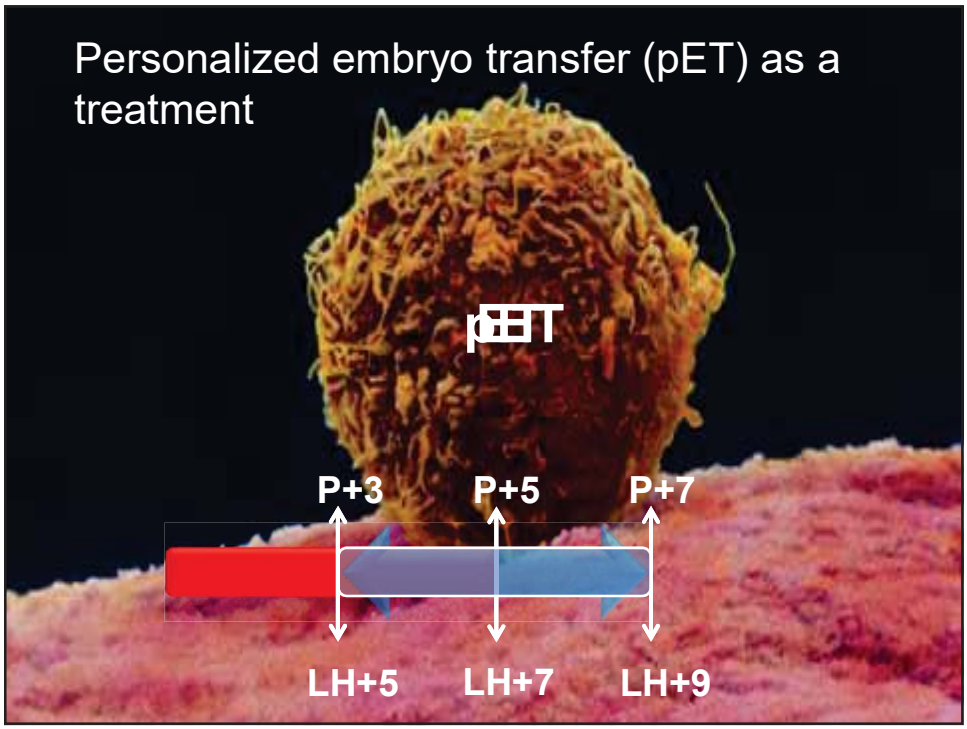
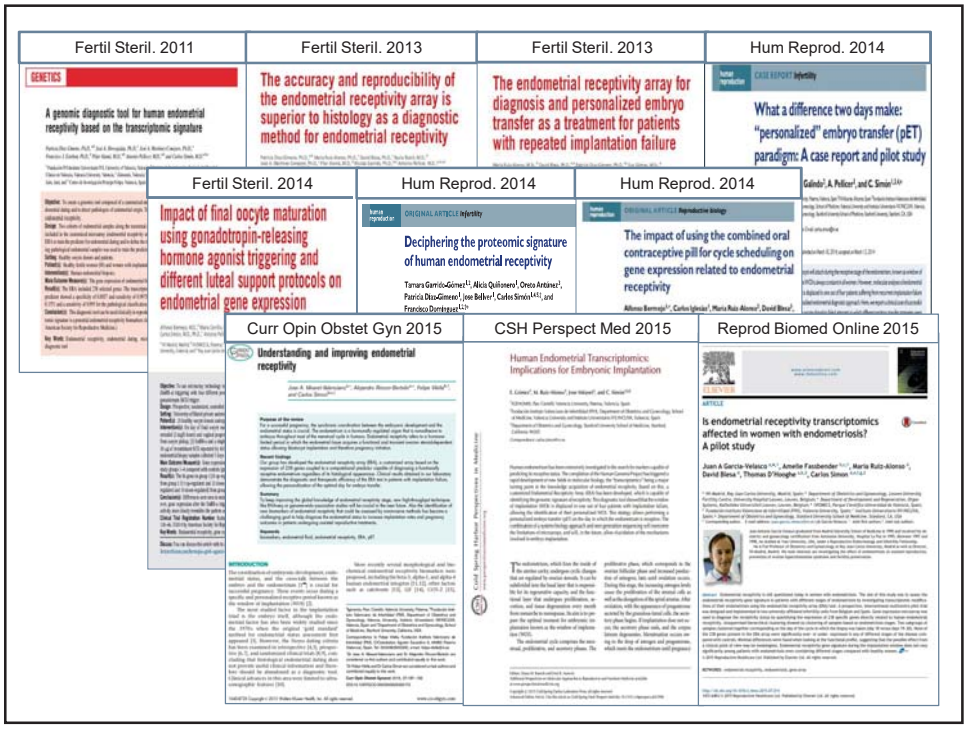
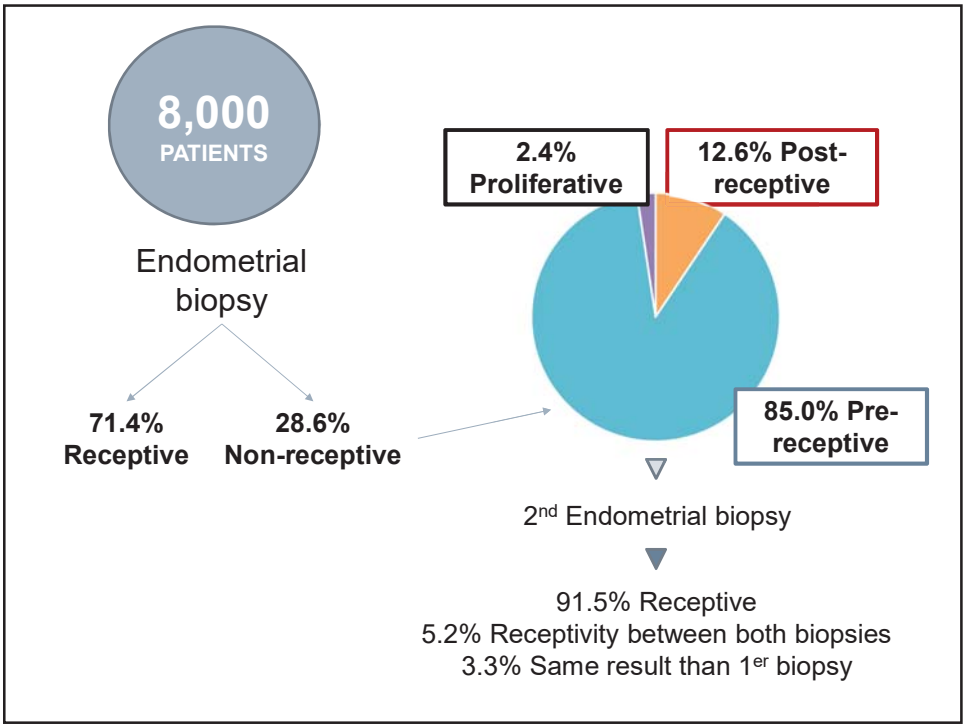
Cumulative	Pregnancy rate	**19.4%** (7/36)	**60.0%** (12/20)
Cumulative	Ongoing pregnancy rate	**0%** (0/7)	**75.0%** (9/12)
Cumulative	Clinical abortion	**71.4%** (5/7)	**0%** (0/12)
Cumulative	Biochemical pregnancy	**28.6%** (2/7)	**25.0%** (3/12)

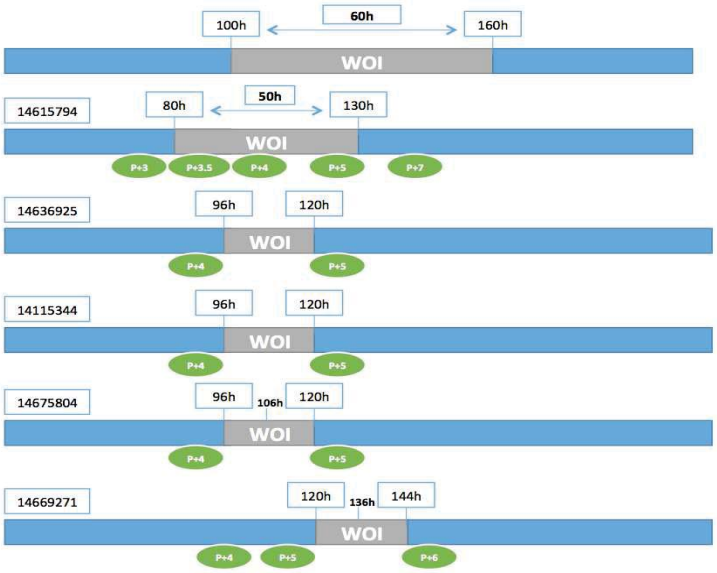
ENDOMETRIAL RECEPTIVITY DIAGNOSIS USING ERA		
	ET	**pET**
Receptive	**0%** (0/17)	**100%** (17/17)
Pre-receptive	**94%** (16/17)	0
WOI delayed 1 day	**19%** (3/16)	0
WOI delayed 2 days	**81%** (13/16)	0
Post-receptive	**6%** (1/17)	0
WOI advanced 1 day	**100%** (1/1)	0




Fertil Steril. 2011
A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature
Fertil Steril. 2013
The accuracy and reproducibility of the endometrial receptivity array is superior to histology as a diagnostic method for endometrial receptivity
Fertil Steril. 2013
The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure
Hum Reprod. 2014
What a difference two days make: "personalized" embryo transfer (pET) paradigm: A case report and pilot study
Fertil Steril. 2014
Impact of final oocyte maturation using gonadotropin-releasing hormone agonist triggering and different luteal support protocols on endometrial gene expression
Hum Reprod. 2014
Deciphering the proteomic signature of human endometrial receptivity
Hum Reprod. 2014
The impact of using the combined oral contraceptive pill for cycle scheduling on gene expression related to endometrial receptivity
Curr Opin Obstet Gyn 2015
Understanding and improving endometrial receptivity
CSH Perspect Med 2015
Human Endometrial Transcriptomics: Implications for Embryonic Implantation
Reprod Biomed Online 2015
Is endometrial receptivity transcriptomics affected in women with endometriosis? A pilot study

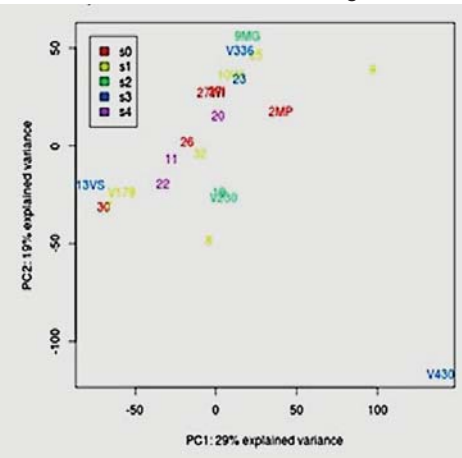

8,000
PATIENTS
Endometrial biopsy
71.4% Receptive
28.6% Non-receptive
2.4% Proliferative
12.6% Post-receptive
85.0% Pre-receptive
2nd Endometrial biopsy
91.5% Receptive
5.2% Receptivity between both biopsies
3.3% Same result than 1er biopsy

Narrow WOI

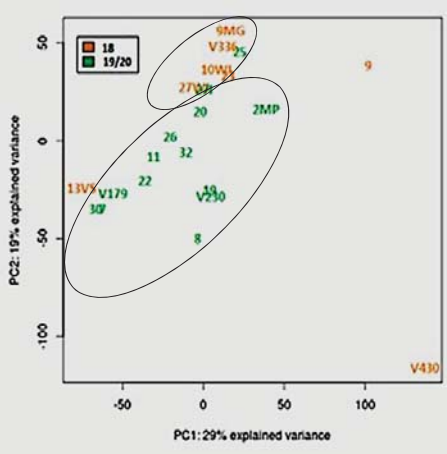


Is endometrial receptivity transcriptomics affected In women with endometriosis? A pilot study

Endometriosis Stage



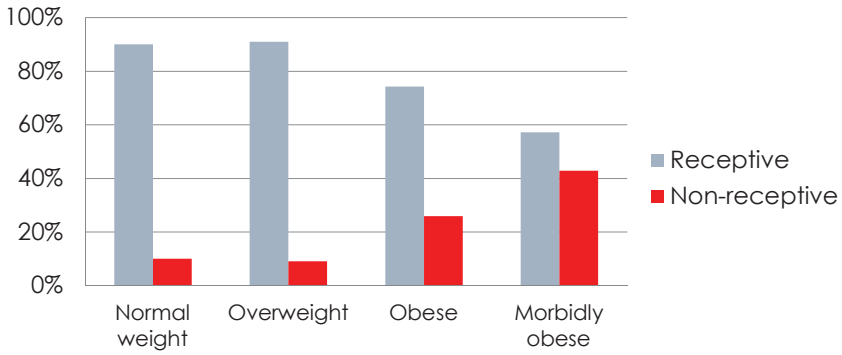
Day of endometrial biopsy



García-Velasco, et al. RBM Online 2016

Is endometrial receptivity affected in obese women?

- Endometrial samples from 73 women included in analysis:
  - 10 normal-weight (BMI 19-24.9 kg/m²)
  - 11 overweight (BMI 25-29.9 kg/m²)
  - 31 obese (BMI 30-34.9 kg/m²)
  - 21 morbidly obese (BMI ≥ 35 kg/m²)



Athanasiadis, et al. Submitted

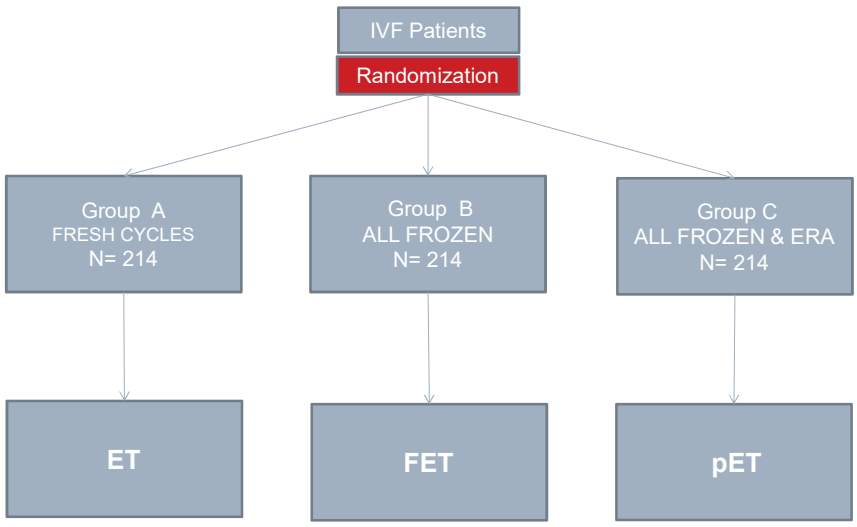
Endometrial Thickness versus Molecular Receptivity

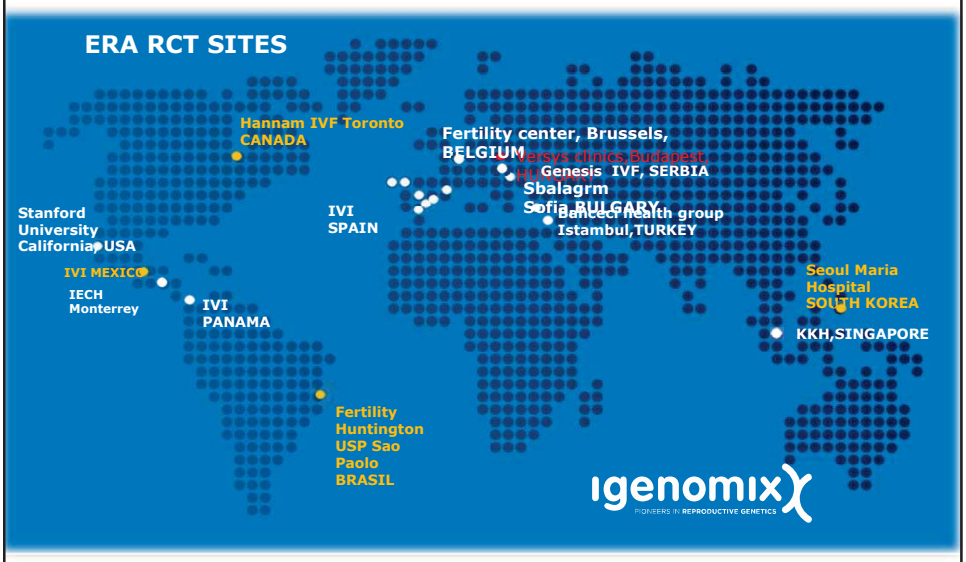
Endometrial thickness (mm)	Receptive (%)	Non Receptive (%)
<6	6/14 **(43%)***	8/14 **(57%)***
6-12	333/431 **(77%)***	98/431 **(23%)***
>12	24/37 **(65%)**	13/37 **(35%)**
TOTAL	363	119

*P= 0,003 by Chi-square test.

Valbuena D. et al. ESHRE 2016

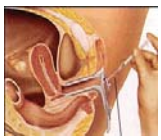
ClinicalTrials.gov Identifier: NCT01954758



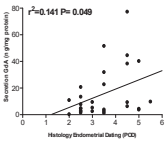


Secretomics of endometrial receptivity

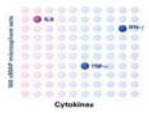
- ✓ Aspiration of endometrial secretion does not affect pregnancy rates
  Van der Gaast, et al. 2002



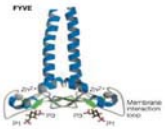
- ✓ Glycodelin levels correlate with the menstrual cycle phase of endometrial aspirations
  Van der Gaast, et al. 2009



- ✓ The profile of cytokines can be determined in endometrial secretions
  Simón, et al. 1996
  Boomsma, et al. 2009



- ✓ The lipidomics is the large-scale study of lipid species present in a cell or biological fluid and their interacting pathways
  Wenk. 2005



ORIGINAL ARTICLE

Endocrine Research

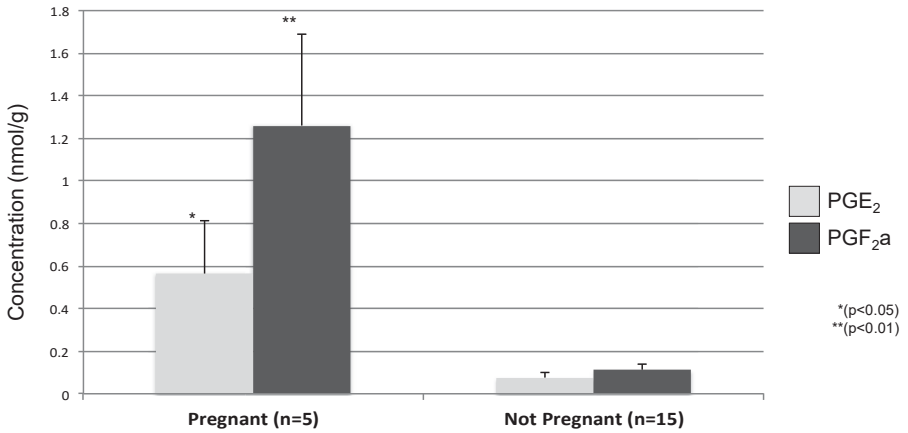
PGE_2 and $PGF_2\alpha$ Concentrations in Human Endometrial Fluid as Biomarkers for Embryonic Implantation

F. Vilella,* L. Ramirez,* O. Berlanga, S. Martínez, P. Alamá, M. Meseguer, A. Pellicer, and C. Simón

Fundación Instituto Valenciano de Infertilidad (F.V., L.R., O.B., S.M., C.S.), Valencia University and Instituto Universitario IVI/INCLIVA, Valencia University, 46980 Valencia, Spain; Instituto Valenciano de Infertilidad (IVI) (P.A., M.M., A.P., C.S.), Valencia University, 46015 Valencia, Spain; and Department of Ob/Gyn (C.S.), Stanford University School of Medicine, Stanford California 94305

Vilella, et al.JCEM 2013

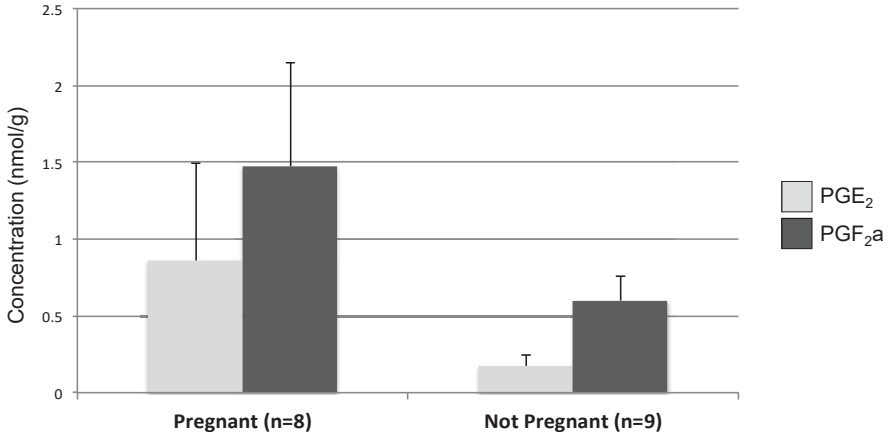
PGs LEVELS IN EF OBTAINED 24H BEFORE DAY 3 ET (S&S)



	PGE_2	$PGF_2\alpha$
ROC curve	0.88	0.973
Sensitivity	80.00%	100%
Specificity	86.70%	93.30%

Vilella, et al.JCEM 2013

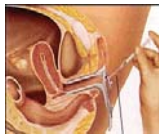
PGs LEVELS IN EF OBTAINED 24H BEFORE DAY 5 ET (S&S)



	PGE_2	$PGF_2\alpha$
ROC curve	0.694	0.653
Sensitivity	75.00%	37.50%
Specificity	77.80%	100%

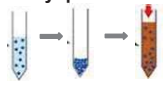
Vilella, et al. JCEM 2013

Endometrial Fluid RNA-seq project



Endometrial fluid

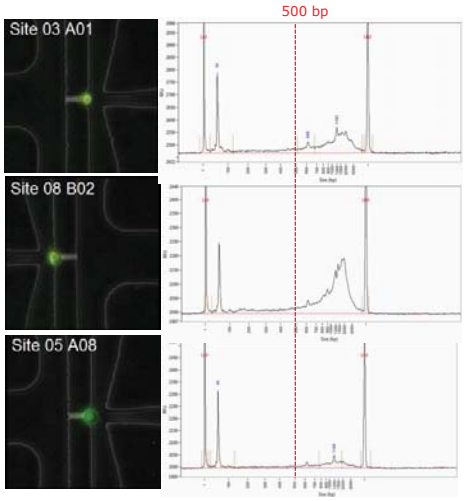
Cryopreservation

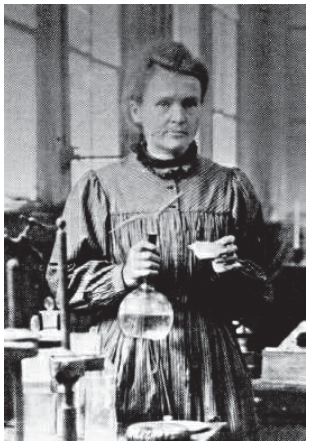


Centrifuge | Remove supernatant | Suspend with 90% FBS and 10% DMSO

Single-cell RNA-seq

Wash cells with DMEM





Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less.

(Marie Curie, 1867-1934)

Conclusions

best day

depends on the patient

endometrial factor

25% of cases

pET normalize clinical results.

RCT answer

ERA will be the first diagnostic line

Carlos Simon Laboratory

Senior Researchers:

Felipe Vilella, PhD (Lab. Manager)
Francisco Dominguez, PhD
Irene Cervelló, PhD
Patricia Diaz, PhD

Post-Doctoral Researchers:

Claudia Gil, PhD
Hortensia Ferrero, PhD

PhD Students &

Nuria Balaguer
Hannes Campo
Alessia Grasso
Jose M. Miguez
Stefania Salsano

Lab. Technicians:

Alicia Quiñonero
Amparo Faus
Maria Herrero
Marta Gonzalez

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Renee Reijo Pera Montana University USA
Steve Quake Stanford University, USA
Ruth Lathi Stanford University USA
Susan Fisher UCSF USA
Ayman Al-Hendy. Georgia Regents University USA

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EC Eurostars-Eureka Programme, No. E!6478-NOTED
Spanish Ministry of Science (SAF 2008-02048 & SAF 2012-31017)
Spanish Ministry of Health (EC11-299 & RD06/0010/1006)
Regional Valencian Ministry of Education (PROMETEO/2008/163 & PROMETEOII/2013/018)

Our Team

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References

1. Hertig AT, Rock J, Adams EC, Menkin MC. Thirty-four fertilized human ova, good, bad and indifferent, recovered from 210 women of known fertility; a study of biologic wastage in early human pregnancy. Pediatrics 1959;23:202-11.
2. Navot D, Scott RT, Droesch K, Veeck LL, Liu HC, Rosenwaks Z. The window of embryo transfer and the efficiency of human concep- tion in vitro. Fertil Steril 1991; 55(1):114–118
3. Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. Fertil Steril 1950;1:3–25
4. Murray MJ, Meyer WR, Zaino RJ, Lessey BA, Novotny DB, Ireland K, et al. A critical analysis of the accuracy, reproducibility, and clinical utility of histologic endometrial dating in fertile women. Fertil Steril 2004;81:1333–43.
5. Coutifaris C, Myers ER, Guzick DS, Diamond MP, Carson SA, Legro RS, et al. Histological dating of timed endometrial biopsy tissue is not related to fertility status. Fertil Steril 2004;82:1264–72.
6. Kliman HJ, Honig S, Walls D, Luna M, McSweet JC, Copperman AB. Optimization of endometrial preparation results in a normal endometrial function test (EFT) and good reproductive outcome in donor ovum recipients. J Assist Reprod Genet. 2006;23(7-8):299-303.
7. Dubowy RL, Feinberg RF, Keefe DL, Doncel GF, Williams SC, McSweet JC, Kliman HJ. Improved endometrial assessment using cyclin E and p27. Fertil Steril. 2003 ;80(1):146-56.
8. Quackenbush J. Microarray analysis and tumor classification. N Engl J Med. 2006 Jun 8;354(23):2463-72.
9. Domínguez F, Avila S, Cervero A, Martín J, Pellicer A, Castrillo JL, **Simón C**. A combined approach for gene discovery identifies insulin-like growth factor-binding protein-related protein 1 as a new gene implicated in human endometrial receptivity. J Clin Endocrinol Metab. 2003;88(4):1849-57.
10. Riesewijk A, Martin J, Horcajadas JA, Polman J, Pellicer A, Mosselman S, **Simón C**. Gene expression profiling of human endometrial receptivity on days LHþ2 versus LHþ7 by microarray technology. Mol Hum Reprod 2003;9:253–64.
11. Horcajadas JA, Riesewijk A, Polman J, van Os R, Pellicer A, Mosselman S, **Simón C**. Effect of controlled ovarian hyperstimulation in IVF on endometrial gene expression profiles. Mol Human Reprod 2005;11:195–205.
12. **Simón C**, Bellver J, Vidal C, Bosch E, Horcajadas JA, Murphy C, et al. Similar endometrial development in oocyte donors treated with high- or low-dose GnRH480 antagonist compared to GnRH-agonist treatment and natural cycles. Hum Reprod 2005;12:3318–27.

13. Horcajadas JA, Sharkey AM, Catalano RD, Sherwin JRA, Domınguez F, Burgos LA, Castro A, PerazaMR, Pellicer A, **Simón C**. Effect of an intrauterine device on the gene expression profile of the endometrium. J Clin Endocrinol Metabol 2006;91:3199–207.
14. Horcajadas JA, Riesewijk A, Mınguez P, Dopazo J, Esteban FJ, Domınguez F, Giudice LC, Pellicer A, Simón C. Controlled ovarian stimulation induces a functional genomic delay of the endometrium with potential clinical implications. J Clin Endocrinol Metab 2008;93:4500–10.
15. Horne AW, Phillips JA 3rd, Kane N, Lourenco PC, McDonald SE, Williams AR, Simon C, Dey SK, Critchley HO. CB1 expression is attenuated in Fallopian tube and decidua of women with ectopic pregnancy. PLoS One. 2008;3(12):e3969.
16. Labarta E, Martínez-Conejero JA, Alamá P, Horcajadas JA, Pellicer A, Simón C, Bosch E. Endometrial receptivity is affected in women with high circulating progesterone levels at the end of the follicular phase: a functional genomics analysis. Hum Reprod. 2011;26(7):1813-25.
17. Garrido-Gómez T, Domínguez F, Ruiz-Alonso M, **Simón C**. The Analysis of Endometrial receptivity. In: Textbook of Assisted Reproductive Techniques. UK; Informa Healthcare; 2012: 366-79.
18. Díaz-Gimeno P, Horcajadas JA, Martínez-Conejero JA, Esteban FJ, Alamá P, Pellicer A, **Simón C**. A Genomic Diagnostic Tool for Human Endometrial Receptivity Based on the Transcriptomic Signature. Fertil Steril 2011;95:50-60.
19. Díaz-Gimeno P, Ruiz-Alonso M, Blesa D, Bosch N, Martínez-Conejero JA, Alamá P, Garrido N, Pellicer A, **Simón C**. The accuracy and reproducibility of the endometrial receptivity array is superior to histological dating as diagnostic method for the endometrial factor. Fertil Steril. 2013;99(2):508-17.
20. Ruiz-Alonso M, Blesa D, Díaz-Gimeno P, Gómez E, Fernández-Sánchez M, Carranza F, Carrera J, Vilella F, Pellicer A, **Simón C**. The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure. Fertil Steril. 2013;100(3):818-24.
21. Wenk MR. The emerging field of lipidomics. Nat Rev Drug Discov. 2005;4(7):594-610. Review. Erratum in: Nat Rev Drug Discov. 2005 Sep;4(9):725.
22. Vilella F, Ramirez L, Berlanga O, Martínez S, Alamá P, Meseguer M, Pellicer A, **Simón C**. PGE2 and PGF2α concentrations in human endometrial fluid as biomarkers for embryonic implantation. J Clin Endocrinol Metab. 2013 Oct;98(10):4123-32.

23. Ruiz-Alonso M, Galindo N, Pellicer A, **Simón C**. What a difference two days make: “personalized” embryo transfer (pET) paradigm: A case report and pilot Study. Hum Reprod. 2014;29(6):1244-7.
24. Bermejo A, Iglesias C, Ruiz-Alonso M, Blesa D, **Simón C**, Pellicer A, García-Velasco J. The impact of using the combined oral contraceptive pill for cycle scheduling on gene expression related to endometrial receptivity. Hum Reprod. 2014; 29(6):1271-8.
25. Bermejo A, Cerrillo M, Ruiz-Alonso M, Blesa D, **Simón C**, Pellicer A, Garcia-Velasco JA. Impact of final oocyte maturation using gonadotropin-releasing hormone agonist triggering and different luteal support protocols on endometrial gene expression. Fertil Steril. 2014; 101(1):138-146.e3.
26. Miravet-Valenciano JA, Rincon-Bertolin A, Vilella F, **Simon C**. Understanding and improving endometrial receptivity. Curr Opin Obstet Gynecol. 2015; 27(3):187-92.
27. Gómez E, Ruíz-Alonso M, Miravet J, **Simón C**. Human Endometrial Transcriptomics: Implications for Embryonic Implantation. Cold Spring Harb Perspect Med. 2015; 5(7):a022996.
28. Garcia-Velasco JA, Fassbender A, Ruiz-Alonso M, Blesa D, D'Hooghe T, **Simon C**. Is endometrial receptivity transcriptomics affected in women with endometriosis? A pilot study. Reprod Biomed Online. 2015 ;31(5):647-54.
29. Ruiz-Alonso M, Díaz-Gimeno P, Gómez E, Rincón-Bertolín A, Vladimirov Y, Garrido N, **Simón C**. Clinical efficiency of embryo transfer performed in receptive vs non-receptive endometrium diagnosed by the endometrial receptivity array (ERA) (70th ASRM Annual Meeting, Honolulu, Hawaii. 2014) **P-466**
30. van der Gaast MH, Beckers NG, Beier-Hellwig K, Beier HM, Macklon NS, Fauser BC. Ovarian stimulation for IVF and endometrial receptivity--the missing link. Reprod Biomed Online. 2002;5 Suppl 1:36-43.
31. van der Gaast MH, Macklon NS, Beier-Hellwig K, Krusche CA, Fauser BC, Beier HM, Classen-Linke I. The feasibility of a less invasive method to assess endometrial maturation--comparison of simultaneously obtained uterine secretion and tissue biopsy. BJOG. 2009 Jan;116(2):304-12.
32. **Simón C**, Mercader A, Frances A, Gimeno MJ, Polan ML, Remohí J, Pellicer A. Hormonal regulation of serum and endometrial IL-1 alpha, IL-1 beta and IL-1ra: IL-1 endometrial microenvironment of the human embryo at the apposition phase under physiological and supraphysiological steroid level conditions. J Reprod Immunol. 1996 Oct;31(3):165-84.
33. Boomsma CM, Kavelaars A, Eijkemans MJ, Amarouchi K, Teklenburg G, Gutknecht D, Fauser BJ, Heijnen CJ, Macklon NS. Cytokine profiling in endometrial secretions: a non-invasive window on endometrial receptivity. Reprod Biomed Online. 2009;18(1):85-9

Immunologic Tests In Reproduction- Do these predict successful Implantation?

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DISCLOSURES
Immunologic Tests In Reproduction

- No conflict of Interest

LEARNING OBJECTIVES
Immunologic Testing in Reproduction

At the end of this presentation, the participant should be able to:

- Define the antiphospholipid syndrome
- Discuss the role of antithyroid antibodies
- Understand Natural Killer cells
- Know the guidelines of ACOG and ASRM

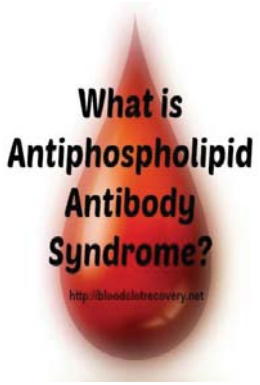
Autoimmune Antibodies
Possible pathophysiologic roles

- Actual pathogenic agents of disease (Causative-Erythroblastosis fetalis)
- Arise as a consequence of another disease process (Tissue damage-Systemic Lupus)
- Merely mark the presence of another etiological agent (Footprint-Hepatitis antibodies)

Kutteh The Endocrinologist 6:462-466,1996

Antiphospholipid Antibodies

- IgG or IgM or IgA isotypes
- Bind to phospholipids
- Includes lupus anticoagulant
- Harmful actions on trophoblast



Kutteh & Hinote. APS. Obstet Gynecol Clin N Am. 2014;41:113-132

Research Diagnostic Criteria for APS

Clinical Criteria	Laboratory Criteria
Recurrent loss <10 wk	Lupus anticoagulant
Fetal death > 10 wk	IgG antiCL (> 99%)
Venous Thrombosis	IgM antiCL (> 99%)
Arterial Thrombosis	IgG anti β2- glycoprotein
	IgM anti β2- glycoprotein

Miyakis et al. J Thromb Haemost 4:295 – 306, 2006

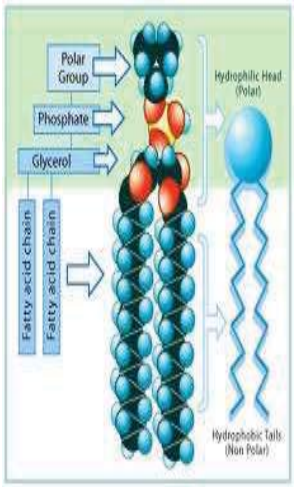
ASRM and ACOG Guidelines

"The three antiphospholipid antibodies that should be tested"

1) lupus anticoagulant
2) anticardiolipin
3) anti-beta-2-glycoprotein 1

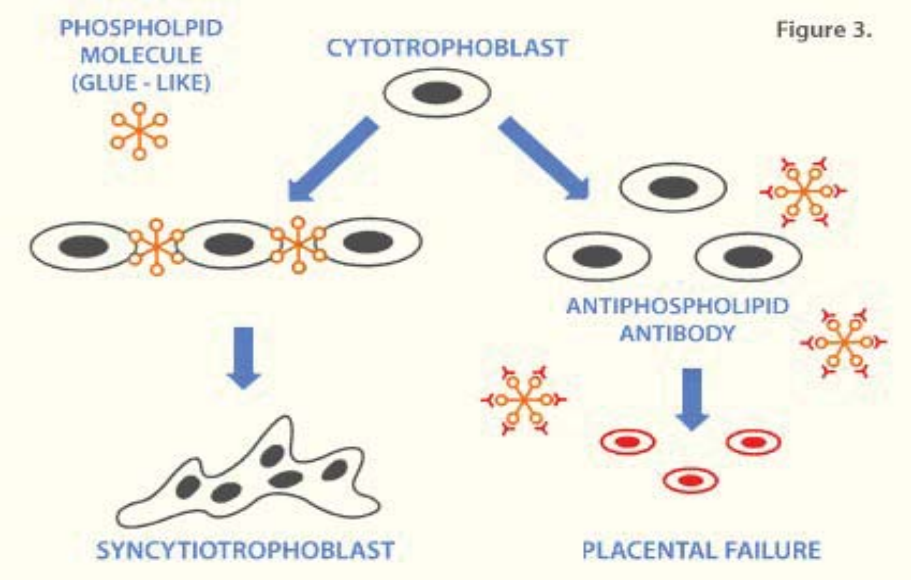
ASRM Practice Committee Fertil Steril 98:1103-1111, 2012
Branch et al., ACOG Bulletin 132 Obstet Gynecol. 120:1514-1521,2012.

What about other aPL Antibodies?



- Phosphatidylinositol
- Phosphatidylglycerol
- Phosphatidylserine
- Diphosphatidylglycerol
- Phosphatidylethanolamine
- Phosphatidylcholine
- Phosphatidic acid

In vitro action of Antiphospholipid Antibodies



ASRM and ACOG Guidelines- Antiphospholipid Antibodies and Recurrent Loss

"The combination of twice daily unfractionated heparin or low molecular weight heparin and low-dose aspirin appears to confer a significant benefit in pregnancies with aPLs and otherwise unexplained recurrent pregnancy loss;

Comparable efficacy of low molecular weight heparin has not been established"

ASRM Practice Committee Fertil Steril 98:1103-1111, 2012
ACOG Bulletin 132 Obstet Gynecol. 120:1514-1521,2012

Antiphospholipid Antibodies do not affect IVF Outcome

TABLE 1

Anti-phospholipid antibodies and IVF outcome.

Outcome	Authors	Odds ratio	(95% CI)
Pregnancy	Birdsall et al (11)	1.65	(0.50, 5.46)
	Denis et al (12)	0.91	(0.42, 1.97)
	El Roiey et al (13)	0.26	(0.04, 1.83)
	Gleicher et al (14)	1.34	(0.36, 4.95)
	Kowalik et al (15)	1.38	(0.52, 3.34)
	Kutteh et al (16)	.85	(0.21, 3.50)
	Sher et al (3)	.55	(0.13, 2.34)
Average for Pregnancy		.99	(0.64, 2.34)
Live Birth	Birdsall et al (11)	1.67	(0.50, 5.56)
	Denis et al (12)	.94	(0.44, 1.98)
	El-Roiey et al (13)	.18	(0.02, 2.14)
	Gleicher et al (14)	1.60	(0.39, 6.53)
	Kowalik et al (15)	1.10	(0.42, 2.90)
Average from Live Birth		1.07	(0.66, 1.75)

ASRM Practice Committee Fertil Steril 2006; 86:S224-S225

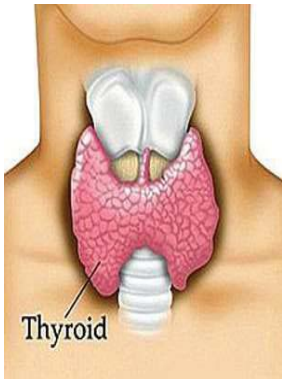
ASRM Guidelines-Antiphospholipid Antibodies and Implantation Failure

1. Antiphospholipid antibody abnormalities were not associated with IVF success or outcome.
2. Assessment of antiphospholipid antibodies is not indicated among couples undergoing IVF.
3. Therapy (with IVIG and anti-thrombogenic therapy) is not justified on the basis of existing data.

ASRM Practice Committee Fertil Steril 2006; 86:S224-S225
ACOG Bulletin 132 Obstet Gynecol. 120:1514-1521,2012

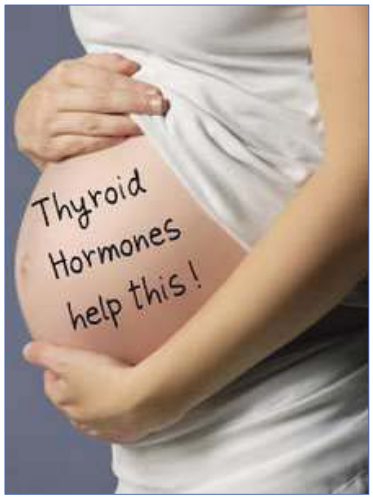
Hypothyroidism and Antithyroid Antibodies

- Overt hypothyroidism is associated with infertility, RPL and adverse pregnancy outcomes
- The normal range for TSH in nonpregnant reproductive-aged women is 1.0 -2.5 mU/L
- Thyroid antibodies may precede the occurrence of hypothyroid disease
- 15-20% of reproductive-aged women may have antithyroid antibodies

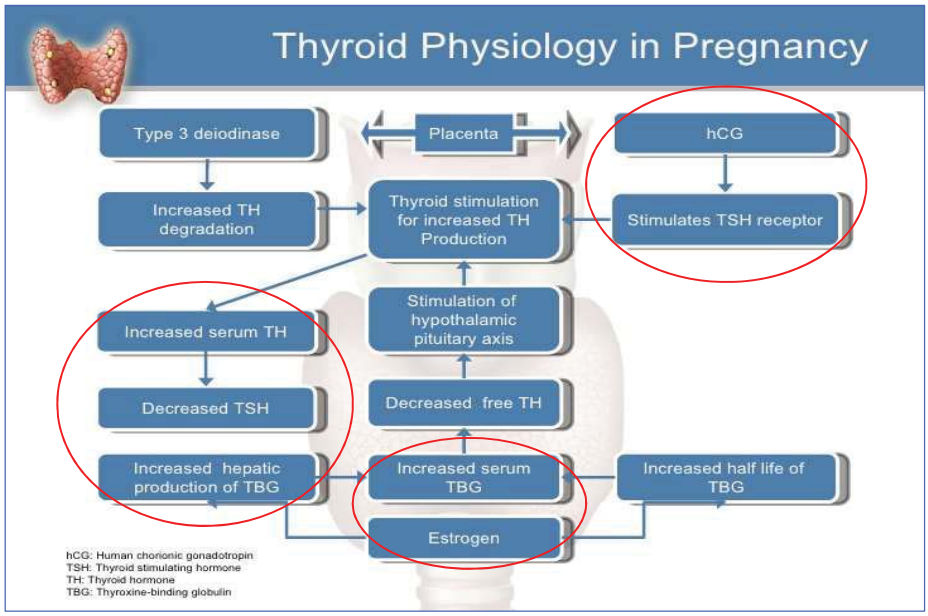


Importance of Thyroid Hormone in Pregnancy

- **Thyroid hormones are important for fetal brain development (cognitive function)**
- **Fetal Thyroid does not begin to function until 12 weeks gestation**
- **Thyroid hormones bind to nuclear receptors in the fetal brain (which apprear prior to the time of fetal thyroid hormone production.**
- **Thyroid hormone crosses the placenta starting in the first trimester**



Burrow, Fisher, Larsen. Maternal and Fetal Thyroid Function. N Engl J Med 1994;331:1077-8

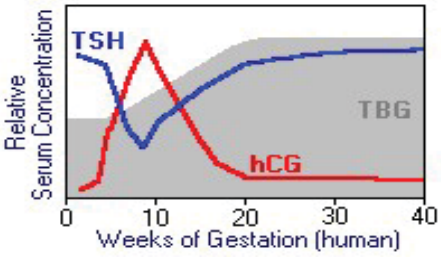


Glinoer D. Endocrin Rev. The regulation of thyroid function in pregnancy 1997;18:404-33.

Trimester-Specific TSH Levels

TRIMESTER	TSH LEVEL
First	2.5 mU/L (0.1 – 2.5)
Second	3.0 mU/L (0.2 – 3.0)
Third	3.5 mU/l (0.3 -3.5)

Stricker et al. Evaluation of maternal thyroid function during pregnancy. Eur J Endocrinol 2007; 157:509-114.



Antithyroid Antibodies Associated with Miscarriage

Increased prevalence of antithyroid antibodies identified in women with recurrent pregnancy loss but not in women undergoing assisted reproduction

William H. Kutteh, M.D., Ph.D., Deborah L. Yetman, B.S., Alexander C. Carr, B.S., Leslie A. Beck, B.S., and Richard T. Scott, Jr., M.D.

Conclusion: Antithyroid antibodies are identified more frequently in women with recurrent pregnancy loss but not in women undergoing ART. These antibodies may be markers of autoimmune activation and have been associated with an increased risk of pregnancy loss and postpartum thyroid disease.

Kutteh, Yetman, Carr, Beck, and Scott Fertil Steril 1999;71:843-848.

Thyroid Immunity and Miscarriage

Table 1 Meta-analysis of case–control studies on the association between miscarriage and the presence of antithyroid autoantibodies.

Reference	Patients No. of Ab +ve (%)	Controls No. of Ab +ve (%)	Odds ratio	95% CI
Pratt *et al.* (8)	≥3 abortions 14/45 (31%)	Blood donors 19/100 (19%)	1.93	0.86–3.37
Bussen & Steck (9)	≥3 abortions 8/22 (36%)	No abortions 3/44 (7%)	7.81	1.82–33.6
Bussen & Steck (10)	≥3 abortions 11/28 (39%)	No abortions 2/28 (7%)	8.41	1.70–42.8
Esplin *et al.* (11)	≥3 abortions 22/74 (29%)	≥3 pregnancies 28/75 (37%)	0.71	0.50–1.01
Kutteh *et al.* (12)	≥2 abortions 158/700 (23%)	Blood donors 29/200 (15%)	1.72	1.12–2.65
Mecacci *et al.* (13)	≥2 abortions or ≥1 fetal death 20/51 (39%)	Unknown 10/69 (15%)	3.81	1.95–9.14
Dendrinos *et al.* (14)	≥3 abortions 11/30 (37%)	≥1 pregnancies 2/15 (13%)	3.76	0.71–19.87
Bagis *et al.* (15)	≥1 abortion 54/162 (33%)	No abortions 54/714 (8%)	5.98	3.98–9.38
Total	298/1112 (27%)	147/1245 (12%)	2.73	2.20–3.40

Prummel & Wiersinga. Eur J Endocrinol 2004;150:751-755.

BMJ

RESEARCH

Association between thyroid autoantibodies and miscarriage and preterm birth: meta-analysis of evidence

Shakila Thangaratinam, senior lecturer/consultant in obstetrics and gynaecology and clinical epidemiology,[1] Alex Tan, academic foundation trainee,[2] Ellen Knox, consultant obstetrician/subspecialist in fetal medicine,[3] Mark D Kilby, professor of fetal medicine,[2,3] Jayne Franklyn, professor of medicine,[2] Arri Coomarasamy, reader/consultant gynaecologist/subspecialist in reproductive medicine and surgery[2]

Conclusion The presence of maternal thyroid autoantibodies is strongly associated with miscarriage and preterm delivery. There is evidence that treatment with levothyroxine can attenuate the risks.

Thangaratinam et al. BMJ 2011; 342: d2616

Increased Miscarriage in Subfertile Women with Thyroid Antibodies and Normal TSH (<4.5 mU/

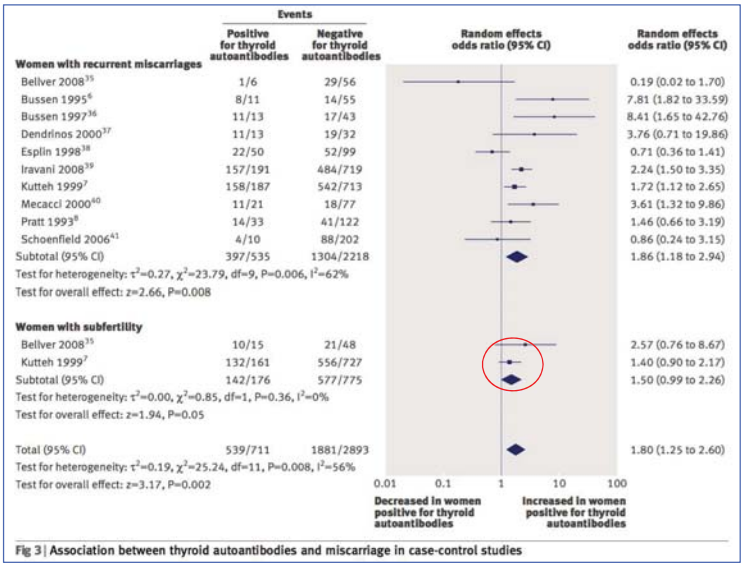


Fig 3 | Association between thyroid autoantibodies and miscarriage in case-control studies

Thangaratinam et al. BMJ 2011;342: d2616

European Journal of Endocrinology (2010) **162** 643–652 ISSN 0804-4643

REVIEW

Risk of spontaneous miscarriage in euthyroid women with thyroid autoimmunity undergoing IVF: a meta-analysis

Konstantinos A Toulis[1], Dimitrios G Goulis[1], Christos A Venetis[2], Efstratios M Kolibianakis[2], Roberto Negro[3], Basil C Tarlatzis[2] and Ioannis Papadimas[1]

[1]Unit of Reproductive Endocrinology and [2]Unit for Human Reproduction, First Department of Obstetrics and Gynecology, Medical School, Papageorgiou General Hospital, Aristotle University, Ring Road, Nea Efkarpia, 56403 Thessaloniki, Greece and [3]Department of Endocrinology, Azienda Ospedaliera, 'V. Fazzi', Piazza F. Muratore, 73100 Lecce, Italy

Conclusion: Based on the currently available evidence, it appears that the presence of TAI is associated with an increased risk for spontaneous miscarriage in ~~subfertile women achieving a~~ pregnancy through an IVF procedure.

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The Journal of Clinical Endocrinology & Metabolism 91(7):2587–2591

doi: 10.1210/jc.2005-1603

Levothyroxine Treatment in Euthyroid Pregnant Women with Autoimmune Thyroid Disease: Effects on Obstetrical Complications

Roberto Negro, Gianni Formoso, Tiziana Mangieri, Antonio Pezzarossa, Davide Dazzi, and Haslinda Hassan

Department of Endocrinology (R.N., G.F.), Azienda Ospedaliera LE/1, 73100 Lecce, Italy; Department of Obstetrics and Gynecology (T.M.), Casa di Cura "Salus", 72100 Brindisi, Italy; Department of Internal Medicine (A.P., D.D.), Azienda Ospedaliera PR, "Di Vaio" Hospital, 43036 Fidenza, Italy; and Endocrine Unit (H.H.), Raja Isteri Pengiran Anak Saleha Hospital, Bandar Seri Begawan, Brunei Darussalam BA 1000

Conclusions: Euthyroid pregnant women who are positive for TPOAb develop impaired thyroid function, which is associated with an increased risk of miscarriage and premature deliveries. Substitutive treatment with LT_4 is able to lower the chance of miscarriage and premature delivery. (***J Clin Endocrinol Metab*** **91: 2587–2591, 2006)**

ASRM PAGES

Subclinical hypothyroidism in the infertile female population: a guideline

Practice Committee of the American Society for Reproductive Medicine
American Society for Reproductive Medicine, Birmingham, Alabama

Summary statement. There is good evidence that thyroid autoimmunity is associated with miscarriage and fair evidence that it is associated with infertility. Levothyroxine treatment may improve pregnancy outcomes in women with positive thyroid antibodies, especially if the TSH level is over 2.5 mIU/L.

Fertility and Sterility® VOL. 104 NO. 3 / SEPTEMBER 2015

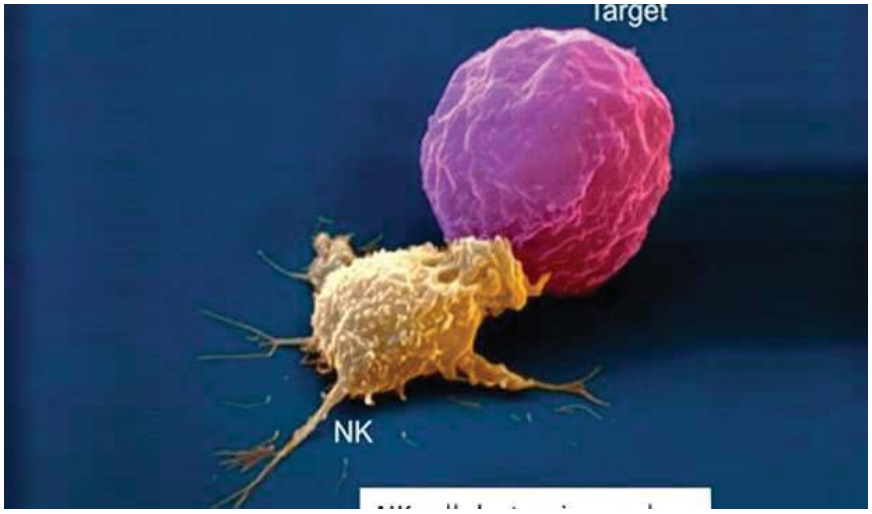
Summary of Recommendations

	TSH Screening	Treat TSH > 2.5 mU/L	ATA Screening	Treating ATA
The Endocrine Society	Targeted	Yes	No	No
American College of Obstetricians and Gynecologists	Targeted	?	No	No
American Thyroid Association	Targeted	Yes	No	No
American Society for Reproductive Medicine	Targeted	yes	No	No

Thyroid Immunity –Take Home Messages

- The presence of TA is associated with RPL and premature delivery
- Screening for TA and treatment with Thyroxine particularly when the TSH is > 2.5 mU/L
- If treated, frequent monitoring is safe and is recommended
- Patients with TA (not treated) should be monitored closely for potential hypothyroidism
- Treatment initiated if TSH rises above trimester-specific range

"NK Cell Destroying Embryo"

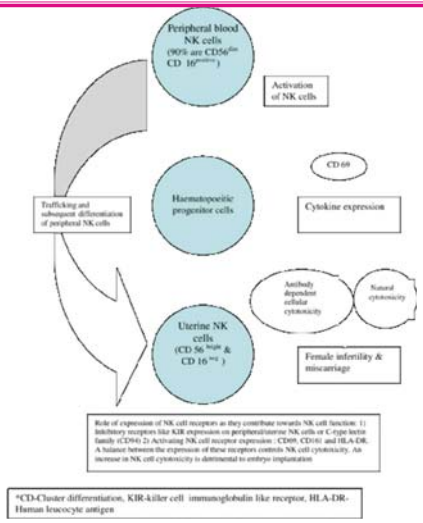


The Fetal Allograft Puzzle

The mysterious lack of rejection of the fetus has puzzled generations of immunologists, and no comprehensive explanation has yet emerged. One problem is that acceptance of the fetal allograft is so much the norm that it is difficult to study the mechanism that prevents rejection; if the mechanism for rejecting the fetus is rarely activated, how can one analyze the mechanism that controls it?

Colucci, Moffett, Trowsdale. Medawar and the immunological paradox of pregnancy: 60 years on. Eur J Immunol 2014;44:1883-5.

Differences of expression of Peripheral Blood and Uterine natural killer (NK) cells .



Srividya Seshadri, and Sesh Kamal Sunkara Hum. Reprod. Update 2014;20:429-438

Natual Killer Cells (NK)-pbNK vs uNK

- NK cells in the peripheral circulation (pbNK) have effector functions in killing target cells
- NK Cells differ in distribution and function in the endometrium and in the circulation
- Immune cells with a similar phenotype to NK cells but poor killers populate the uterine lining at implantation (uNK) and during early pregnancy
- Knock-out mice genetically engineered to lack uNK endometrial cells are unable to reproduce

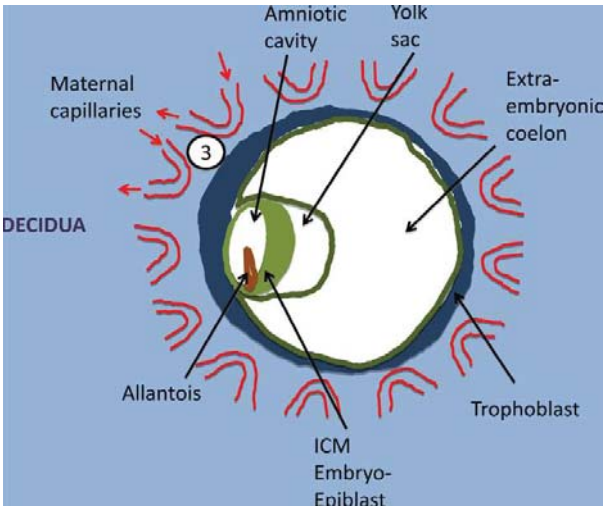
Moffet and Shreeve. Hum Reprod. 2015;30:1519-1525.

Natural Killer (NK) Cells: pbNK vs uNK

- Paternal MHC Class 1a antigens are expressed on extravillous and endovascular trophoblasts
  - Thought to regulate uterine natural killer (uNK) cells in the decidua
- pbNK cells have activating receptors that can trigger cytolytic activity and secrete cytokines
  - Thought to be vital in eliminating pathogens, especially viruses and cells infected with viruses
  - Can be triggered by foreign HLA
- uNK cells are the most represented lymphomyeloid cells in the human decidua in the first trimester
  - CD 56^{bright} and CD 16^{-}

Clark DA. Popular myths in reproductive immunology. J Reprod Immunol 2014;104-105:54-62.
Sharma S. Natural killer cells and regulatory T cells in early pregnancy loss. Int J Dev Biol. 2014;58(2-4):219-29.

uNK Cells are Important in Early Embryo Implantation



Clark DA. Popular myths in reproductive immunology. J Reprod Immunol. 2014 Oct;104-105:54-62.

Uterine Natural Killer (uNK) Cells

- Embryo survival depends on maintenance of immune tolerance at the maternal-fetal interface
- uNK cells are key immune cells that populate uterus
- Not "Killers" in normal pregnancy
  - Necessary for healthy development
- Many cells are involved with "cross-talk" between placenta and trophoblast
  - Vital to establish tolerance
  - Regulatory T cells (Tregs) vital in this interaction

Clark DA. Popular myths in reproductive immunology. J Reprod Immunol 2014;104-105:54-62.
Sharma S. Natural killer cells and regulatory T cells in early pregnancy loss. Int J Dev Biol. 2014;58(2-4):219-29.
l.

Function of uNK in Implantation

- uNK play a role in building health placenta
- Angiogenic factors secreted by uNK
- uNK cells play a role in regulation of decidualization
- uNK maintain a balance of excessive trophoblast intrusion and defective placentation
- Summary: uNK do not need to be suppressed

Clark DA. Popular myths in reproductive immunology. J Reprod Immunol. 2014 Oct;104-105:54-62.
Moffett and Shreeve. First do no harm: uNK cells in ART Human Reprod. 2015;30:1519-1525

Proposed Testing & Treatment

- Often considered "propriatary" and panels differ from center to center
- IVIg is commonly used treatment every 28 days
- Treatment lengths vary
  - Can start as early as 2-3 weeks before conception
  - Can continue as late as 35 weeks
- Typically, costs are $3,000 per IVIG infusion

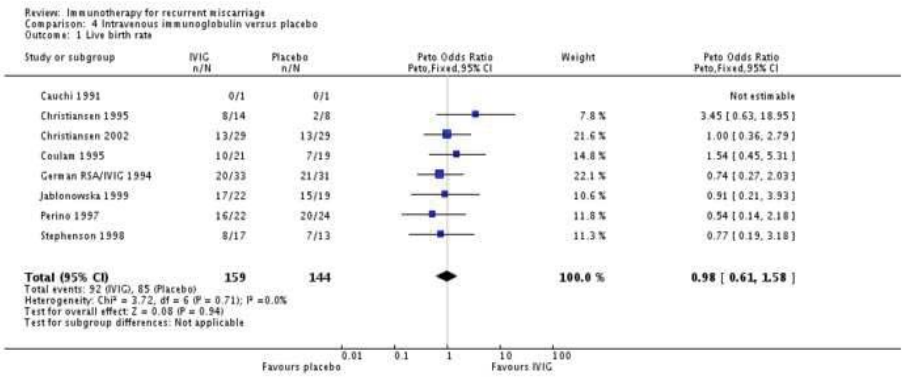
http://www.stirrup-queens.com/2006/07/testing-for-recurrent-pregnancy-loss/
Clark DA. Popular myths in reproductive immunology. J Reprod Immunol. 2014 Oct;104-105:54-62.

Agents used for Immunomodulation in ART

DRUG	COST (USD)	CLINICAL USES	SIDE EFFECTS/ADVERSE EVENTS
Lipid Emulsion (Intralipid)	$425 Per infusion	Parenteral nutrition Given with propofol	Liver and spleen dz, thrombocytopenia
Intravenous Immunoglobulin (IVIG)	$2,500 Per infusion	Immunoglobulin deficiency states, hematologic and neurologic disorders, transplants	Meningitis, renal failure, thrombosis, enteritis, infections
Corticosteroids	$2.50 Per 28 tabs	Inflammation, allergy, asthma, autoimmune disease	Diabetes, osteoporosis Ulcers, Cushings dz
Anti-TNF	$500 40mg inject	Autoimmune disease, rheumatic and inflammatory disease	Infection, lymphoma, CHF, lupus-like dz
Granulocyte-CSF	$75 300mcg inj	Neutropenia, recurrent and HIV infection	Liver/spleenomegaly, osteoporosis, gout

Moffett & Shreeve. First do no harm: uNK cells in ART. Hum Reprod 2015; 30:1519-1525

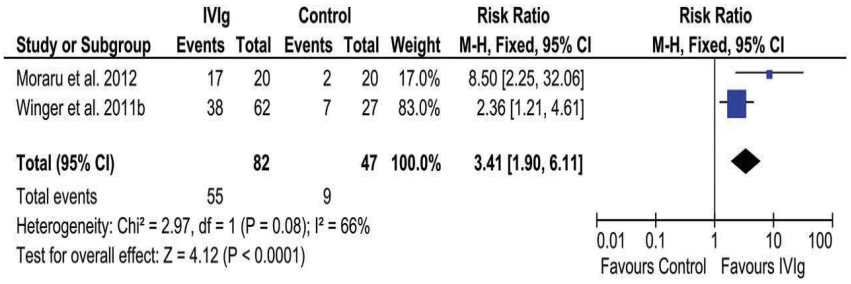
Cochrane Review : No effect of IVIG treatment on outcome—live birth rate.



"Paternal cell immunization and IVIG provide no beneficial effect over placebo in improving live birth rate"

Wong, Porter, Scott. Immunotherapy for recurrent miscarriage. Cochrane Database Syst Rev 2014;10:CD000112

Clinical pregnancy rates in ART cycles where IVIg was administered.



Polanski et al. Interventions in women with elevated NK cells undergoing ART. Hum. Reprod. 2014;29:65-75

Summary NK Cells

- Conflicting data
  - No standardization of testing and treatments
  - Protocols vary from center to center
- ASRM Practice Committee Opinion (2012)
  - NK Cell testing/treatment is not recommended
- ASRM Fact Sheet (2014)
  - "There is no proof that intravenous (IV) infusions of blood products (such as intravenous immunoglobulin [IVIG]) or intralipids decrease the risk of miscarriage."

ASRM Practice Committee. Evaluation and treatment of RPL. Fertil Steril. 2012 Nov;98(5):1103-11.
Sharma S. NKcells and regulatory T cells in early pregnancy loss. Int J Dev Biol. 2014;58(2-4):219-29.
https://www.asrm.org/FACTSHEET_Treatment_of_recurrent_pregnancy_loss/

Summary Testing & Treatment for Implantation Failure

TEST CONSIDERED	TEST RECOMMENDED	TREATMENT RECOMMENDED
Antiphospholipid Antibodies	NO	NO
Antithyroid antibodies	NO	NO
TSH	YES	YES, if TSH > 2.5
Natural Killer Cells	NO	NO

References Immune Testing (1)

- Kutteh The Endocrinologist 1996;6:462-466
- Kutteh & Hinote. APS. Obstet Gynecol Clin N Am. 2014;41:113-132
- Miyakis et al. J Thromb Haemost 2006;4:295-306
- ASRM Practice Committee Fertil Steril 98:1103-1111, 2012
- Branch et al., ACOG Bulletin 132 Obstet Gynecol. 120:1514-1521,2012
- *ASRM Practice Committee Fertil Steril 2006; 86:S224-S225*
- Burrow, Fisher, Larsen. Maternal and Fetal Thyroid Function. N Engl J Med 1994;331:1077-8
- Glinoer D. The regulationof thyroid function in pregnancy. Endocrin Rev. 1997;18:404-33.
- Stricker et al. Evaluation of maternal thyroid function during pregnancy. Eur J Endocrinol 2007; 157:509-114.
- Kutteh et al. Fertil Steril 1999;71:843-848.
- Prummel & Wiersinga. Eur J Endocrinol 2004;150:751-755.
- Thangaratinam et al. BMJ 2011;342: d2616
- Toulis et al. Eur J Endocrin. 2010;162:643-52.
- Negro et al. JCEM. 2006;91:2587-91.
- Colucci, Moffett, Trowsdale. Medawar and the immunological paradox of pregnancy: 60 years on. Eur J Immunol 2014;44:1883-5.

References Immune Testing (2)

- Practice Committee of the ASRM Subclinical hypothyroidism. Fertil Steril 2015;104:545-553.
- Clark DA. Popular myths in reproductive immunology. J Reprod Immunol 2014;104-105:54-62.
- Sharma S. Natural killer cells and regulatory T cells in early pregnancy loss. Int J Dev Biol. 2014;58(2-4):219-29.
- Moffett & Shreeve. First do no harm:uterine natural killer cells in assisted reproduction. Hum Reprod 2015; 30:1519-1525
- Seshadri, Sunkara NK cells in female infertility and recurrent miscarriage: a systematic review and meta-analysis Hum. Reprod. Update 2014;20:429-438
- Wong, Porter, Scott. Immunotherapy for recurrent miscarriage. Cochrane Database Syst Rev 2014;10:CD000112
- Polanski et al. Interventions in women with elevated NK cells undergoing ART. Hum. Reprod. 2014;29:65-75
- Sharma S. Natural killer cells and regulatory T cells in early pregnancy loss. Int J Dev Biol. 2014;58(2-4):219-29.

Sperm DNA fragmentation: Does it impact live birth rate after IVF or ICSI?

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Conflict of interest

- None to declare

Learning objectives

- To understand the biological basis for sperm DNA fragmentation
- To appreciate the biological consequences of DNA fragmentation
- To understand the level of existing evidence on the relationship between DNA fragmentation and outcome of IVF/ICSI
- To appreciate the limited utility of the currently available DNA fragmentation tests

Outline

- Introduction
- Do sperm DNA integrity test predict IVF outcome?
- Does the extent of sperm DNA fragmentation affect IVF or ICSI outcome?

Sperm DNA Fragmentation- Biological Basis

- The main pathway that leads to sperm DNA breaks is a process of apoptosis triggered by testicular conditions and by oxidative stress during the transit in the male genital tract (Muratori et al., 2015)
- Once the sperm nucleus has been introduced into the ooplasm, the condensed nucleus undergoes rapid de-condensation to release the DNA for formation of a paternal pronucleus. Any abnormal change in the structural organization can cause delays or defects in the delivery of the paternal DNA.

Sperm DNA Fragmentation- Biological Basis

- Any damage to the DNA during the transition from the testicle to the egg cannot be repaired until the DNA is accessible for DNA repair systems in the ooplasm.
- The risk of error during the repair process increases with the number of DNA strand breaks in an individual sperm nucleus.

Sperm DNA Fragmentation-Biological Basis

- In animals, induced sperm chromatin fragmentation severely delayed the replication of the paternal pronucleus and severe damage led to arrested embryo development

(Gawecka et al., 2013)

Sperm DNA Fragmentation-Biological Basis

- When the DNA damage is less severe (mostly single-stranded breaks), there is no detectable delay in the DNA synthesis but chromosomal breaks are detected at mitosis demonstrating that DNA synthesis is possible in the zygote with some breaks

(Gawecka et al., 2013)

- In both cases, embryo development might be compromised. These are the reasons why the injection of a spermatozoa with fragmented DNA can be detrimental.

Impact of Sperm DNA fragmentation on the IVF/ICSI outcome

- Contradictory evidence:
  - sperm DNA fragmentation in predicting fertilization, embryo development, implantation, birth defects in the offspring and early pregnancy loss

(Gandini et al., 2004; Huang et al., 2005; Borini et al., 2006; Bungum et al., 2007; Simon et al., 2011; Sakkas, 2013; Palermo et al., 2014)

Impact of Sperm DNA fragmentation on the IVF/ICSI outcome

- However, some studies found that sperm with DNA damage were capable of fertilizing an oocyte because they only found a modest effect on conception rates with conventional IVF and little, if any, effect with intracytoplasmic sperm injection (ICSI)

Bungum, et al Hum Reprod, 19 (2004), pp. 1401–1408

Virro, et al.,Fertil Steril, 81 (2004), pp. 1289–1295
Check, et al., Arch Androl, 51 (2005), pp. 121–124
Benchaib, Jet al., Fertil Steril, 87 (2007), pp. 93–100
Bungum et al.Hum Reprod, 22 (2007), pp. 174–179
Frydman et al.Fertil Steril, 89 (2008), pp. 92–97

Impact of Sperm DNA fragmentation on the IVF/ICSI outcome

- Two major categories of selection method are currently used:
  - those aiming to enhance the number of spermatozoa with intact DNA in the sperm population used for ICSI and
  - those aiming to isolate the single spermatozoon with the lowest chance of having fragmented DNA for the injection.

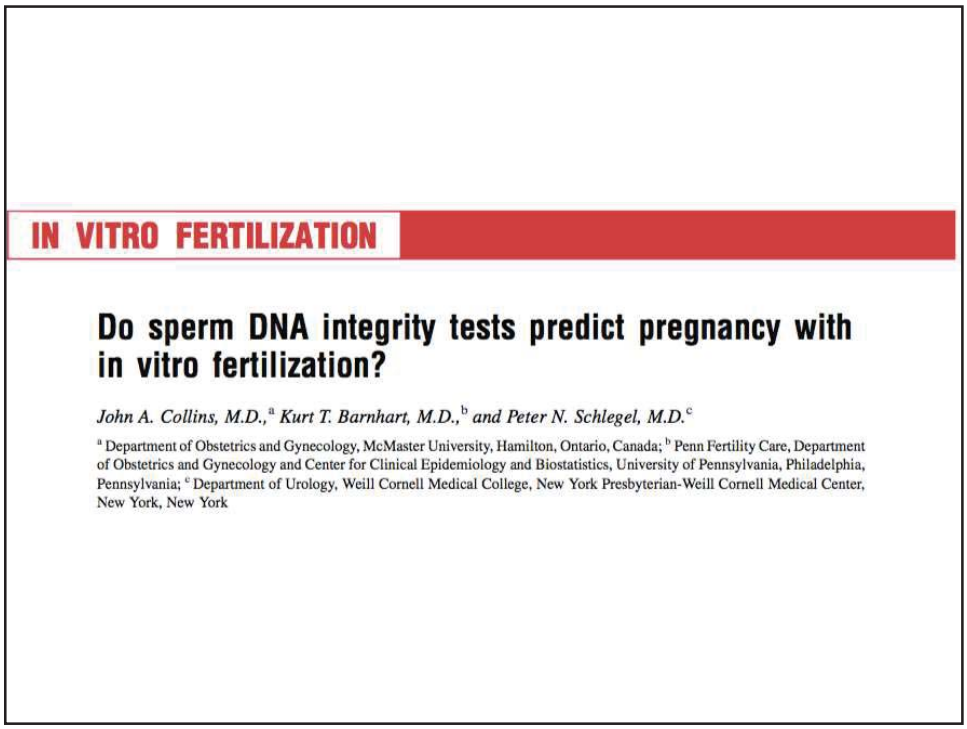
Impact of Sperm DNA fragmentation on the IVF/ICSI outcome

- To date, there is no reliable approach to completely filter out spermatozoa with DNA strand breaks from an ejaculate

(Zini et al., 2000; Gandini et al., 2004; Stevanato et al., 2008; Ebner et al., 2011).

Ultimately a single sperm is selected in ICSI, and these assays destroy the gametes they interrogate.

Even if a DNA integrity test can tell us that the sperm of an individual may be more or less likely to result in pregnancy or miscarriage,
we still have no way of translating that knowledge into a truly useful clinical outcome.



IN VITRO FERTILIZATION

Do sperm DNA integrity tests predict pregnancy with in vitro fertilization?

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[a] Department of Obstetrics and Gynecology, McMaster University, Hamilton, Ontario, Canada; [b] Penn Fertility Care, Department of Obstetrics and Gynecology and Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania, Philadelphia, Pennsylvania; [c] Department of Urology, Weill Cornell Medical College, New York Presbyterian-Weill Cornell Medical Center, New York, New York

TABLE 1

Methodological features: studies of the association between sperm DNA fragmentation and pregnancy.

Study	Treatment	Assay	Normal range	Cycles	Pregnancy outcome	Outcome rates (%)
Boe-Hanson et al., 2006 (46)	IVF	SCSA	DFI <27%	139	Clinical	28
	ICSI	SCSA	DFI <27%	47	Clinical	30
Borini et al., 2006 (52)	IVF	TUNEL	<10%	82	Clinical	22
	ICSI	TUNEL	<10%	50	Clinical	24
Bungum et al., 2007 (27)	IVF	SCSA	DFI <30%	388	Delivery	28
	ICSI	SCSA	DFI <30%	223	Delivery	38
Check et al., 2005 (47)	IVF	SCSA	DFI <30%	106	Ongoing	17
Gandini et al., 2004 (48)	ICSI	SCSA	DFI <30%	22	Full term	41
Host et al., 2000 (53)	IVF	TUNEL	≤4%	175	Biochemical	29
	ICSI	TUNEL	≤4%	61	Biochemical	34
Huang et al., 2005 (54)	IVF	TUNEL	≤4%	217	Pregnancy	55
	ICSI	TUNEL	≤4%	86	Pregnancy	51
Larson et al., 2000 (24)	IVF, ICSI	SCSA	DFI <27%	24	Pregnancy	29
Larson-Cook et al., 2003 (25)	IVF, ICSI	SCSA	DFI <27%	89	Clinical	31
Payne et al., 2005 (49)	IVF, ICSI	SCSA	DFI <27%	94	Clinical	33
Seli et al., 2004 (14)	IVF, ICSI	TUNEL	<20%	49	Clinical	47
Virro et al., 2004 (50)	IVF, ICSI	SCSA	DFI <30%	249	Ongoing	41
Zini et al., 2005 (51)	ICSI	SCSA	DD ≤30%	60	Clinical	52

Note: DD, sperm DNA denaturation; DFI, DNA fragmentation index; SCSA, sperm chromatin structure assay; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end labeling assay.

Collins. Sperm DNA integrity tests. Fertil Steril 2008.

TABLE 2

Diagnostic test properties: studies of the association between sperm DNA fragmentation and pregnancy.

Study	Treatment	Sens	Spec	Sens + Spec	Abnormal tests (%)	DOR	(95% CI)
Boe-Hanson et al., 2006 (46)	IVF	0.06	0.97	1.03	5	2.04	(0.38, 11.0)
	ICSI	0.36	0.57	0.94	38	0.76	(0.21, 2.73)
Borini et al., 2006 (52)	IVF	0.17	0.89	1.06	16	1.57	(0.38, 6.51)
	ICSI	0.71	0.75	1.46	60	6.55	(1.77, 24.3)
Bungum et al., 2004 (26)	IVF	0.17	0.85	1.02	16	1.16	(0.64, 2.12)
	ICSI	0.30	0.63	0.93	33	0.74	(0.42, 1.31)
Check et al., 2005 (47)	IVF	0.30	0.83	1.13	27	1.90	(0.61, 5.89)
Gandini et al., 2004 (48)	ICSI	0.38	0.44	0.83	45	0.52	(0.10, 2.74)
Host et al., 2000 (53)	IVF	0.34	0.80	1.14	30	1.91	(0.93, 3.91)
	ICSI	0.58	0.38	0.96	59	0.84	(0.29, 2.43)
Huang et al., 2005 (54)	IVF	0.22	0.83	1.04	19	1.30	(0.66, 2.56)
	ICSI	0.64	0.50	1.14	57	1.78	(0.76, 4.16)
Larson et al., 2000 (24)	IVF, ICSI	0.58	0.94	1.59	42	10.17	(1.77, 58.4)
Larson-Cook et al., 2003 (25)	IVF, ICSI	0.17	0.98	1.16	11	5.08	(1.24, 20.8)
Payne et al., 2005 (49)	IVF, ICSI	0.16	0.71	0.87	20	0.44	(0.15, 1.27)
Seli et al., 2004 (14)	IVF, ICSI	0.46	0.61	1.07	43	1.32	(0.43, 4.07)
Virro et al., 2004 (50)	IVF, ICSI	0.35	0.81	1.17	29	2.27	(1.30, 3.96)
Zini et al., 2005 (51)	ICSI	0.17	0.81	0.98	18	0.87	(0.24, 3.19)

Note: CI, confidence interval; DOR, diagnostic odds ratio; Sens, sensitivity; Spec, specificity.

Collins. Sperm DNA integrity tests. Fertil Steril 2008.

A DOR greater than 1.0 means that with abnormal DNA integrity test results the chance of disease (in this case nonpregnancy) with IVF or ICSI is

higher.

TABLE 3

Likelihood ratios: studies of the association between sperm DNA fragmentation and pregnancy.

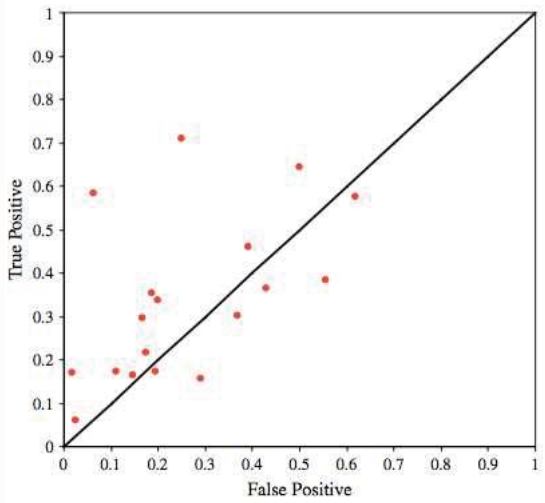
Study	Treatment	LR (+)	95% CI	LR (–)	(95% CI)
Boe-Hanson et al., 2006 (46)	IVF	2.34	(0.27, 20.1)	0.96	(0.05, 18.1)
	ICSI	0.85	(0.24, 3.03)	1.11	(0.56, 2.22)
Borini et al., 2006 (52)	IVF	1.55	(0.31, 7.72)	0.93	(0.28, 3.08)
	ICSI	2.84	(0.65, 12.5)	0.39	(0.27, 0.54)
Bungum et al., 2004 (26)	IVF	1.14	(0.61, 2.11)	0.98	(0.56, 1.71)
	ICSI	0.82	(0.46, 1.45)	1.11	(0.76, 1.60)
Check et al., 2005 (47)	IVF	1.77	(0.47, 6.65)	0.85	(0.48, 1.50)
Gandini et al., 2004 (48)	ICSI	0.69	(0.12, 3.89)	1.38	(0.51, 3.79)
Host et al., 2000 (53)	IVF	1.68	(0.77, 3.69)	0.83	(0.56, 1.23)
	ICSI	0.93	(0.32, 2.74)	1.12	(0.63, 1.98)
Huang et al., 2005 (54)	IVF	1.25	(0.63, 2.45)	0.95	(0.53, 1.69)
	ICSI	1.29	(0.54, 3.06)	0.71	(0.49, 1.05)
Larson et al., 2000 (24)	IVF, ICSI	9.33	(0.46, 190)	0.44	(0.27, 0.74)
Larson-Cook et al., 2003 (25)	IVF, ICSI	9.82	(0.55, 174)	0.85	(0.23, 3.13)
Payne et al., 2005 (49)	IVF, ICSI	0.54	(0.19, 1.50)	1.19	(0.41, 3.41)
Seli et al., 2004 (14)	IVF, ICSI	1.18	(0.38, 3.68)	0.88	(0.51, 1.53)
Virro et al., 2004 (50)	IVF, ICSI	1.90	(1.04, 3.47)	0.79	(0.58, 1.10)
Zini et al., 2005 (51)	ICSI	0.89	(0.24, 3.31)	1.03	(0.28, 3.77)

Note: CI, confidence interval; LR(+), likelihood ratio of a positive (abnormal) test result; LR(–), likelihood ratio of a normal test result.

Collins. Sperm DNA integrity tests. Fertil Steril 2008.

FIGURE 2

Receiver operating characteristics (ROC) curve: sperm DNA integrity and pregnancy.



Collins. Sperm DNA integrity tests. Fertil Steril 2008.

This association was not adequate by itself to discriminate which couples would conceive after treatment. The sensitivity and specificity of the test in different studies were scattered around the nondiscriminatory diagonal of the ROC space.

The sensitivity and specificity of the test in different studies were scattered around the nondiscriminatory diagonal of the ROC space. In general, likelihood ratios less than 0.5

TABLE 4

Subgroup analyses among studies of sperm DNA integrity and pregnancy.

	Number of studies	Number of cycles	DOR	95% CI	*P* value
Treatment					
IVF	6	1107	1.53	(0.77, 3.02)	
ICSI	7	549	1.12	(0.59, 2.15)	.41
IVF, ICSI	5	505	1.91	(0.79, 4.57)	
Assay type					
SCSA	11	1441	1.31	(0.81, 2.11)	.48
TUNEL	7	720	1.67	(0.89, 3.11)	

Note: CI, confidence interval; SCSA, sperm chromatin structure assay; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end labeling assay.

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Note: CI, confidence interval; SCSA, sperm chromatin structure assay; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end labeling assay.

Collins. Sperm DNA integrity tests. Fertil Steril 2008.

Recent evidence

Tests: SCSA, COMET, TUNEL

Does the extent of sperm DNA fragmentation affect IVF or ICSI outcome?
A systematic review and meta analysis

www.sciencedirect.com
www.rbmonline.com

REVIEW

The effect of sperm DNA fragmentation on live birth rate after IVF or ICSI: a systematic review and meta-analysis

A Osman *, H Alsomait, S Seshadri, T El-Toukhy, Y Khalaf

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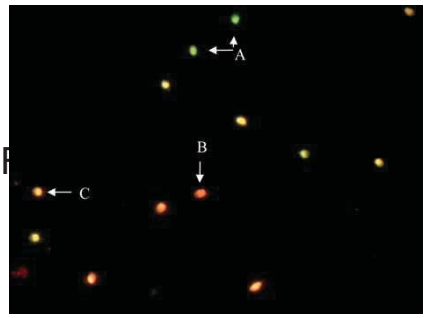
Dr Amira Osman received her medical training (MBBCh) in 2003, MSc in 2007 and MD in 2012 from the Department of Obstetrics and Gynaecology in Cairo University, Egypt. She became a member of the Royal College of Obstetricians and Gynaecologists, UK, in 2010. She joined Guys and St Thomas NHS trust in 2011 and for the last 2 years has specialized in reproductive medicine and assisted conception.

Sperm chromatin structure assay SCSA

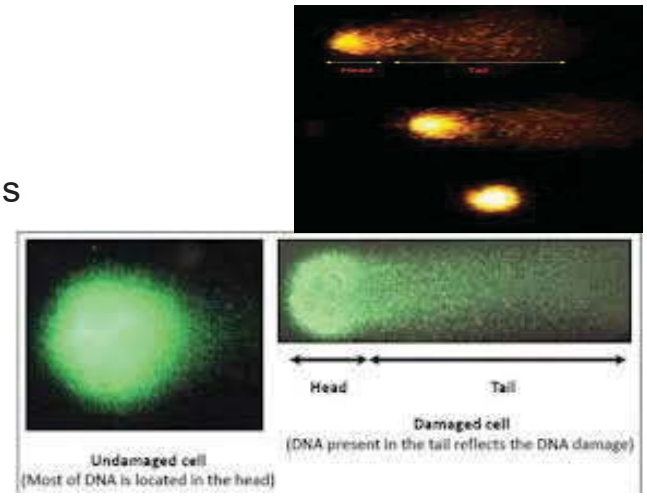
- Indirect test
- DNA fragmentation index(DF
- DFI >30% poor fertility



(A) Green fluorescence represented sperm with normal double stranded DNA. (B) Red or orange fluorescence represented sperm with single-stranded DNA.

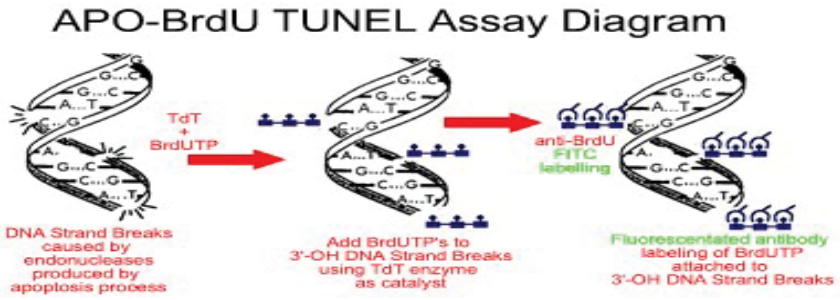
Comet

Single cell gel electrophoresis



TUNEL

deoxynucleotidyl transferase –mediated Dutp nick end labeling



Objective

To evaluate the effect of sperm DNA

fragmentation on IVF and ICSI outcome

Guy's and St Thomas' NHS
NHS Foundation Trust

INCLUSION CRITERIA

- Sperm DNA damage detected by SCSA,TUNEL or COMET
- SDF in raw/prepared semen in men undergoing IVF/ICSI
- Clinical pregnancy rate and live birth rate as outcome

Methodology

1304 citations

1200 not relevant

104 papers reviewed

57 not fulfilling inclusion criteria

7 no clear cut off

15 unable to construct 2x2 table

25 studies included

25 Studies
3360 couples

SCSA	TUNEL	COMET
14 (1621 couples)	8 (1233 couples)	3 (506 couples)

IVF 2	5	6
ICSI 2	8	4
IVF + ICSI 1	6	1

Methods

Study design:

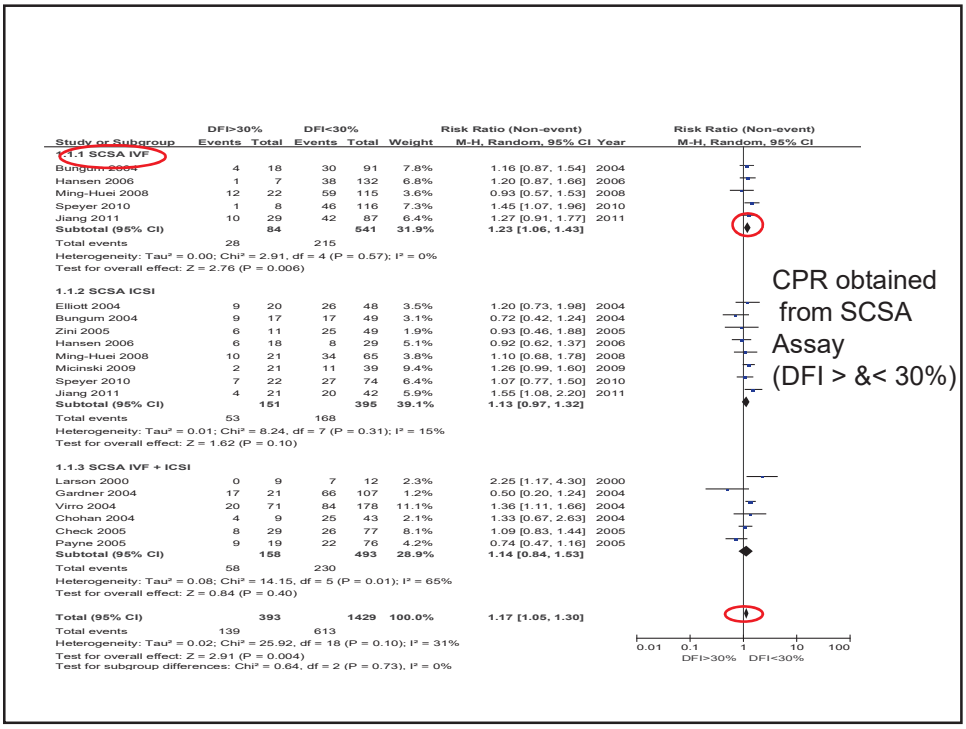
- 13 Prospective studies
- 4 Retrospective studies
- 8 Unclear

Threshold levels for assays

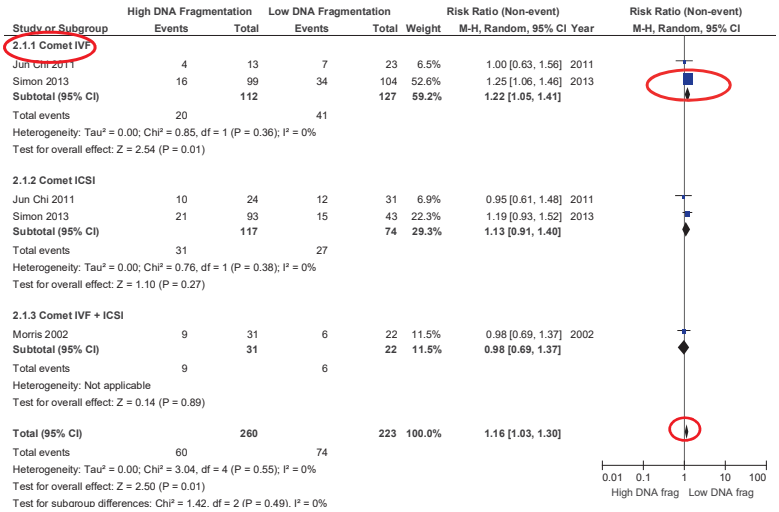
SCSA > 30%

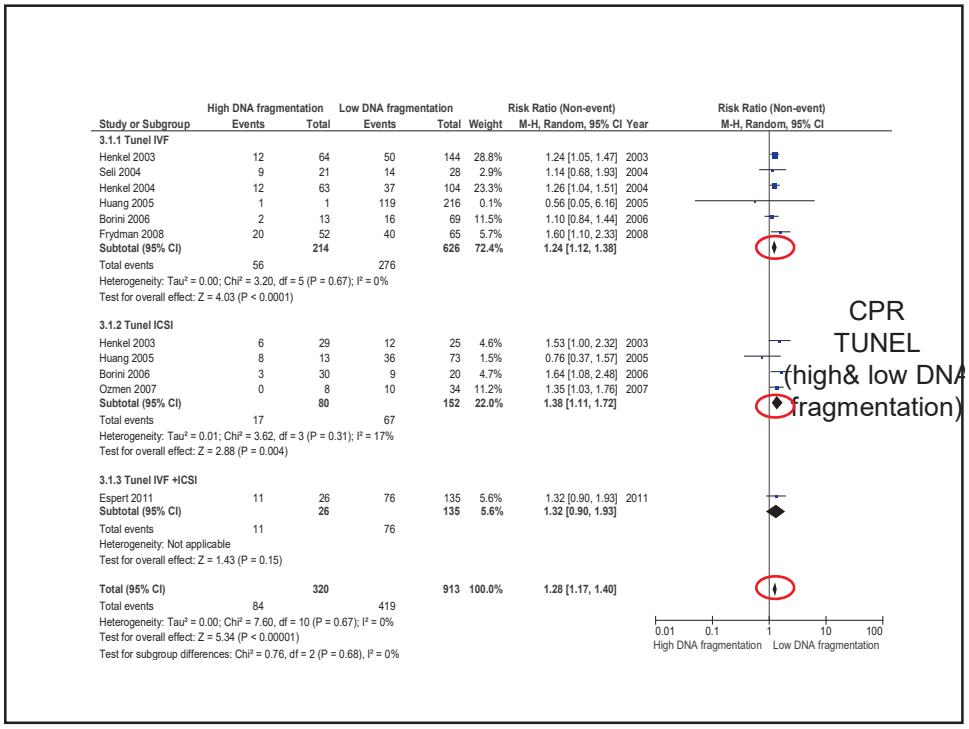
TUNEL >10%

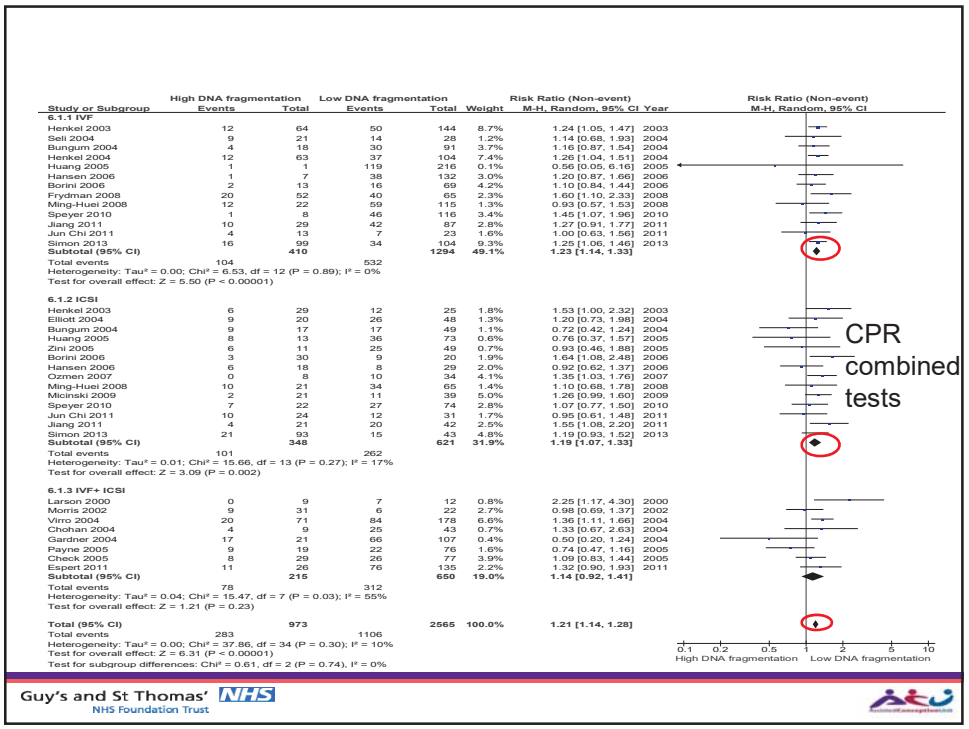
COMET >14%



CPR obtained from **COMET** (high & low DNA fragmentation)







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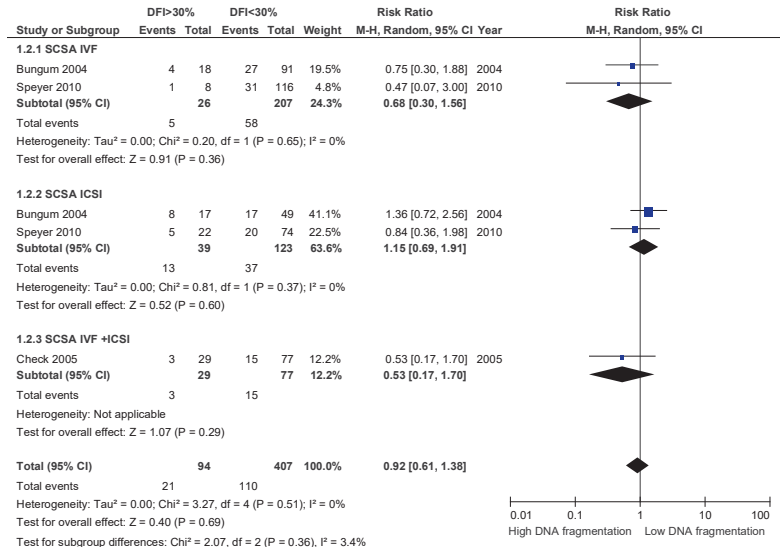
LBR obtained by **SCSA** (high & low DNA fragmentation)



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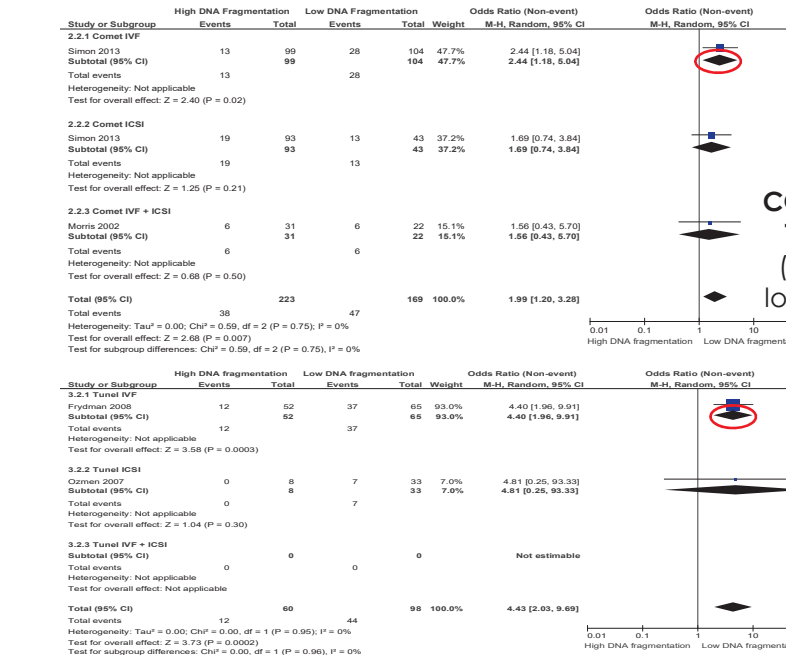
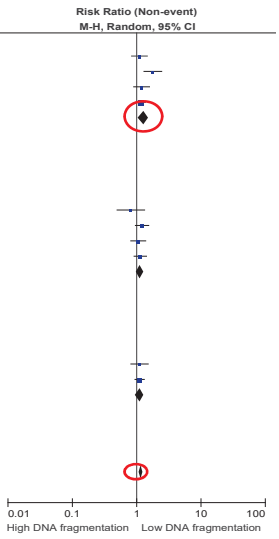
NHS Foundation Trust



LBR COMET & TUNEL (high & low DNF)

LBR obtained from combined tests

Study or Subgroup	High DNA fragmentation Events	Total	Low DNA fragmentation Events	Total	Weight	Risk Ratio (Non-event) M-H, Random, 95% CI	Year
6.2.1 IVF							
Bungum 2004	4	18	27	91	7.5%	1.11 [0.84, 1.46]	2004
Frydman 2008	12	52	37	65	6.0%	1.79 [1.30, 2.45]	2008
Speyer 2010	1	8	31	116	7.3%	1.19 [0.90, 1.59]	2010
Simon 2013	13	99	28	104	23.0%	1.19 [1.03, 1.37]	2013
Subtotal (95% CI)		**177**		**376**	**43.7%**	**1.27 [1.05, 1.52]**	
Total events	30		123				
Heterogeneity: Tau² = 0.02; Chi² = 6.79, df = 3 (P = 0.08); I² = 56%							
Test for overall effect: Z = 2.52 (P = 0.01)							
6.2.2 ICSI							
Bungum 2004	8	17	17	49	2.6%	0.81 [0.50, 1.33]	2004
Ozmen 2007	0	8	7	33	9.9%	1.21 [0.95, 1.54]	2007
Speyer 2010	5	22	20	74	8.2%	1.06 [0.81, 1.38]	2010
Simon 2013	19	93	13	43	11.2%	1.14 [0.91, 1.42]	2013
Subtotal (95% CI)		**140**		**199**	**32.0%**	**1.11 [0.97, 1.27]**	
Total events	32		57				
Heterogeneity: Tau² = 0.00; Chi² = 2.60, df = 3 (P = 0.46); I² = 0%							
Test for overall effect: Z = 1.56 (P = 0.12)							
6.2.3 IVF+ ICSI							
Morris 2002	6	31	6	22	6.3%	1.11 [0.81, 1.51]	2002
Check 2005	3	29	15	77	18.0%	1.11 [0.94, 1.31]	2005
Subtotal (95% CI)		**60**		**99**	**24.3%**	**1.11 [0.96, 1.29]**	
Total events	9		21				
Heterogeneity: Tau² = 0.00; Chi² = 0.00, df = 1 (P = 0.98); I² = 0%							
Test for overall effect: Z = 1.43 (P = 0.15)							
Total (95% CI)		**377**		**674**	**100.0%**	**1.17 [1.08, 1.26]**	
Total events	71		201				
Heterogeneity: Tau² = 0.00; Chi² = 10.46, df = 9 (P = 0.31); I² = 14%							
Test for overall effect: Z = 3.72 (P = 0.0002)							
Test for subgroup differences: Chi² = 1.50, df = 2 (P = 0.47), I² = 0%							



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Weaknesses

- Heterogeneity
  - Threshold level
  - Study design
  - Specimen tested
  - Patient selection
- Small sample size in individual studies

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NHS Foundation Trust

Summary

Clinical pregnancy rate

	SCSA	COMET	TUNEL	COMBINED
IVF	S	S	S	S
ICSI	NS	NS	S	S
IVF + ICSI	NS	NS	NS	NS
TOTAL	S	S	S	S

Live birth rate

	SCSA	COMET	TUNEL	COMBINED
IVF	NS	S	S	S
ICSI	NS	NS	NS	NS
IVF + ICSI	NS	NS	-	NS
TOTAL	NS	S	S	S

S : significant

NS: non significant

Summary

Clinical pregnancy rate

	SCSA	COMET	TUNEL	COMBINED
IVF	**S**	**S**	**S**	**S**
ICSI	NS	NS	S	S
IVF + ICSI	NS	NS	NS	NS
TOTAL	S	S	S	S

Live birth rate

	SCSA	COMET	TUNEL	COMBINED
IVF	NS	S	S	S
ICSI	NS	NS	NS	NS
IVF + ICSI	NS	NS	-	NS
TOTAL	NS	S	S	S

S : significant

NS: non significant

Summary

Clinical pregnancy rate

	SCSA	COMET	TUNEL	COMBINED
IVF	S	S	S	S
ICSI	**NS**	**NS**	**S**	**S**
IVF + ICSI	NS	NS	NS	NS
TOTAL	S	S	S	S

Live birth rate

	SCSA	COMET	TUNEL	COMBINED
IVF	NS	S	S	S
ICSI	NS	NS	NS	NS
IVF + ICSI	NS	NS	-	NS
TOTAL	NS	S	S	S

S : significant

NS: non significant

Summary

Clinical pregnancy rate

	SCSA	COMET	TUNEL	COMBINED
IVF	S	S	S	S
ICSI	NS	NS	S	S
IVF + ICSI	NS	NS	NS	NS
TOTAL	S	S	S	S

Live birth rate

	SCSA	COMET	TUNEL	COMBINED
IVF	NS	S	S	S
ICSI	**NS**	**NS**	**NS**	**NS**
IVF + ICSI	NS	NS	-	NS
TOTAL	NS	S	S	S

S : significant

NS: non significant

Summary

Clinical pregnancy rate

	SCSA	COMET	TUNEL	COMBINED
IVF	S	S	S	S
ICSI	NS	NS	S	S
IVF + ICSI	**NS**	**NS**	**NS**	**NS**
TOTAL	S	S	S	S

Live birth rate

	SCSA	COMET	TUNEL	COMBINED
IVF	NS	S	S	S
ICSI	NS	NS	NS	NS
IVF + ICSI	**NS**	**NS**	**-**	**NS**
TOTAL	NS	S	S	S

S : significant

NS: non significant

Conclusion

1- SDF appears to influence IVF and possibly ICSI outcome by 17-21%

2- Need for standardized criteria in a large prospective study

- LBR as outcome
- Agreed threshold level and specimen
- Standardized inclusion and exclusion criteria for patient
- IVF and ICSI separately

3- Need to consider intervention studies to lower SDF

Guy's and St Thomas' NHS Foundation Trust

Bibliography

1-Practice Committee of the American Society for Reproductive Medicine. The clinical utility of sperm DNA integrity testing: a guideline.

Fertil Steril. 2013 Mar 1;99(3):673-7. doi: 10.1016/j.fertnstert.2012.12.049. Epub 2013 Feb 1.

2-Palermo GD, Neri QV, Cozzubbo T, Rosenwaks Z. Perspectives on the assessment of human **sperm** chromatin integrity

Fertil Steril. 2014 Dec;102(6):1508-17.

3-Collins JA, Barnhart KT, Schlegel PN

Do sperm **DNA** integrity tests predict pregnancy with in vitro fertilization?

Fertil Steril. 2008 Apr;89(4):823-31.

4-Osman A, Alsomait H, Seshadri S, El-Toukhy T, Khalaf Y.

The effect of sperm **DNA fragmentation** on live birth rate after IVF or ICSI: a systematic review and meta-analysis.

Reprod Biomed Online. 2015 Feb;30(2):120-7.

Thank you

MICRODISSECTION TESTICULAR SPERM EXTRACTION (MICRO TESE): DOES IT IMPROVE LOCALIZATION OF SPERM?

(COMPARED TO CONVENTIONAL TESE)

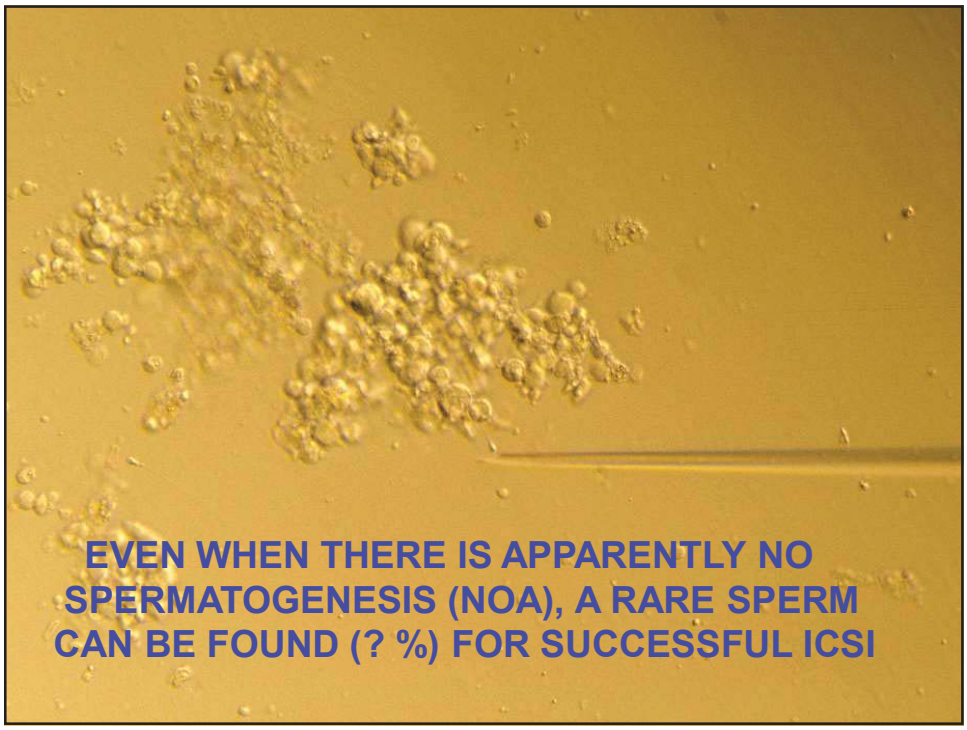
SHERMAN SILBER, M.D.
ST LUKES HOSPITAL
ST LOUIS, MISSOURI

NO CONFLICT OF INTEREST

LEARNING OBJECTIVES

- UNDERSTAND THE CAUSES OF AZOOSPERMIA.
- UNDERSTAND SPERM RETRIEVAL TECHNIQUES FOR OBSTRUCTIVE VS. NON-OBSTRUCTIVE AZOOSPERMIA.
- BE ABLE TO SELECT THE MOST EFFECTIVE AND SAFE SPERM RETRIEVAL TECHNIQUES.
- UNDERSTAND THE DIFFERENCES IN IVF SUCCESS WITH EPIDIDYMAL VS TESTIS SPERM.
- UNDERSTAND TESTIS ANATOMY AND SPERMATOGENIC STEM CELL BIOLOGY.
- UNDERSTAND FERTILITY PRESERVATION FOR PREPUBERTAL BOYS.
- UNDERSTAND IPS CELLS AND CREATION OF SPERMATOZOA FROM SKIN CELLS.


EVEN WHEN THERE IS APPARENTLY
NO SPERMATOGENESIS (NOA)

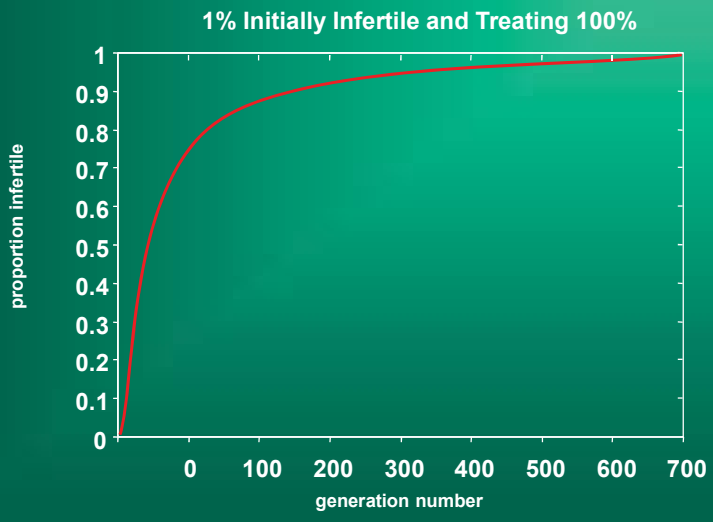

EVEN WHEN THERE IS APPARENTLY NO
SPERMATOGENESIS (NOA), A RARE SPERM
CAN BE FOUND (? %) FOR SUCCESSFUL ICSI

Mathematical Model for Decrease in Male Fertility in Subsequent Generations

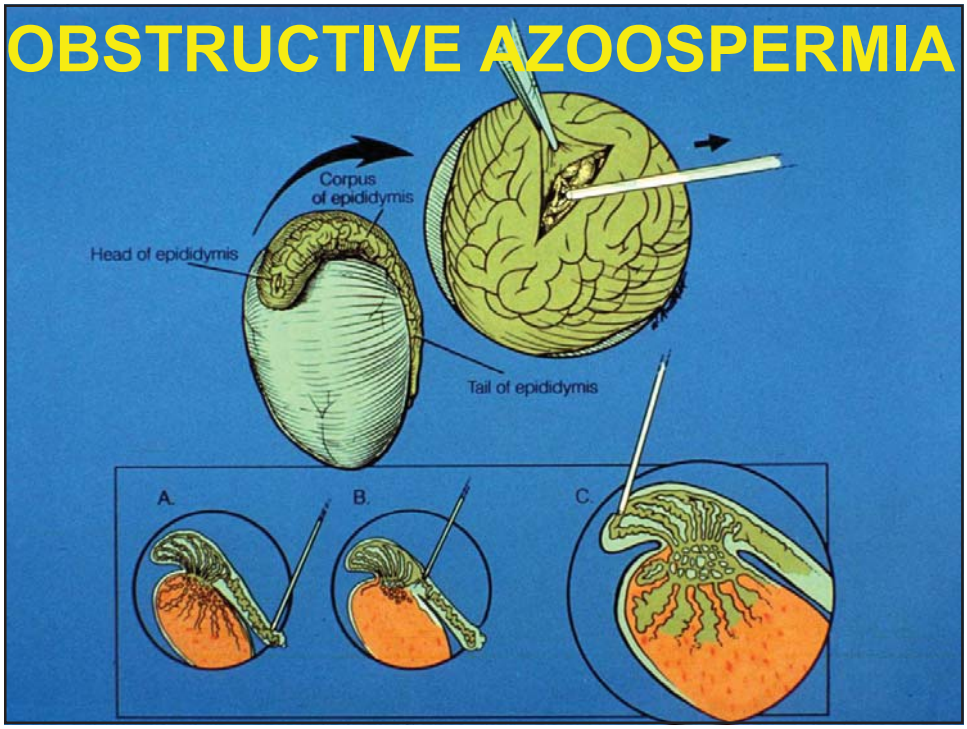
$$P_{i+1} = \frac{[\,(1-p_i) \times 0.01 + p_i \times \theta\,]}{[\,(1-p_i) + p_i \times \theta\,]}$$

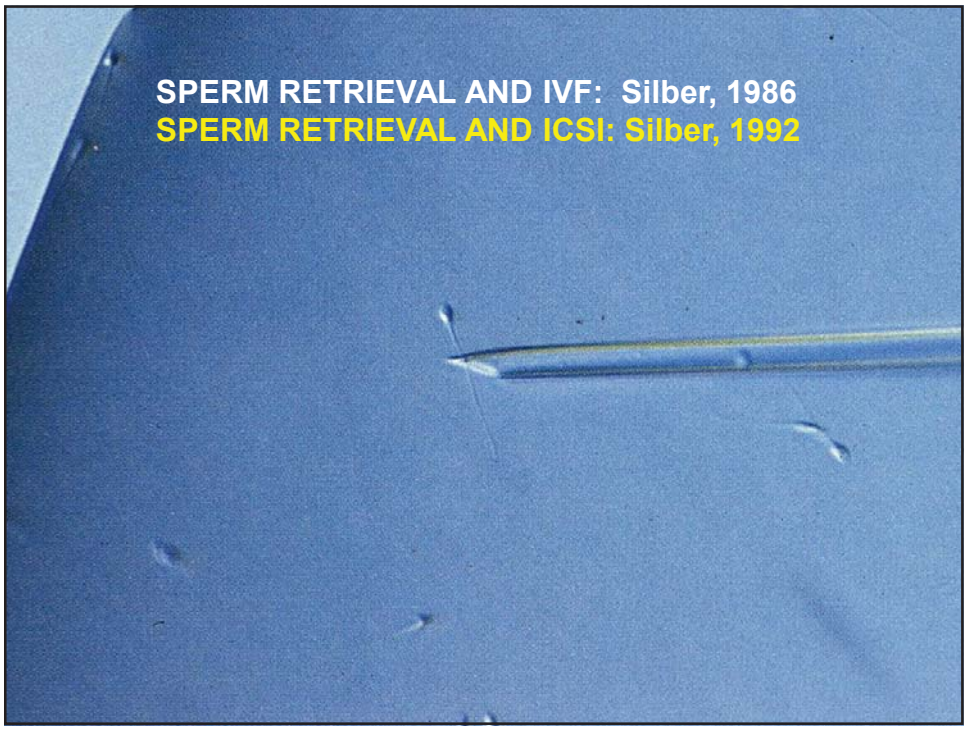
WHAT WILL HAPPEN IF AZOOSPERMIC MEN NOW CAN ALL HAVE OFFSPRING WITH THE SAME PROBLEM, AND ALL UNDERGO SUCCESSFUL ICSI?

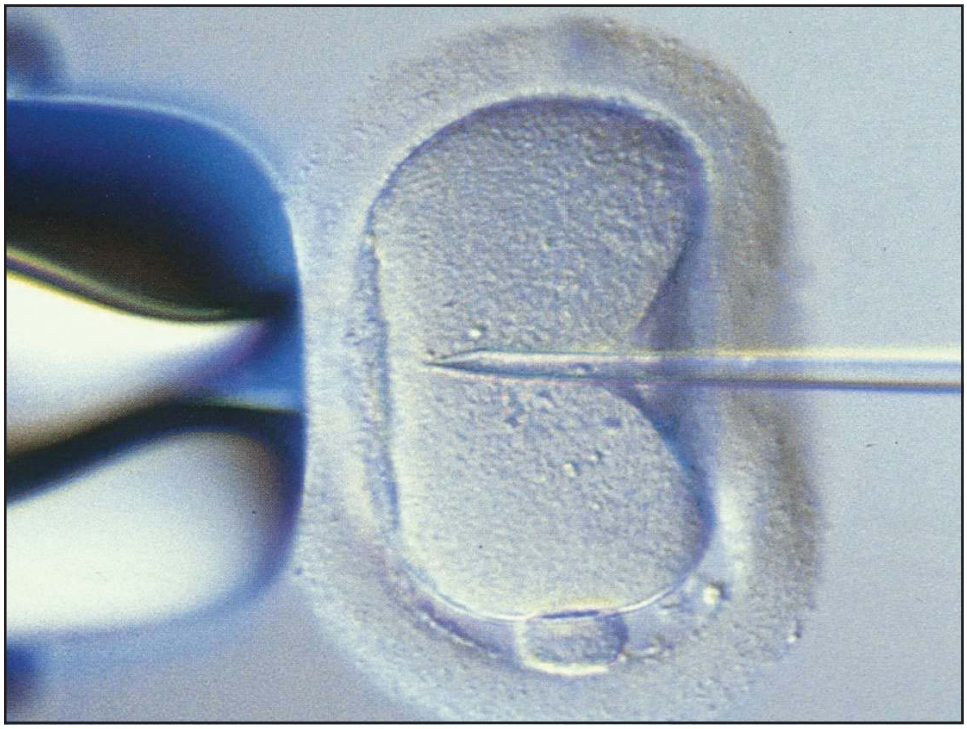
Transmission of Severe Male Infertility to Future Generations via ICSI

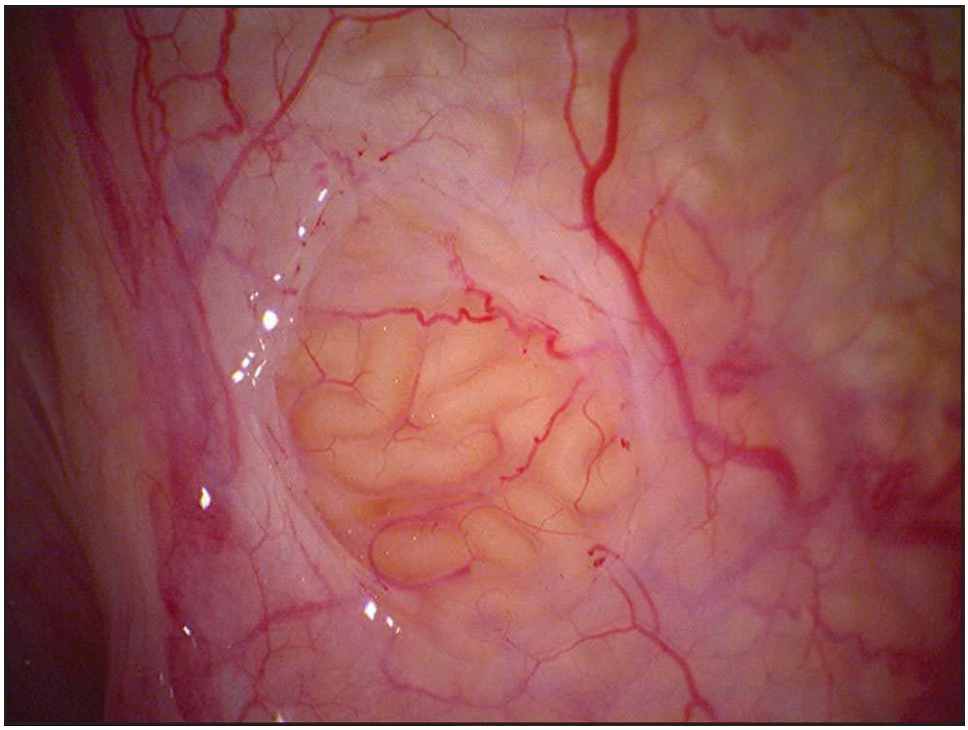


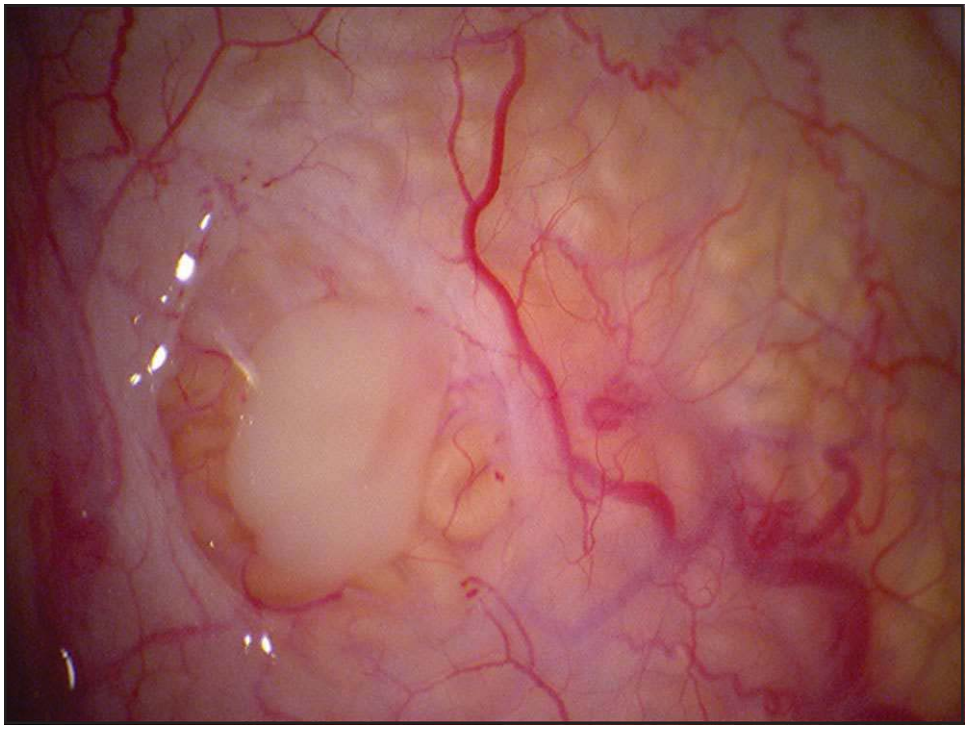
OBSTRUCTIVE AZOOSPERMIA

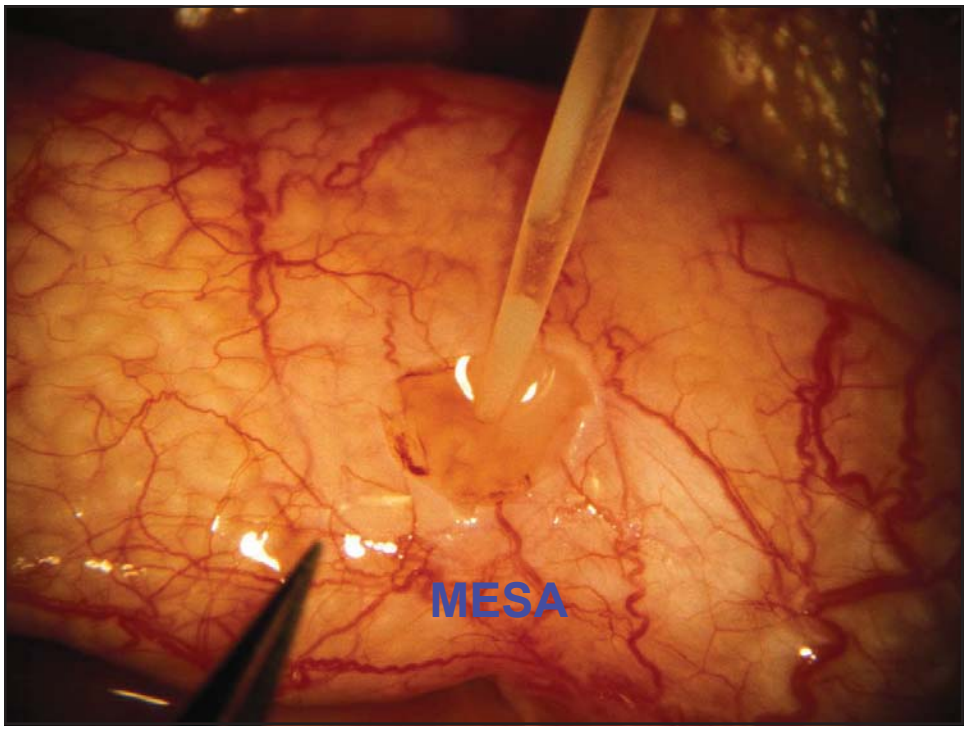




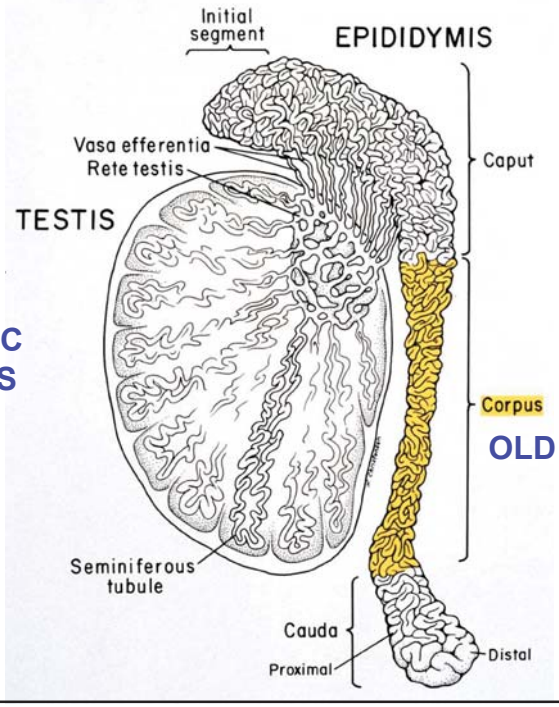




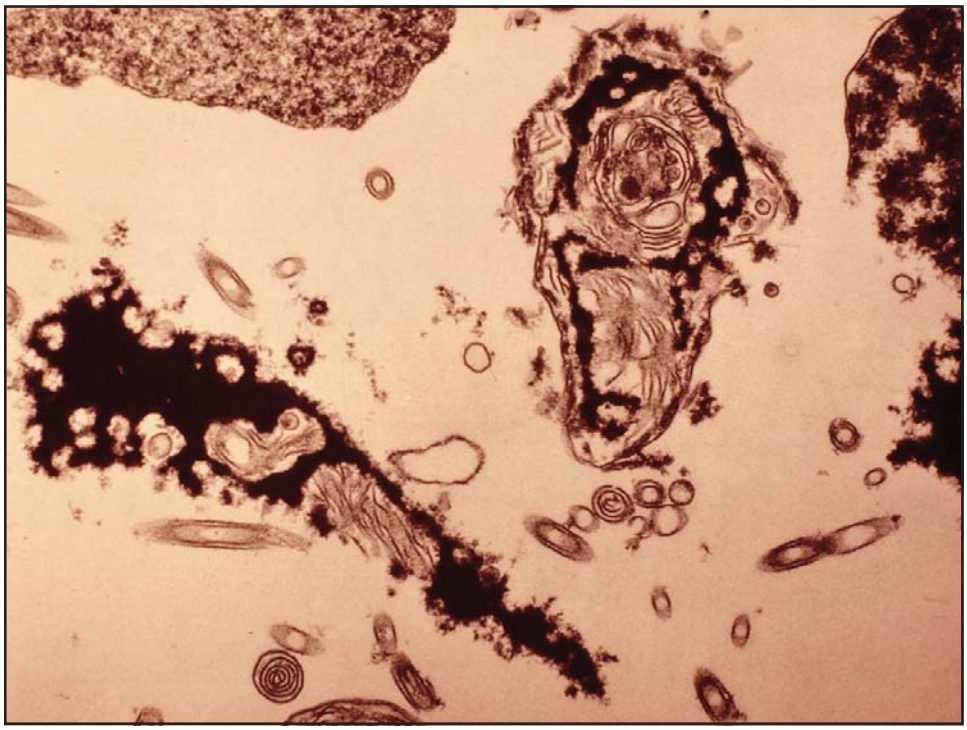


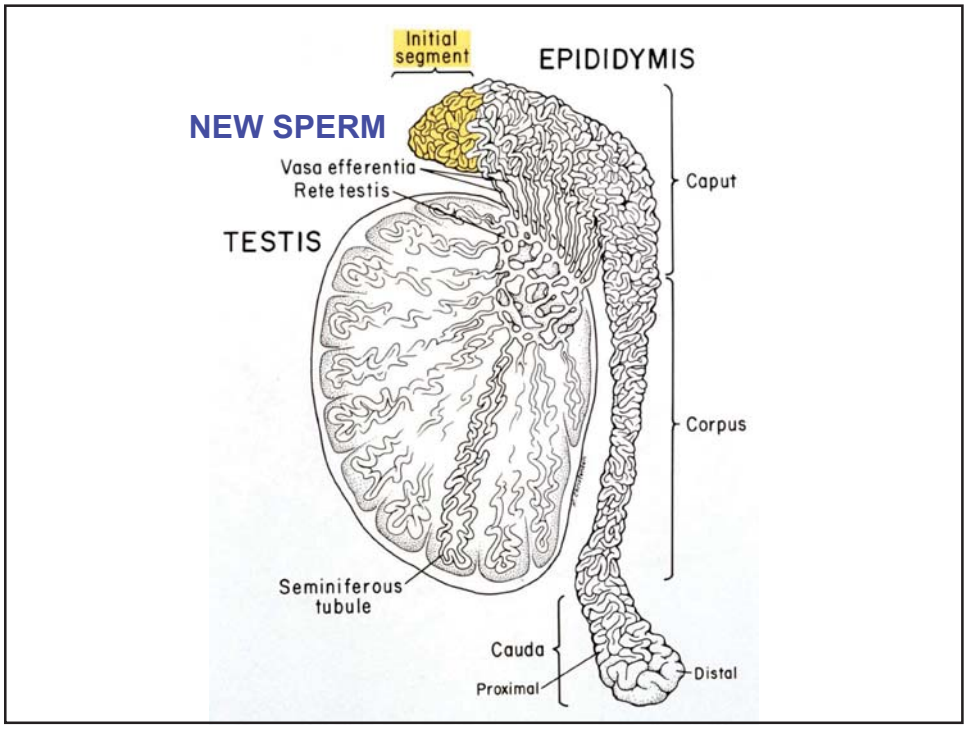

MESA

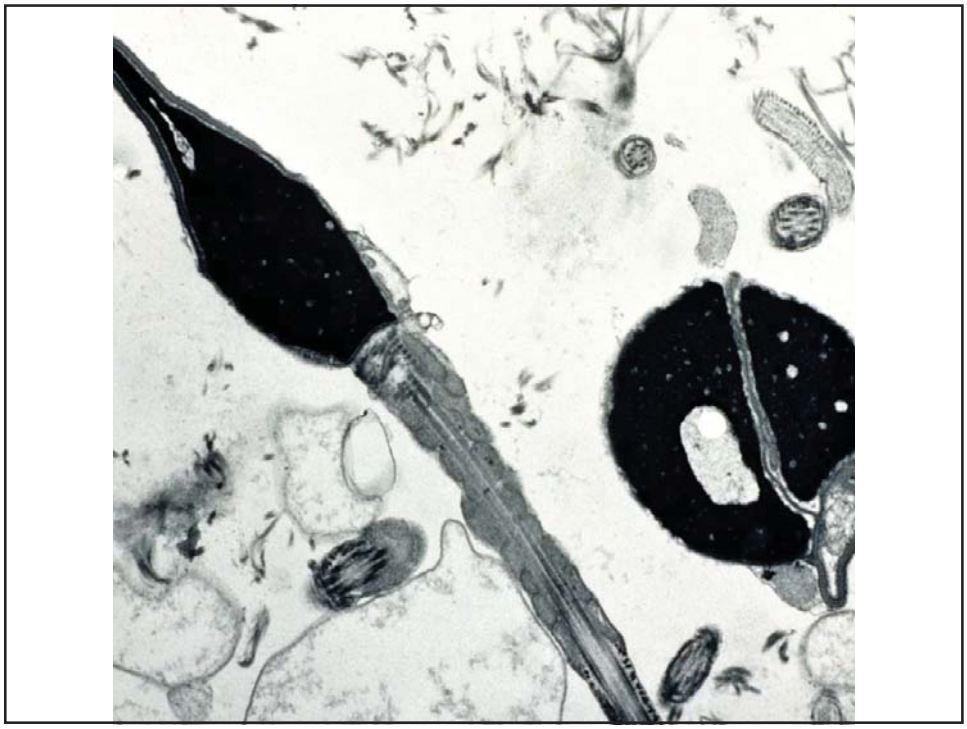
NOTE:
ANATOMIC
LOBULES



OLD SPERM




Initial segment
EPIDIDYMIS
NEW SPERM
Vasa efferentia
Rete testis
TESTIS
Caput
Corpus
Seminiferous tubule
Cauda
Proximal
Distal



Comparison of MESA with ICSI to Conventional MESA with IVF in a Similar Patient Population

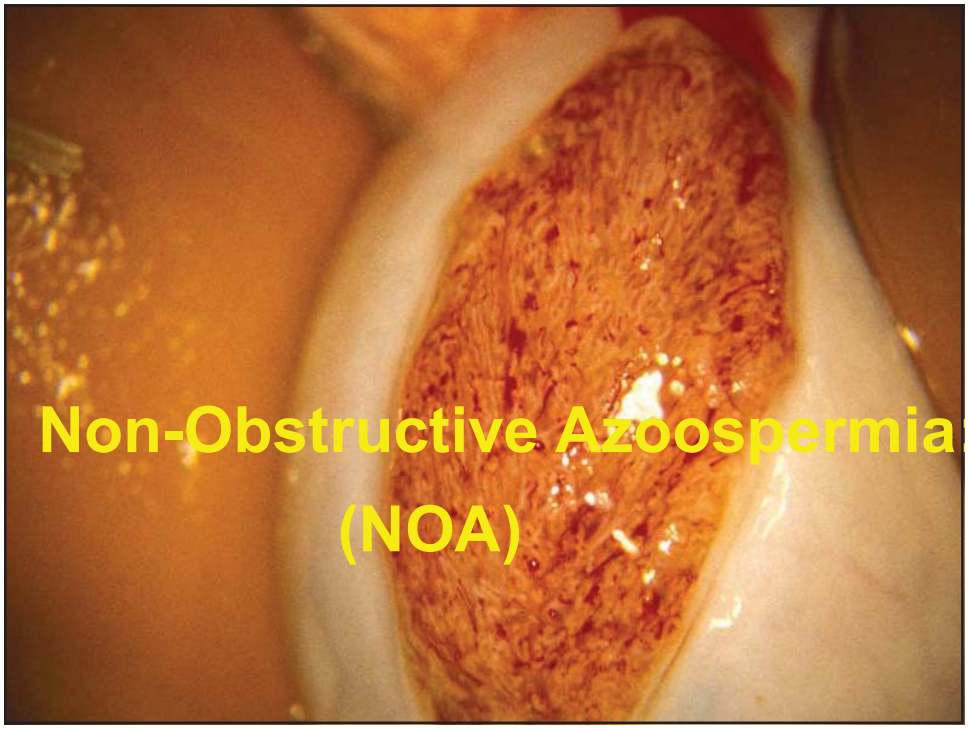
Cycles	Mature Eggs	2PN	Fertilization Rate	Transfers	Pregnancy Rate (Delivered)
IVF-MESA					
67	1,427	98	7%	13/67 (19%)	3/67 (4.5%)
ICSI-MESA**					
33	431	201	47%	31/33 (94%)	12/33 (36.3%)

Silber et al, Fertility and Sterility 1994

Obstructive azoospermia: effect of age of wife

Age of Wife (years)	No. of cycles (% of total)	No. of eggs at MII	No. of 2PN oocytes (% of MII eggs)	No. delivered pregnancies per cycle (% per cycle)	Implantation rate % (per embryo)
<30	50 (27%)	735	392 (53%)	22 (44%)	22%
30–36	87 (47%)	1111	610 (55%)	30 (34%)	19%
37–39	24 (13%)	207	113 (55%)	3 (12%)	4%
40+	25 (13%)	281	147 (52%)	1 (4%)	7%
Totals	186 (100%)	2334	1262 (54%)	56 (30%)	16.2%

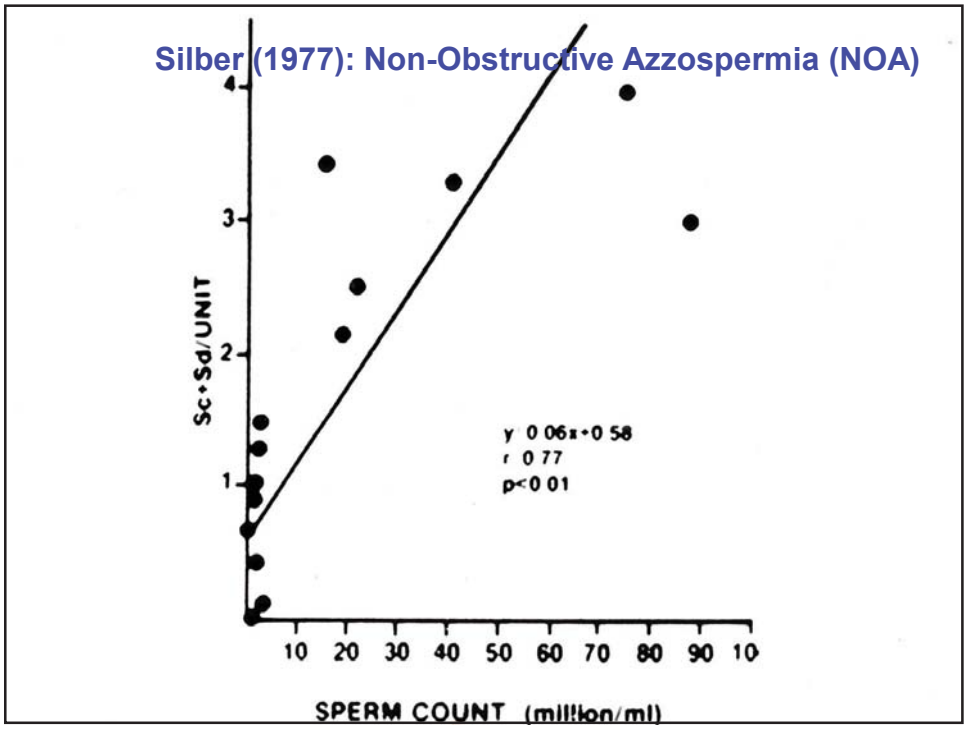
Silber et al. Human Reproduction, 1997



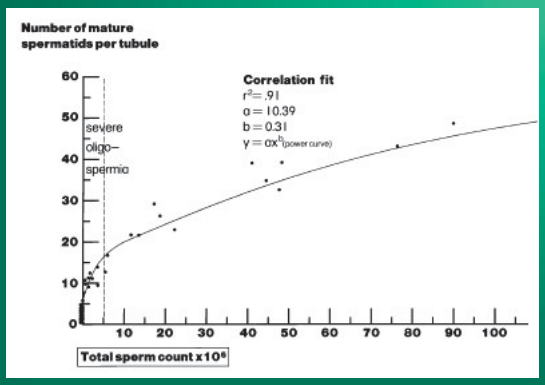
TESE (Testicular Sperm Extraction)

Micro TESE
Vs
Conventional TESE

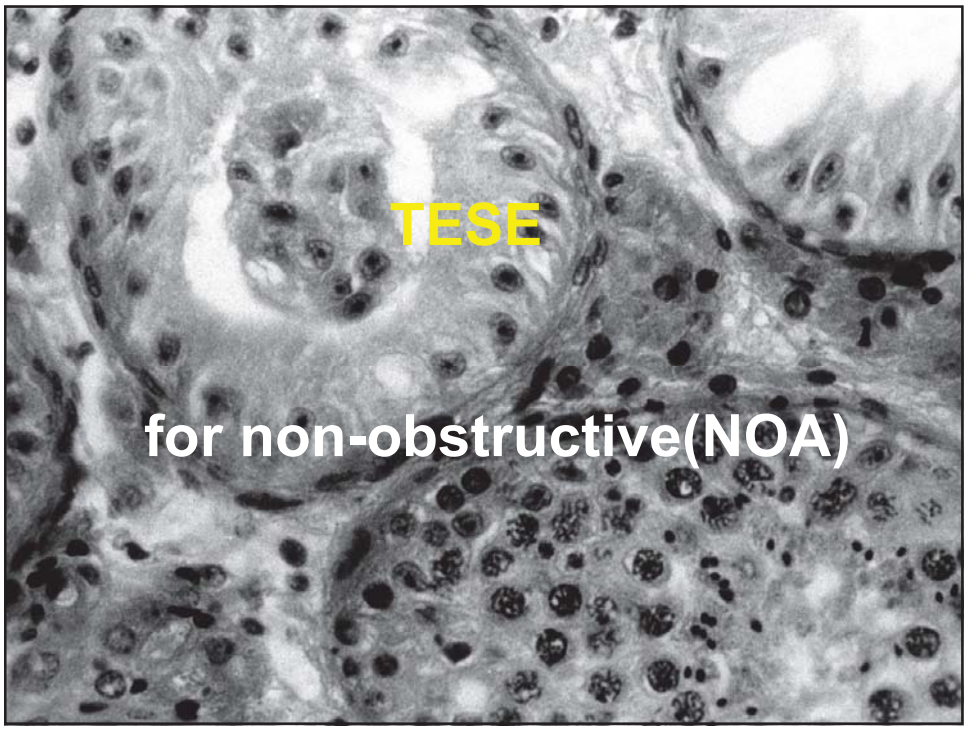
Silber (1977): Non-Obstructive Azzospermia (NOA)

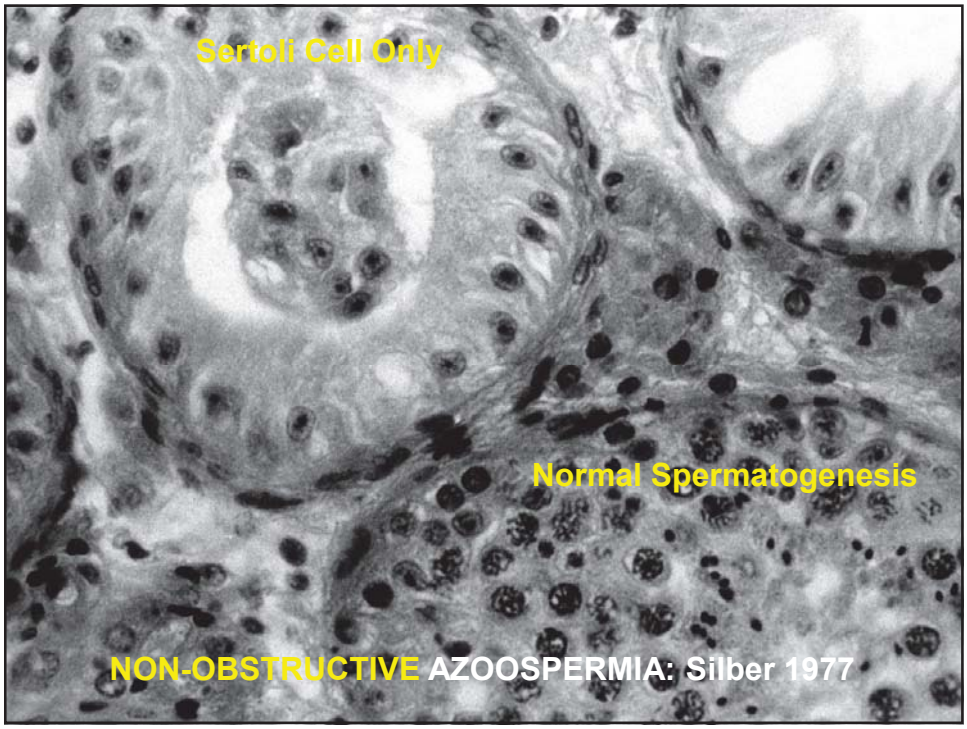


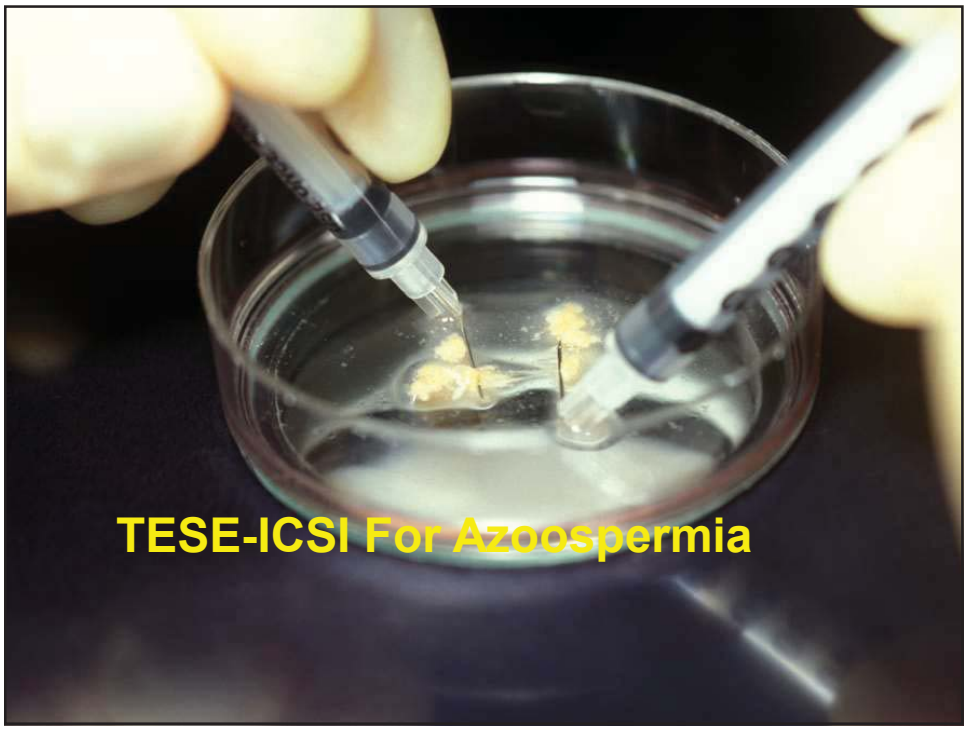
Graph depicting quantitative testicle biopsy and sperm count

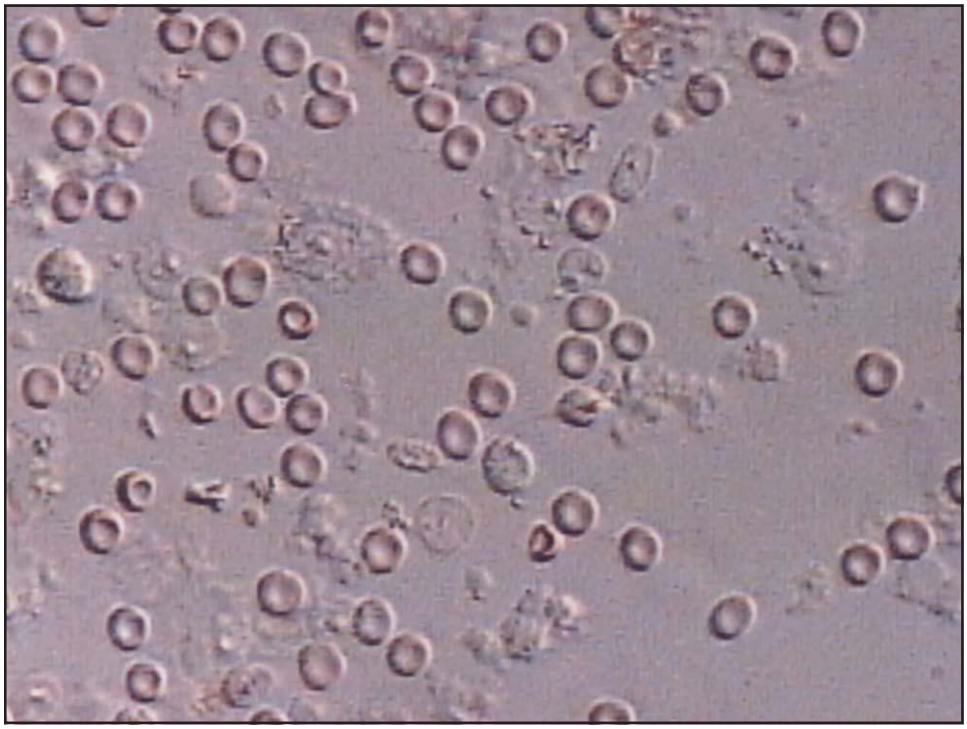


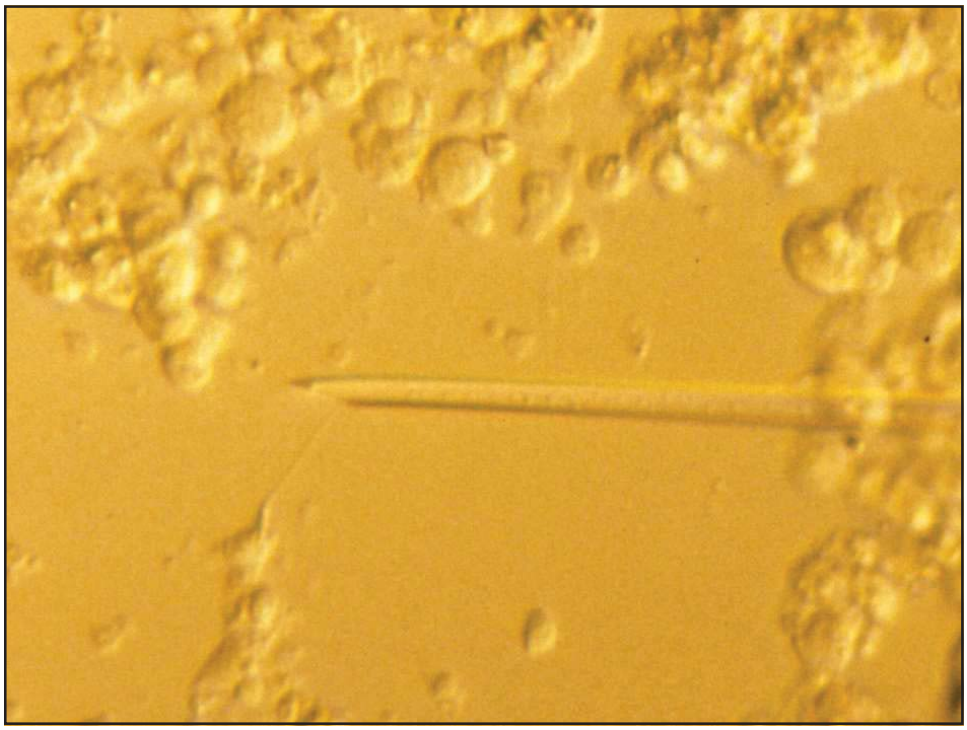
Human Reproduction (1997) Vol. 12 No. 11




Sertoli Cell Only
Normal Spermatogenesis
NON-OBSTRUCTIVE AZOOSPERMIA: Silber 1977


TESE-ICSI For Azoospermia





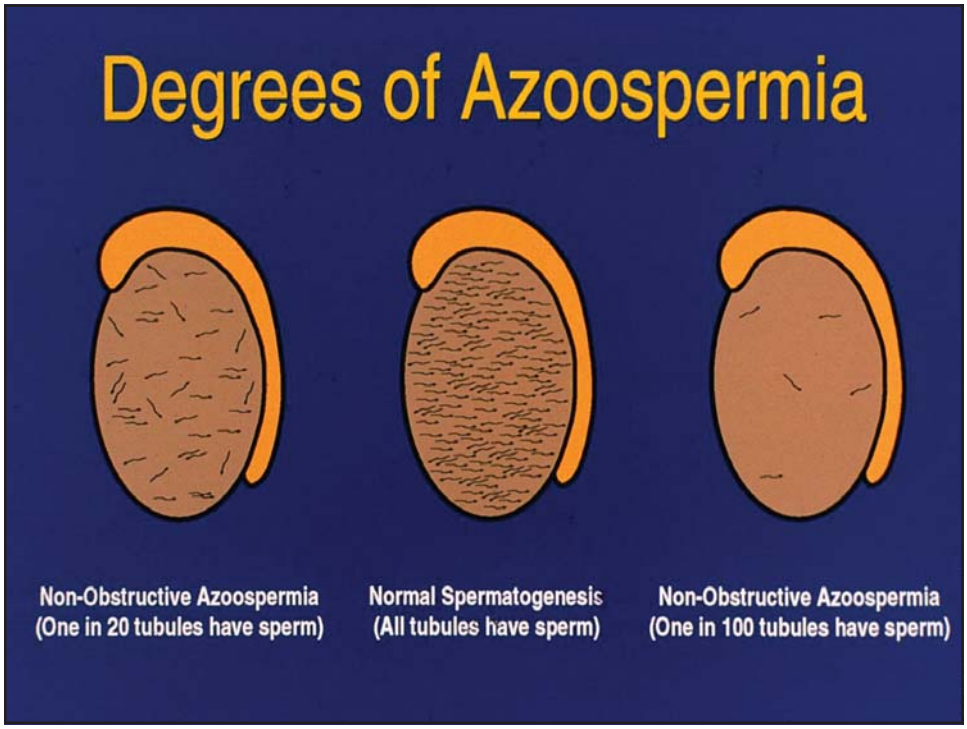
Micro-TESE FOR NOA : Many Techniques for TESE

- Needle aspiration.
- Conventional testis biopsy.
- Multiple conventional biospies.
- Anatomic lobule micro-TESE: Silber
- Mapping: Tureck
- Micro-dissection: Schlegel

- MUCH DEBATE AND CONFUSION ON WHAT IS THE BEST APPROACH

What Does Micro TESE Even Mean?

- Testis biopsy with a microscope.
- Type of Incision? Needle Aspiration?
- “Micro dissection”.
- Sampling of All Anatomic Lobules.
- Spermatogonial Stem Cells.
- Tunica Albuginea Closure and Hemostasis.



Confounding Categories:

These are not NOA, and will skew your results more favorably, as they did NOT need TESE

- "hypospermatogenesis"
- "azoospermia"
- "spermatid arrest"
- "crypt-azoospermia"

DEVELOPED MY MYSELF AND PAUL DEVROEY 1993:

- All the data and directions for doing TESE successfully are in the literature from 23 years ago (Silber and Devroey).
- But a competitive and confusing literature has followed from countless “me-too” urologists and gynecologists who each wanted to grab credit for a “better” method, not citing my original papers.
- In this lecture, I will go through the voluminious literature on TESE success claims, and in the end give a clearer picture of the best approach.

Human Reproduction vol.9 no.9 pp.1705–1709, 1994

Conventional in-vitro fertilization versus intracytoplasmic sperm injection for patients requiring microsurgical sperm aspiration

Sherman J.Silber[1,3], Zsolt P.Nagy[2], Jiaen Liu[2], Hugo Godoy[2], Paul Devroey[2] and André C.Van Steirteghem[2]

[1]St Luke's Hospital, 224 South Woods Mill Road, Saint Louis, MO 63017, USA and [2]Centre for Reproduction Medicine, Academisch Ziekenhuis, Vrije Universiteit Brussel, Laarbeeklaan 101, B-1090 Brussels, Belgium

[3]To whom correspondence should be addressed

Human Reproduction (1994) Vol. 9 No. 9

Human Reproduction vol.10 no.1 pp.148–152, 1995

High fertilization and pregnancy rate after intracytoplasmic sperm injection with spermatozoa obtained from testicle biopsy

S.J.Silber[1], A.C.Van Steirteghem, J.Liu, Z.Nagy, H.Tournaye and P.Devroey

[1]St. Luke's Hospital, 224 South Woods Mill Road, St. Louis, MO 63017 USA; and Centre for Reproductive Medicine, University Hospital, Dutch-Speaking Brussels Free University (Vrije Universiteit Brussel), Laarbeeklaan 101, 1090 Brussels, Belgium

[1]To whom correspondence should be addressed

Human Reproduction (1995) Vol. 10 No. 1

Human Reproduction vol.10 no.8 pp.2031–2043, 1995

The use of epididymal and testicular spermatozoa for intracytoplasmic sperm injection: the genetic implications for male infertility

Sherman J.Silber[1,2], Zsolt Nagy, Jian Liu, Herman Tournaye, Willy Lissens[3], C.Ferec[4], Ingeborg Liebaers[3], Paul Devroey and André C.Van Steirteghem

[1]St Luke's Hospital, 224 South Woods Mill Road (Chesterfield), St Louis, MO 63017, USA, Centre for Reproductive Medicine and [3]Centre for Medical Genetics, University Hospital and Medical School, Dutch-speaking Brussels Free University, Brussels, Belgium and [4]Centre de Biogenetique C.D.T.S., BP 454, 29275 Brest Cedex, France

[2]To whom correspondence should be addressed

Human Reproduction (1995) Vol. 10 No. 8

FERTILITY AND STERILITY®

Vol. 66, No. 1, July 1996
Printed on acid-free paper in U. S. A.

Normal pregnancies resulting from testicular sperm extraction and intracytoplasmic sperm injection for azoospermia due to maturation arrest

Sherman J. Silber, M.D.*
Andre Van Steirteghem, M.D., Ph.D.†
Zsolt Nagy, M.D.†
Jiaen Liu, M.D.†
Herman Tournaye, M.D., Ph.D.†
Paul Devroey, M.D., Ph.D.†

St. Luke's Hospital, St. Louis, Missouri, and Centre for Reproductive Medicine, Academisch Ziekenhuis, Vrije Universiteit Brussel, Brussels, Belgium

Fertility and Sterility (1996) Vol. 66 No. 1

Human Reproduction vol.12 no.11 pp.2422–2428, 1997

Distribution of spermatogenesis in the testicles of azoospermic men: the presence or absence of spermatids in the testes of men with germinal failure

Sherman J.Silber[1,3], Zsolt Nagy[2], Paul Devroey[2], Herman Tournaye[2] and Andre C.Van Steirteghem[2]

[1]Director, Infertility Center of St Louis, St Luke's Hospital, 224 South Woods Mill Road, St Louis, Missouri 63017, USA and [2]Centre for Reproductive Medicine, Academisch Ziekenhuis, Vrije Universiteit Brussel, Brussels, Belgium

[3]To whom correspondence should be addressed

Human Reproduction (1997) Vol. 12 No. 11

Non-obstructive azoospermia (Sertoli cell only, maturation arrest, post-chemotherapy, and cryptorchidism)

Age of wife (years)	No. cycles	No. cycles with sperm found (% of cycles)
<30	19	14(74)
30-36	29	16(55)
37-39	9	4(44)
40+	6	5(83)
Totals	63	39(62)

Silber et al Human Reproduction 1997

Long History of TESE-ICSI: Tournaye

- "The first patient series on this approach were published more than 20 years ago."
- (I still have the napkin from the operating room on which we coined the term "TESE" after doing the first case.)
- Sperm retrieval rates often boasted about are subject to the (pre)selection of patients: biased either by including patients showing "hypospermatogenesis" or crypt-azoospermia.
- e.g. 10 % of NOA cases will have sperm in the ejaculate the morning of the TESE procedure.

Human Reproduction (2011) Vol. 26 No. 12

Results for TESE-ICSI?

- Retrieval rates after testicular surgery reported in the literature differ considerably
- Retrieval rates reported in the literature for NOA men may vary from about 30% to even more than 80%.
- Larger case studies in well-defined NOA populations report sperm recovery rates after a first TESE attempt around 50%

Tournaye
Human Reproduction (2011) Vol. 26 No. 12

How successful is TESE-ICSI in couples with non-obstructive azoospermia?

NOA TESE By DX	
All Patients	Positive Sperm Retrieval
Total	289 (40.5%)
MA	80 (45.7)
SCO	178 (38.4)
Sclerosis and/or atrophy	31 (41.3%)

Vloeberghs et al (2015) Human Reproduction

Conventional TESE and NOA: Results from a non-academic community hospital

- **Sixty-three NOA patients were referred for Conventional TESE.**
- **In 47.6%, sperm were found.**

Sacca et al. Andrology (2016): Bergamo, Italy

Microdissection TESE after Conventional Testicular Biopsy in NOA

- To analyze 86 TESE procedures for ICSI in NOA patients who had a previous conventional TESE
- Testicular motile spermatozoa were successfully retrieved in 39 out of 47 men who had spermatozoa found in the previous biopsy
- In 6 out of 39 men with no sperm in the previous biopsy

Karacan et al., Istanbul, Turkey
European Journal of Obstetrics & Gynecology and Reproductive Biology (2014) Vol. 183 No. 1

Fertility with Conventional TESE in NOA

Diagnosis	Cases	Sperm Found
M.A.	14	8(57%)
Hypo-Spermato Genesis	10	3(30%)
SCO	5	3(60%)
TOTAL	29	14(48%)

Kahraman et al, Ankara, Turkey
Human Reproduction (1996) Vol. 11 No. 4

Microdissection TESE

Histologic Category	Sperm Retrieval Rate with TESE (%)
Hypospermatogenesis	79% (31/39)
Maturation Arrest (n =19)	47% (9/19)
Sertoli Cell-only (n =21)	24% (5/21)
TOTAL	57%

But exclude hypospermatogenesis: then only 35% had sperm!

Cornell Website, 2016

A novel stepwise micro-TESE approach in non obstructive azoospermia

- First, a single TESE sample was taken from one testicle and, after this, a micro-TESE was performed extending the same testicular incision
- Contralateral conventional multiple biopsies in case of negative sperm retrieval on the first testis
- Compare the efficiency of micro-TESE with conventional TESE

Franco et al BMC Urology (2016)

Micro-TESE: A Novel Stepwise Approach

Histology	Positive Sperm Retrieval
MA (15 cases)	10/15 (67%)
SCO (37 cases)	7/37 (18.9%)
Sclerosis (12 cases)	1/12 (0.8%)
TOTAL	18/64 (28%)

Rome, Italy
Franco et al. 2016

Sperm Retrieval Rate With Biopsy VS micro-TESE: Franco et al. 2016

In patients with poor prognosis NOA, micro-TESE did not improve chance for finding sperm.

In all patients with successful sperm retrieval, the initial less invasive conventional biopsy was enough.

The same result was obtained in the initial conventional TESE as in the subsequent micro-TESE.

	Previous TESE	No previous TESE	Chi square
Positive sperm retrieval	6/23 (26%)	12/41 (29%)	P=0.552

Re-evaluation of Microdissection Testicular Sperm Extraction

- In well-designed studies with well-defined men with NOA, the reported successful SRRs after a first TESE attempt is about 50%
- All seminiferous tubules (ST) must be inspected to recognize small foci of normal spermatogenesis
- The space between the tubules and the tunica is very vascular, thus hemorrhage that would be very difficult to control can happen if dissection is made in t his plane.
- Postoperative hemorrhage and hematoma formation after micro-TESE can result in scar formation within the testis.

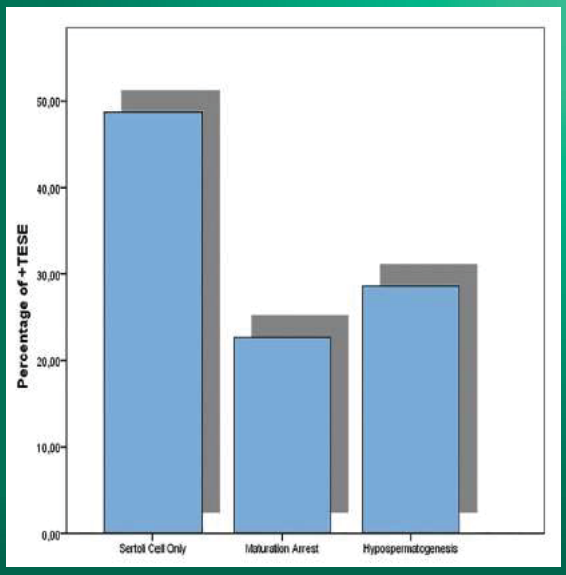
Safarinejad et al. 2015

Re: evaluation of Microdissection Testicular Sperm Extraction Results in Patients with Non-Obstructive Azoospermia: Independent Predictive Factors and Best Cutoff Values for Sperm Retrieval

- To avoid separation of STs from their blood supply and thus devascularization of the STs,
- Postoperative hemorrhage and hematoma formation after micro-TESE can result in scar formation within the testis.

Safarinejad et al. 2015

Percentage of positive Testicular Sperm Extraction (RESE) according to the histologic classification



TOTAL: 54.5%

Cetinkaya et al 2015

Predictive Factors of Successful MD-TESE

Diagnosis	# Cases	Sperm Found	%	Pregnancy
SCO	148	35	23.6%	20(57.1%)

Modarresi et al 2013.
International J Fertil Steril

Initial Micro-Dissection

Kalsi et al 2011

	Successful
N (%)	50 (50 %)
Histology (%)	
SCO	42.85 %
MA	26.70 %
Hypospermatogenesis	75.86 %

Re: Salvage Micro-Dissection Testicular Sperm Extraction; Outcome in Men with Non-Obstructive Azoospermia with Previous Failed Sperm Retrievals

J. S. Kalsi, P. Shah, Y. Thum, A. Muneer, D. J. Ralph and S. Minhas

Frimley Health Foundation Trust, Berkshire and Imperial College, University College London Hospitals and Lister Fertility Clinic, London, United Kingdom

*BJU Int 2015; **116**: 460–465. doi: 10.1111/bju.12932*

Abstract available at http://www.ncbi.nlm.nih.gov/pubmed/25220441

Editorial Comment: Of those men who had azoospermia due to spermatogenic dysfunction, and in whom no sperm was retrieved from biopsy style excisional retrievals or aspirations, approximately half had sperm successfully retrieved from a subsequent microdissection procedure. You may be wondering, why not perform the microdissection technique from the start? Exactly. It is time to sunset all approaches other than microdissection for retrieval of sperm in cases of azoospermia due to spermatogenic dysfunction.

Craig Niederberger, MD

THIS ONE IS REALLY SILLY!

Sperm retrieval outcomes with microdissection testicular sperm extraction (micro-TESE) in men with cryptozoospermia

[1]K. Alrabeeah, [2]A. Wachter, [2]S. Phillips, [2]B. Cohen, [1]N. Al-Hathal and [1]A. Zini

[1]Department of Surgery, McGill University and [2]OVO Fertility Clinic, Montreal, QC, Canada

Alrabeeah et al Andrology (2015)

Microdissection (micro-TESE) with Cryptozoospermia:

THIS IS REALLY SILLY!

- 24 consecutive micro-TESEs in men with cryptozoospermia
- Sperm recovery was successful in 96% (23/24) of the men who underwent micro-TESE and 43% (3/7) of the men who underwent TESA.
- The ICSI pregnancy rates (per embryo transfer) in the micro-TESE and TESA groups were comparable [33% (6/18) and 50% (1/2), respectively]

Slrabeeah et al Andrology (2014)

Outcome of microdissection TESE compared with conventional TESE in non-obstructive azoospermia: a systematic review

[1]Y. Deruyver, [2]D. Vanderschueren and [1]F. Van der Aa

[1]Departments of Urology, and [2]Endocrinology, UZ Leuven, Leuven, Belgium

Andrology (2013) Deruyver et al.

Microdissection TESE compared with Conventional: "a systematic review"

- 62 articles.
- Overall sperm retrieval ranged from 16.7% to 45% in the conventional TESE vs. 42.9 to 63% in the microTESE group
- MicroTESE in men with Sertoli cell only syndrome and hypospermatogenesis carried a small but significantly more favorable outcome

Deruyver et al. 2014 Andrology

Sperm Recovery and IVF after (TESE): Mixes OA with NOA.

- One hundred and thirty men undergoing testicular sperm extraction and 76 couples undergoing 123 in vitro fertilization cycles with testicular sperm for azoospermia.
- Testicular sperm recovery from azoospermic males with all diagnoses was high (70 to 100%) except non-obstructive azoospermia (31%).

Omurtag et al. PLOS ONE (2013)

A Novel Stepwise micro-TESE Approach in NOA

- In the literature, there are many reports indicating micro-TESE performed after previous failed conventional TESE
- The reported rates of successful sperm retrieval with micro-TESE varies between 47 and 66%.
- However in our view, it is reasonable to believe that many of these successful micro-TESE cases might have benefited from a less invasive approach of sperm retrieval

Franco et al BMC Urology (2016)

A novel stepwise micro-TESE approach in non obstructive azoospermia

- Our study indicated that:
  - 1.) in patients with poor prognosis NOA, micro-TESE did not improve the chance of retrieving sperm.
  - 2.) In all patients with successful sperm retrieval, the initial, less invasive single conventional biopsy would have been enough to obtain sperm.
  - 3.) However, micro-TESE was optimally tolerated by patients, and left minimal if no scars.

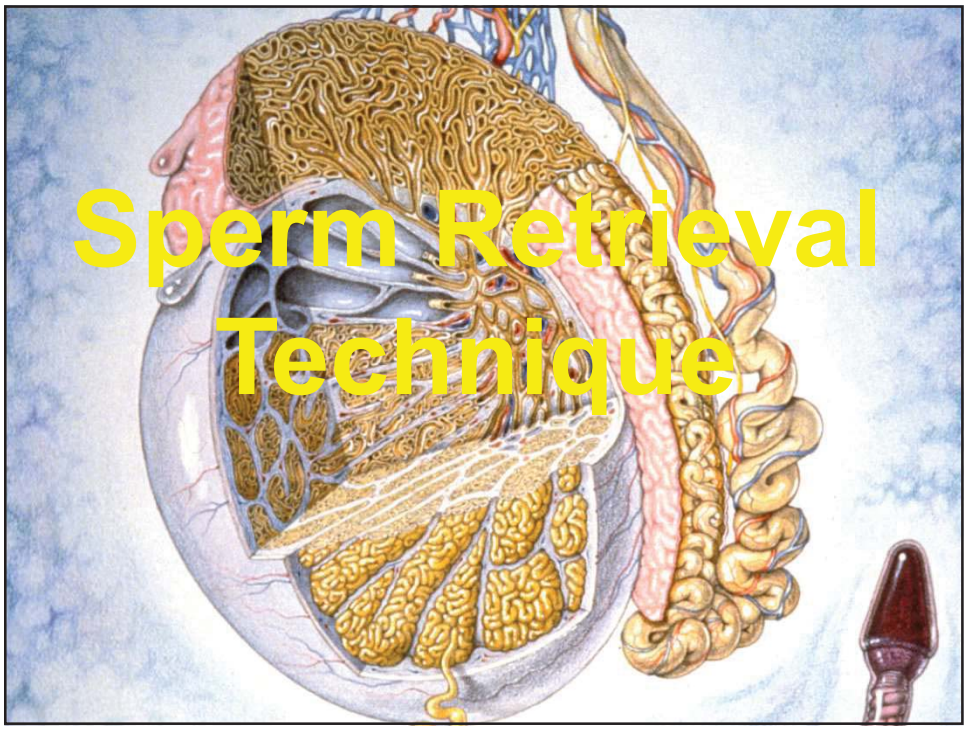
Franco et al BMC Urology (2016)

St Louis NOA Patients-Etiology		
	DIAGNOSIS	Percent
MA	65/212	30%
SCO	100/212	47%
SCO/MA	9/212	4%
Klinefelters	15/212	7%
Male Turners	1/212	1%
Cryptorchidism	4/212	2%
Post Chemo	18/212	9%

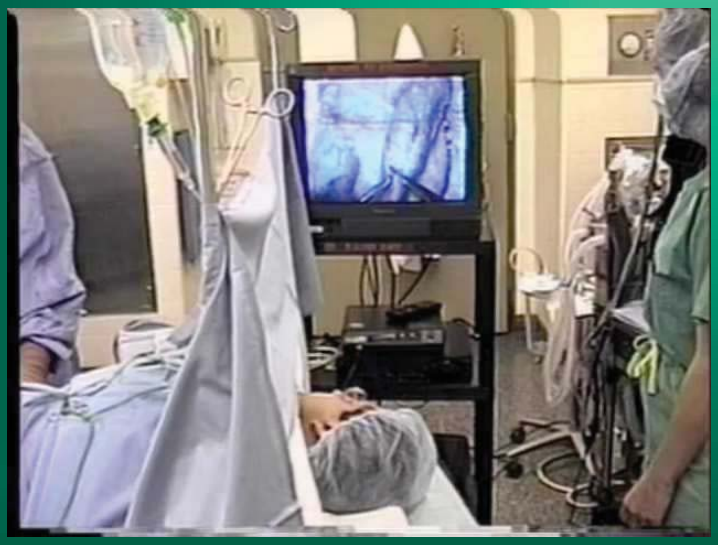
Silber (2016)

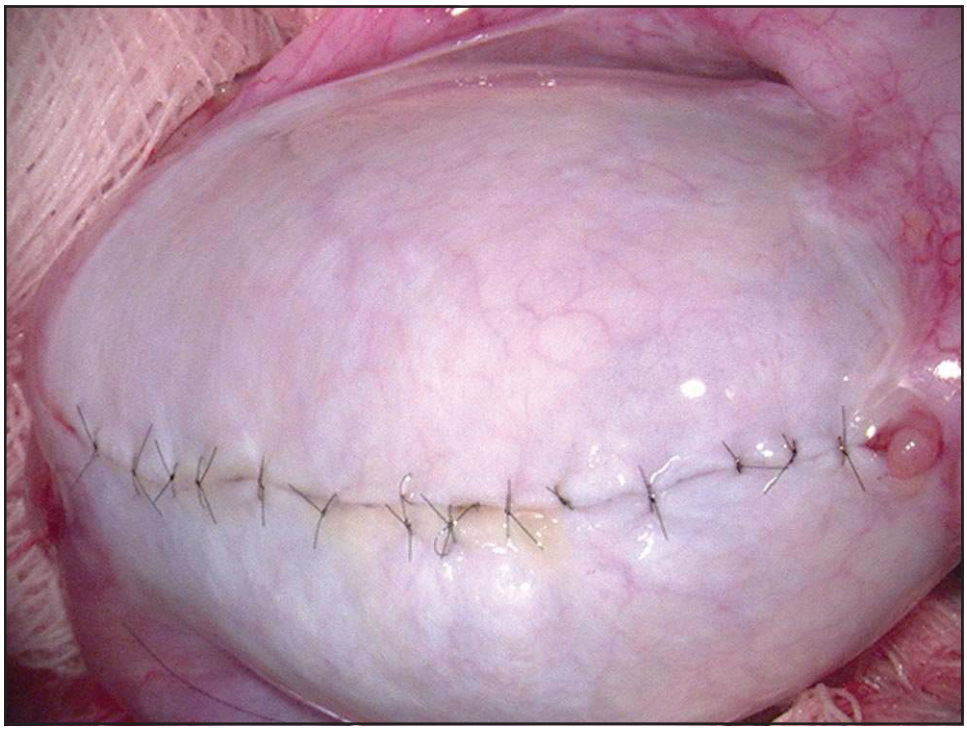
% Sperm Found Via TESE For NOA (St Louis)		
	Number of Cases Sperm Found	Percent
MA	42	44%
SCO	45	32%
SCO/MA	3	27%
Klinefelters	11	48%
Male Turners	3	100%
Cryptorchidism	10	83%
Post Chemo	6	28%
TOTAL	207/444	47%

Silber (2016)



Extensive Micro-TESE or MESA: always under local anesthesia





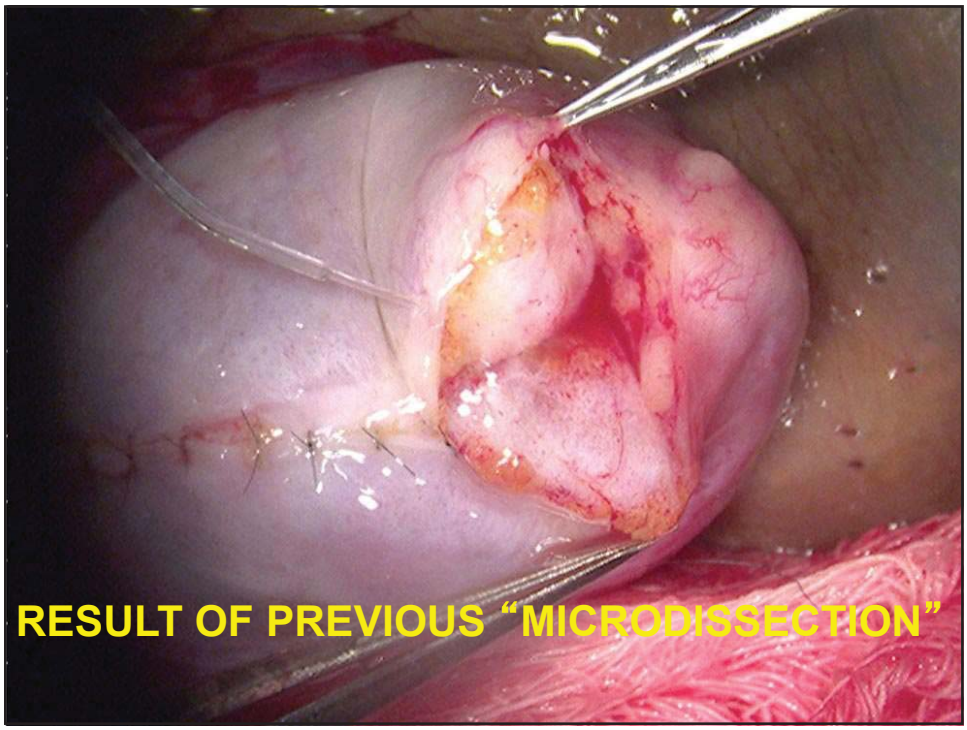
The Patient Gets Up And Walks Away Comfortably After Surgery

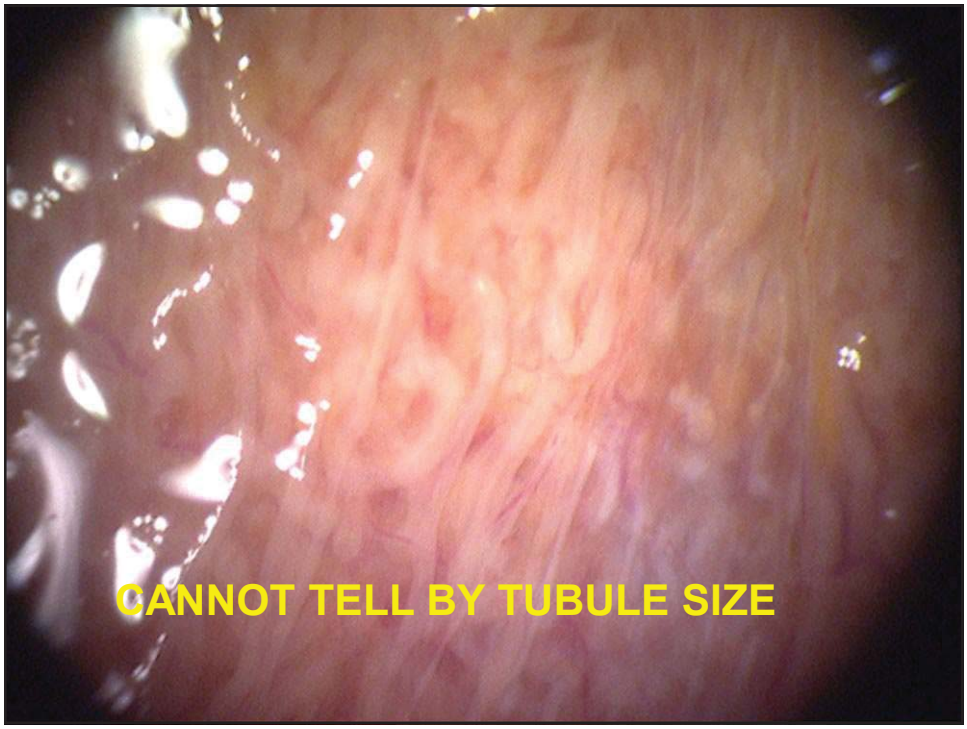
DO NO HARM:

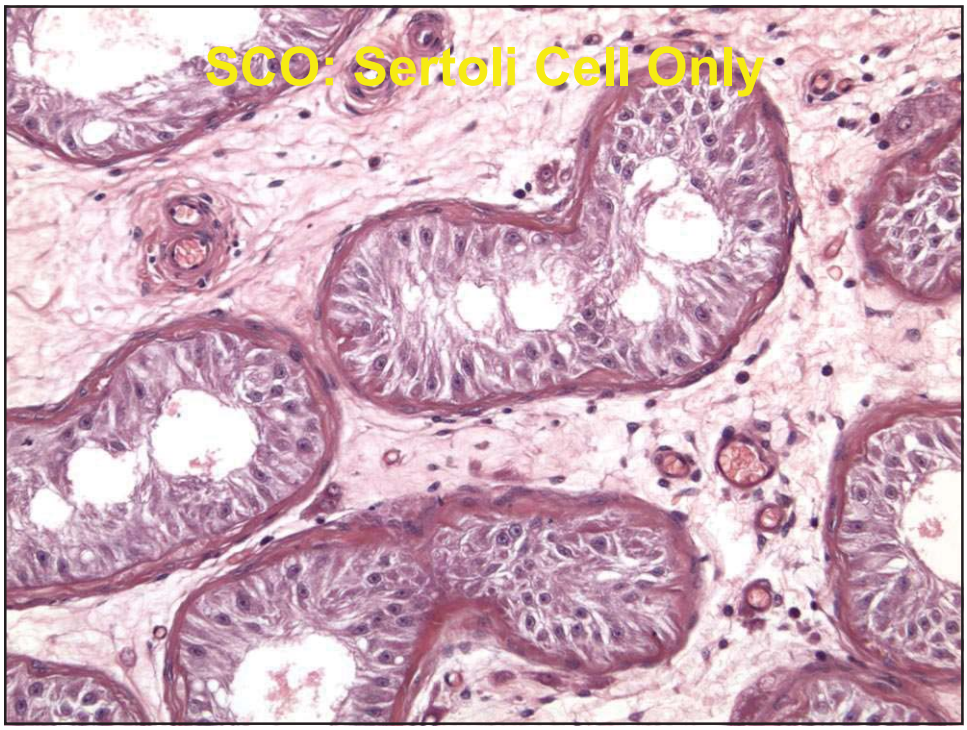
MicroTese vs Microdissection

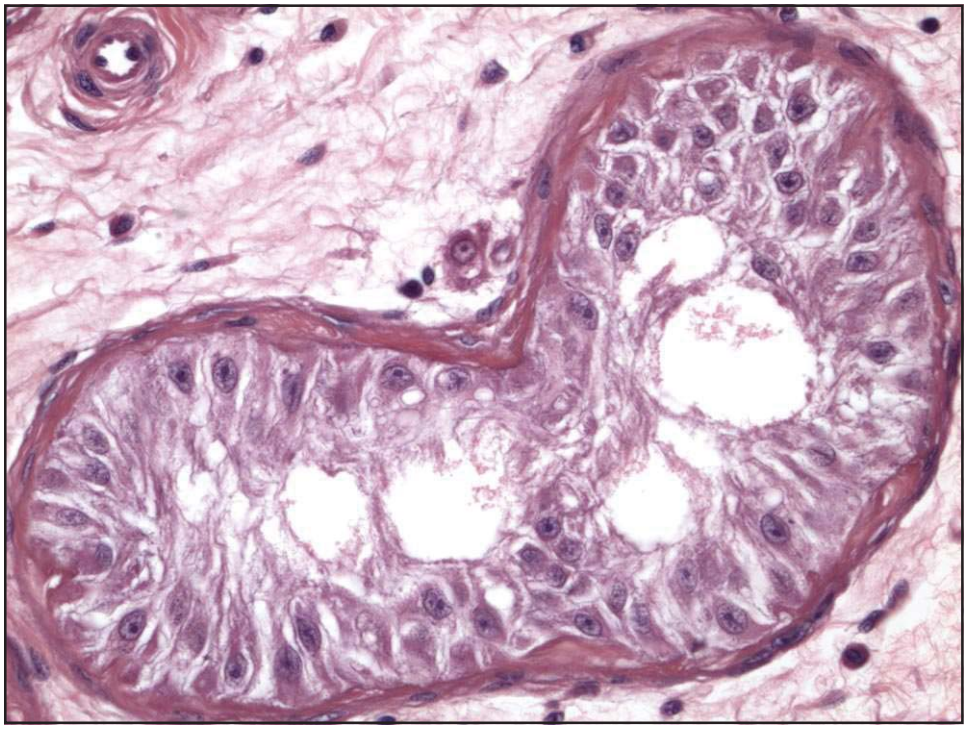
TESE "Micro-dissection" Patient

- 37 year old physician with 33 year old wife.
- Pre-operative testosterone normal: 371.
- 15 months ago underwent bilateral "microdissection" TESE
- No sperm found and has noted increasing fatigue, and muscle weakness since surgery.
- Testosterone now is 84, with LH of 50.5 and FSH of 75.3.
- Essentially he was castrated by this procedure.



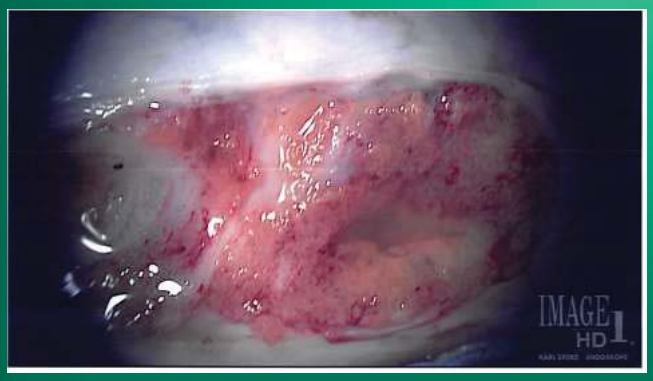

CANNOT TELL BY TUBULE SIZE


SCO: Sertoli Cell Only



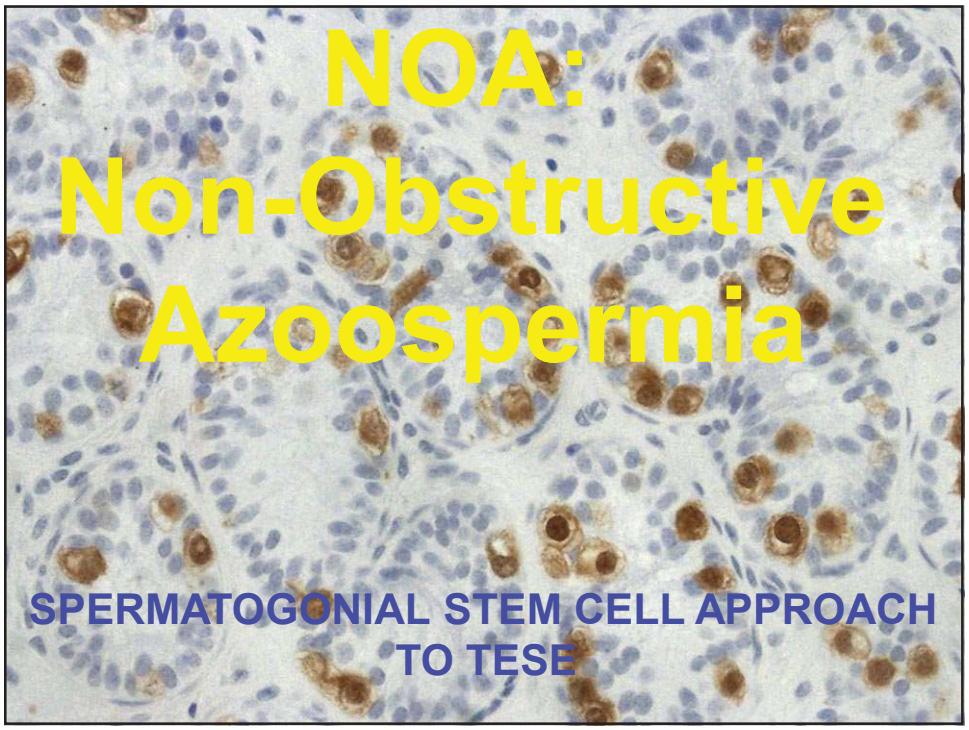

Incomplete Maturation Arrest

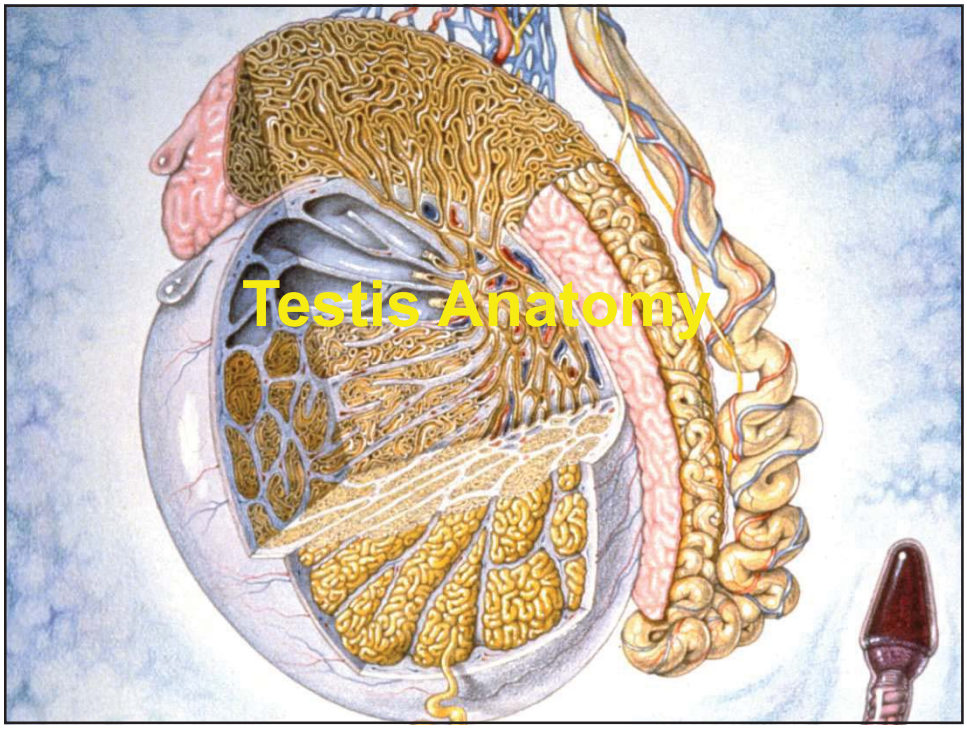
RESULT OF PREVIOUS “MICRODISSECTION



RESULT OF PREVIOUS “MICRODISSECTION

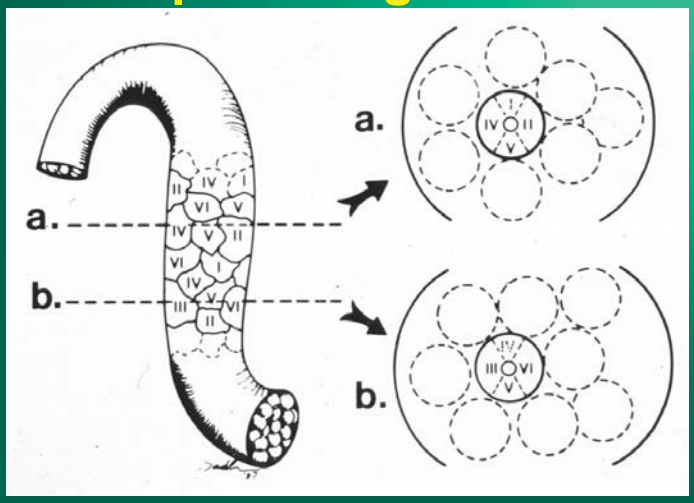

DO NO HARM:
MicroTese vs Microdissection


NOA:
Non-Obstructive
Azoospermia
SPERMATOGONIAL STEM CELL APPROACH
TO TESE


Testis Anatomy

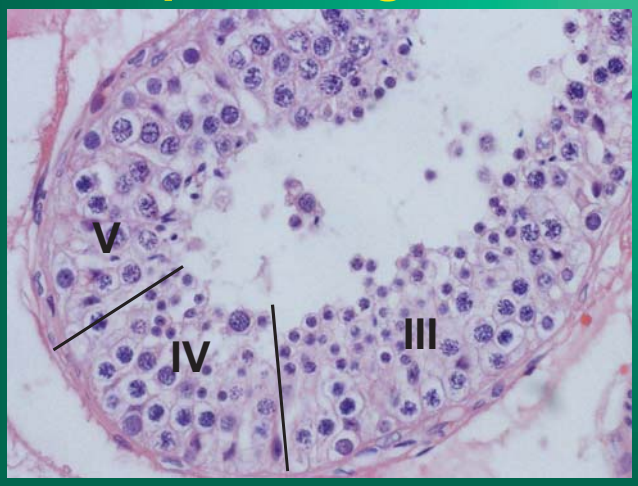

MICRO-TESE:
Anatomic Lobule
Stem Cell Approach

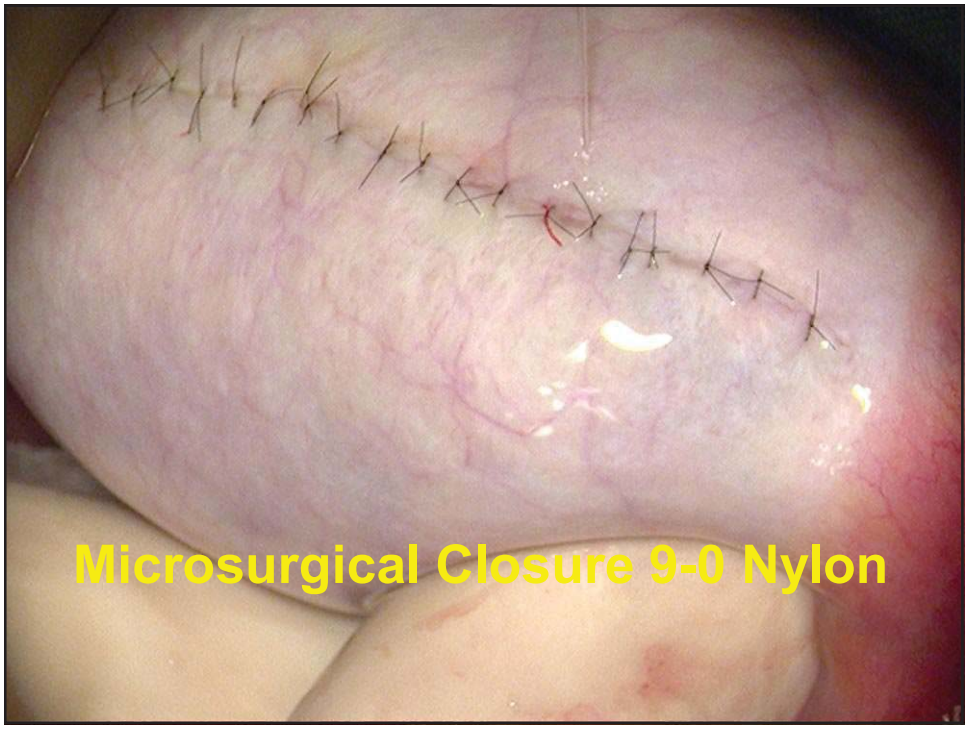
Spermatogenesis



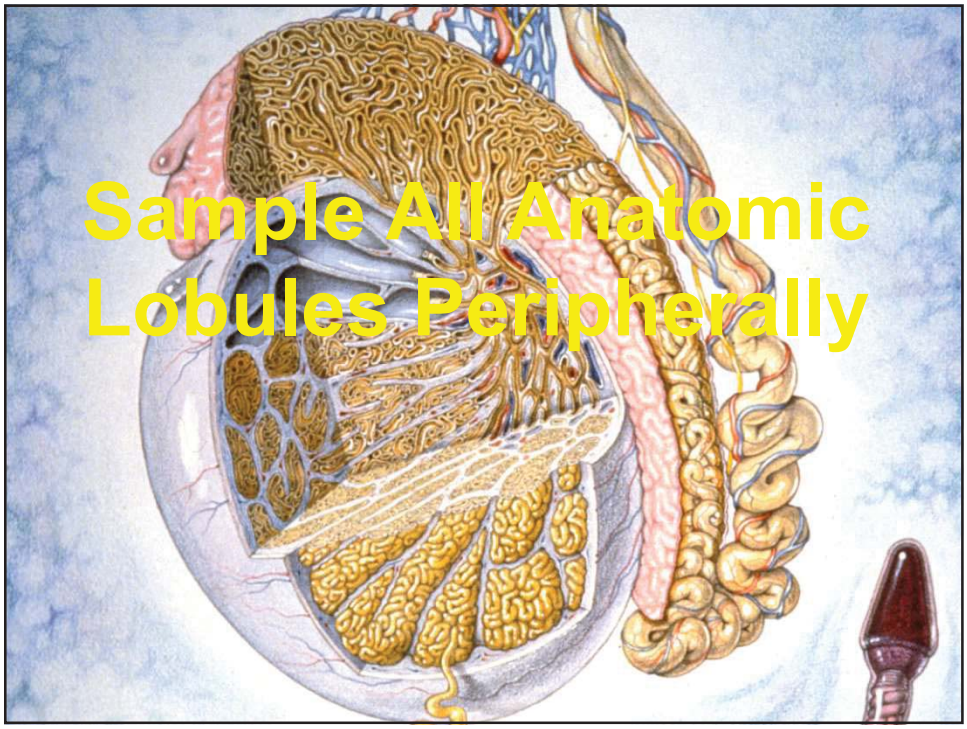
Working, et al. (1988)

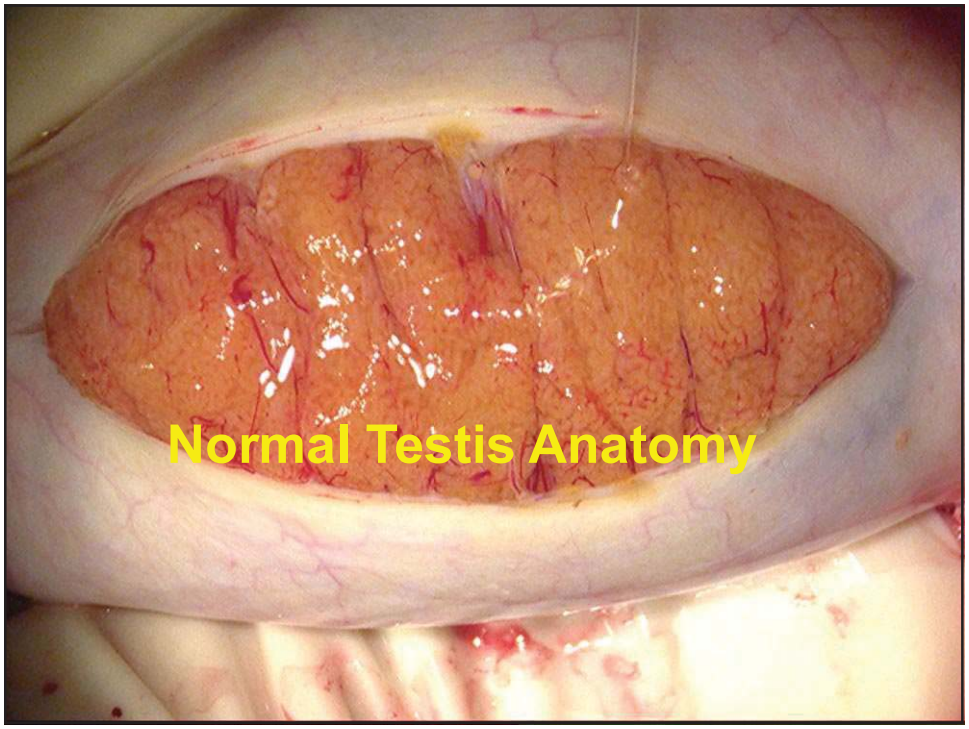
Spermatogenesis

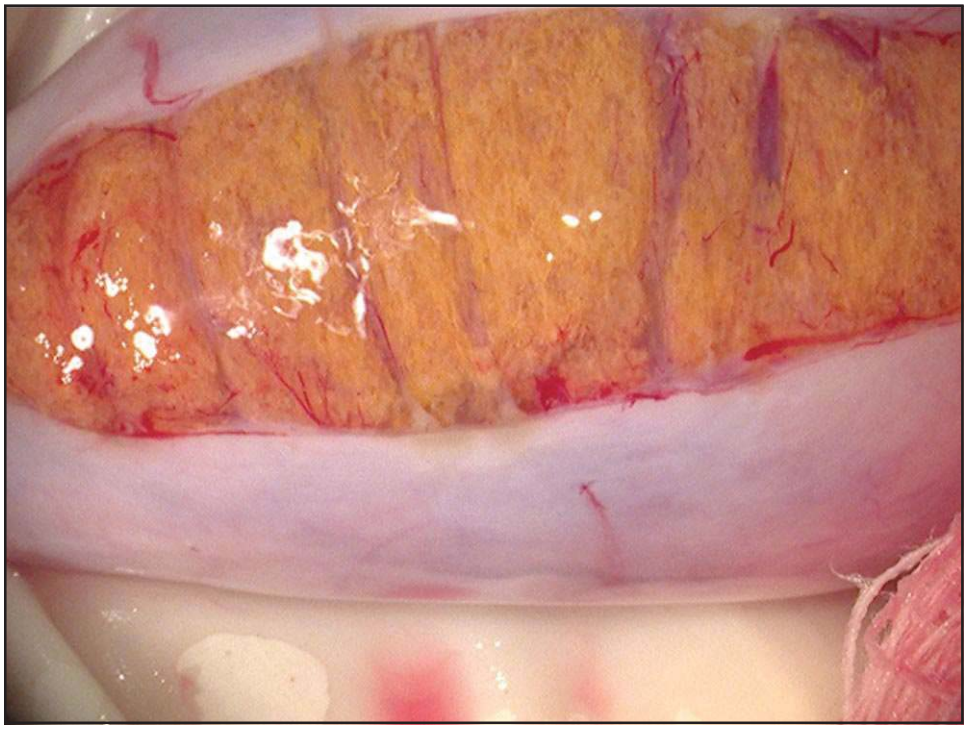


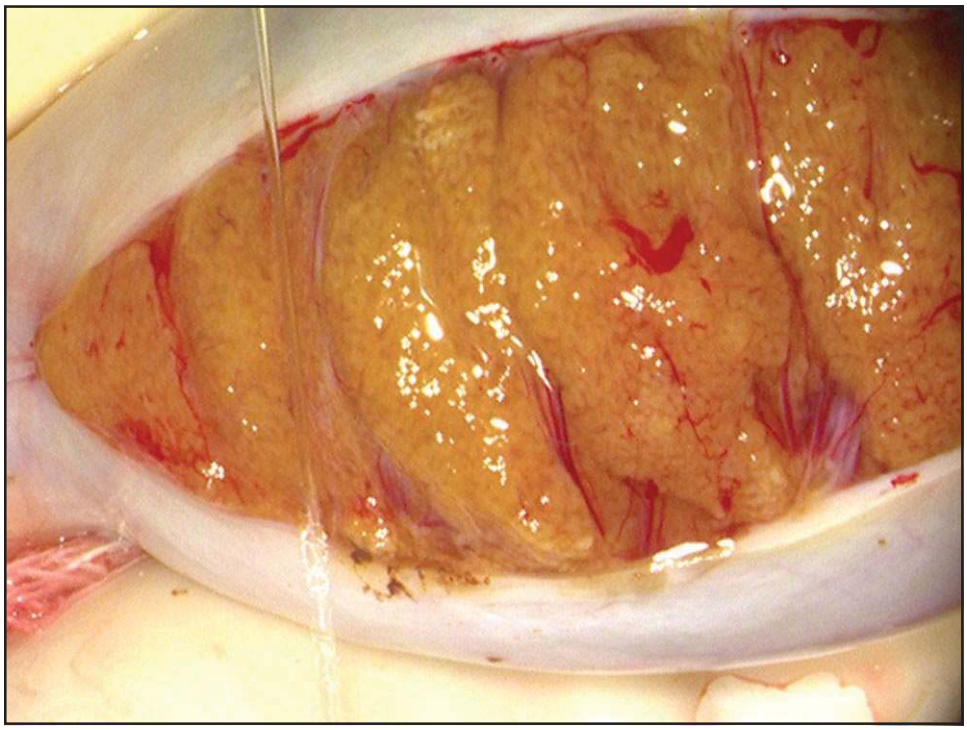

Microsurgical Closure 9-0 Nylon

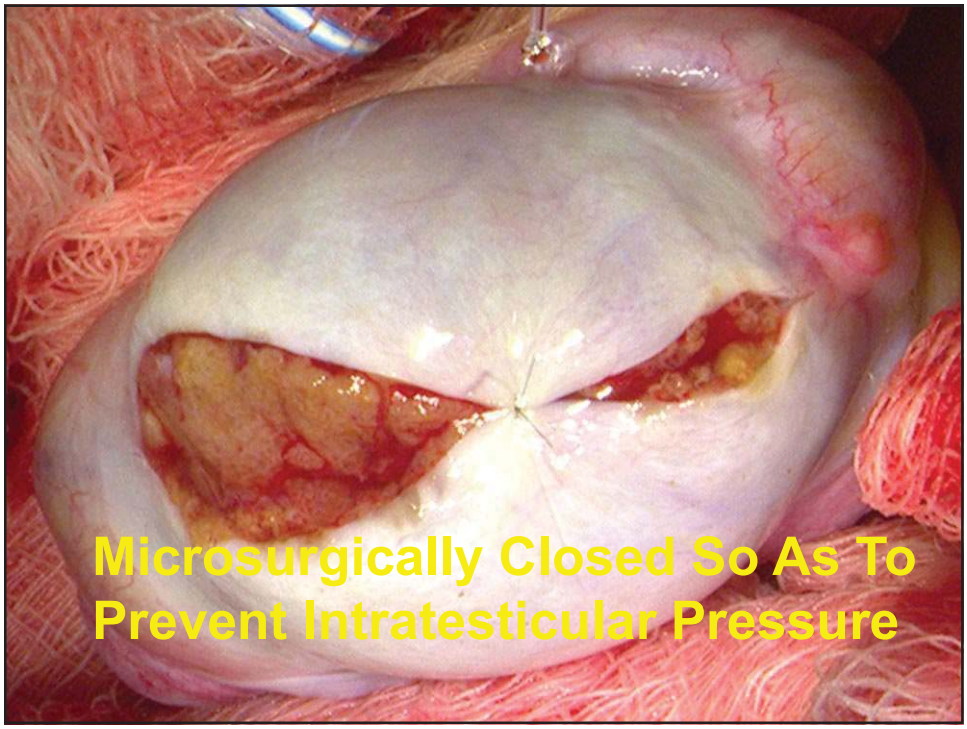

No Adhesions 6 Months Post-Op

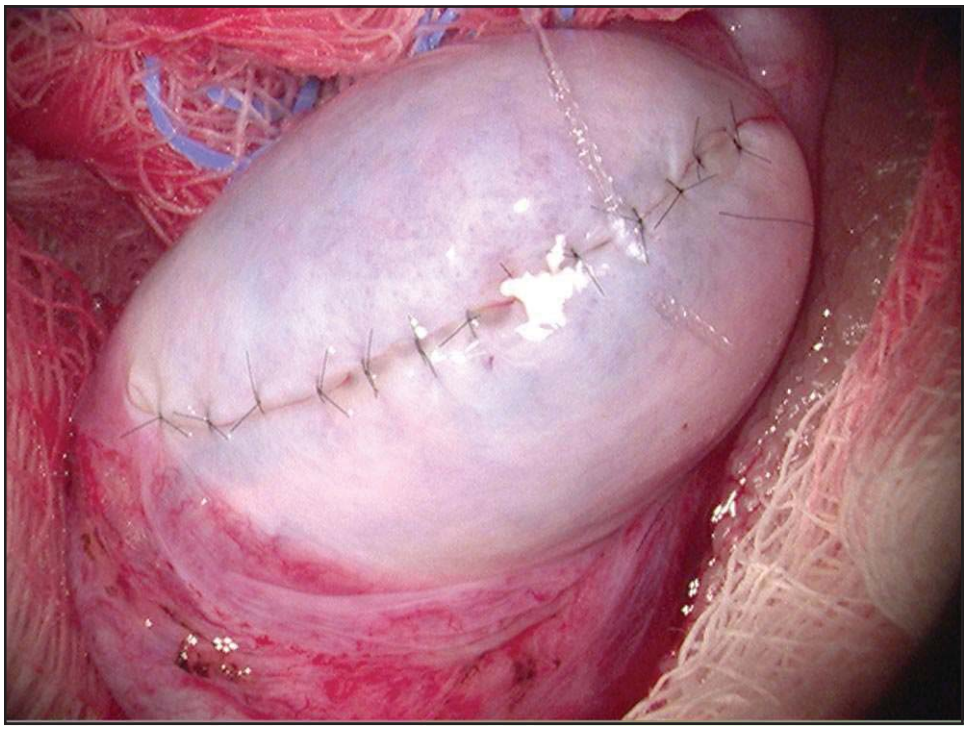

Sample All Anatomic Lobules Peripherally


Normal Testis Anatomy

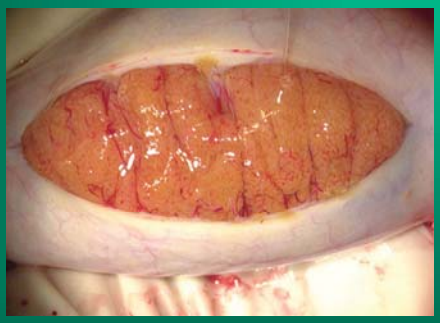


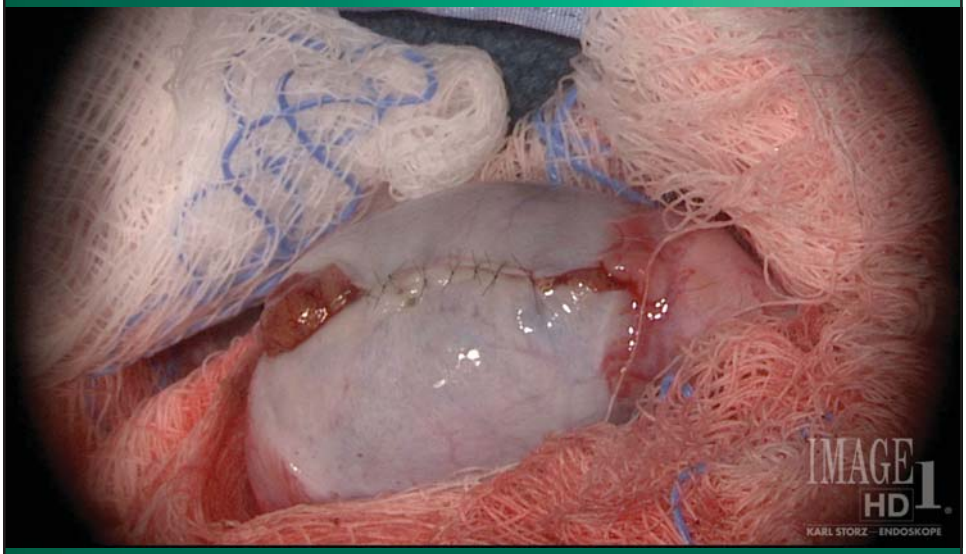



Microsurgically Closed So As To
Prevent Intratesticular Pressure



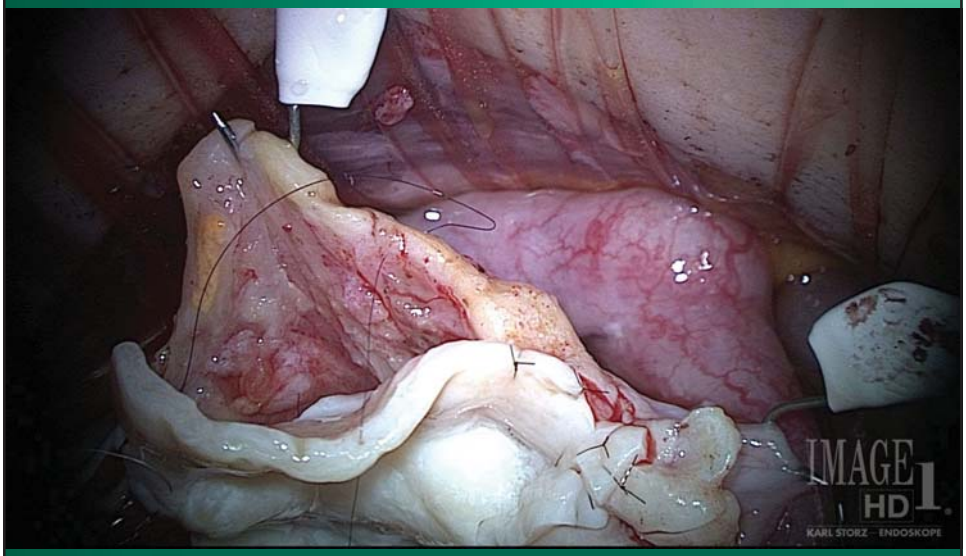
Fibrous Tunica Albuginea of Testis: Same Embryologic Structure as Ovarian Cortex





TESE Closure of Tunica Albugionea

Ovarian Cortex Is Tunica Albuginea of Testis



Ovarian Cortical Transplant

No Predictive Test Parameters for Finding of Sperm

- In normal testis there are 100's of millions of sperm.
- In NOA we are looking for just 10-20 sperm.
- A million fold decline in sperm will still permit successful TESE.

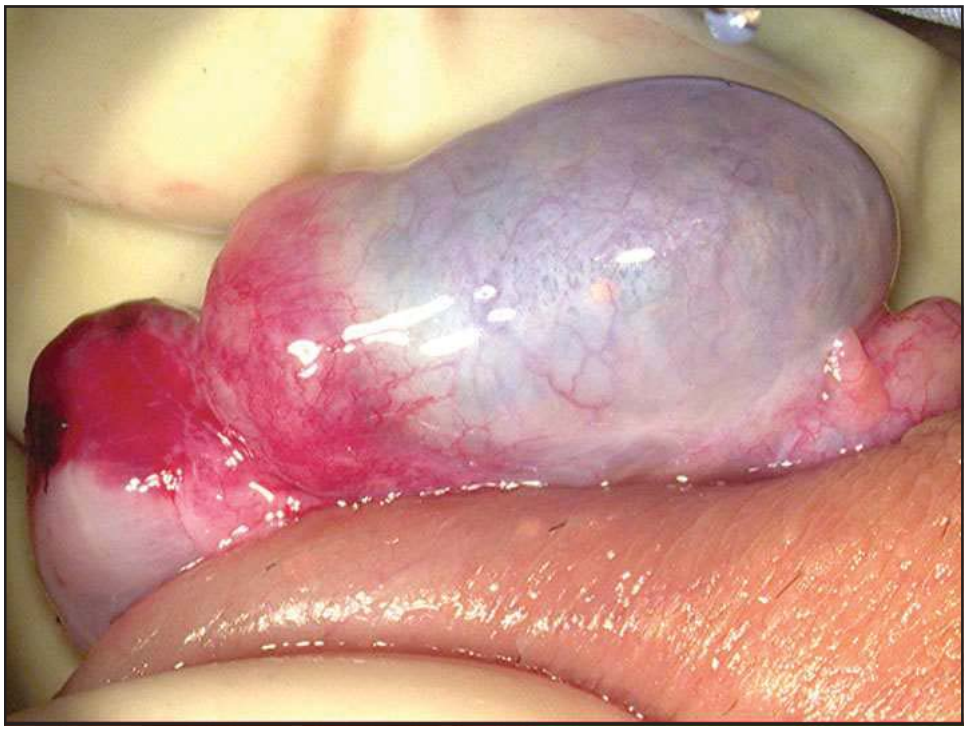
Silber et al (Distribution of Spermatogenesis) Human Reprod 1997
Silber et al (Maturation Arrest) Fertil Steril 1996

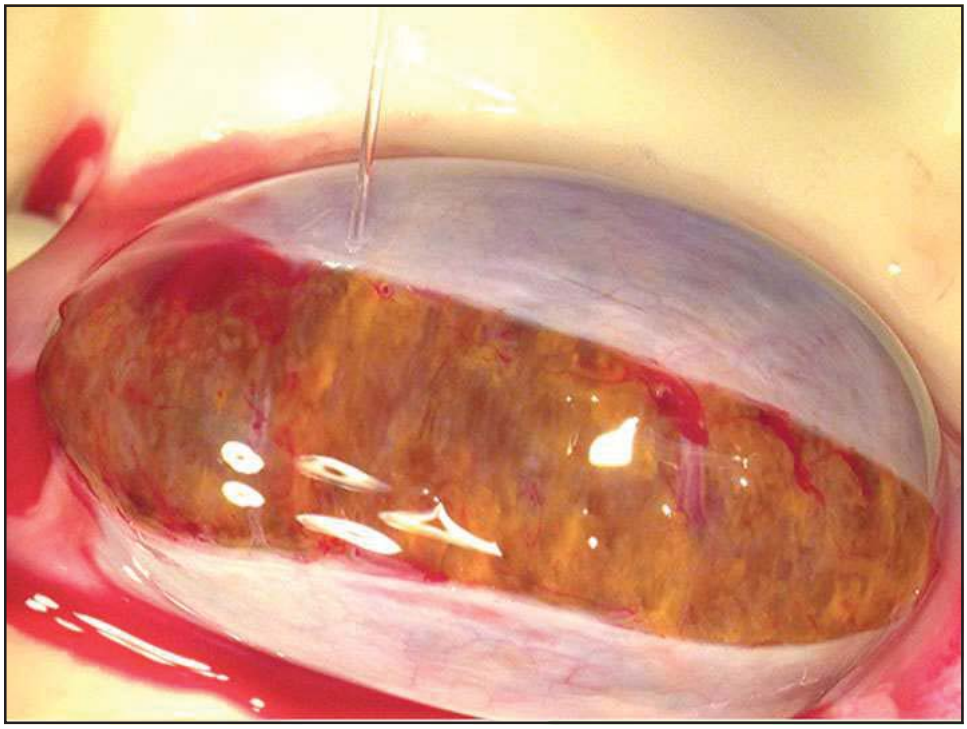
ASRM MEFS AZOOSPERMIA
Oocyte Clip 1

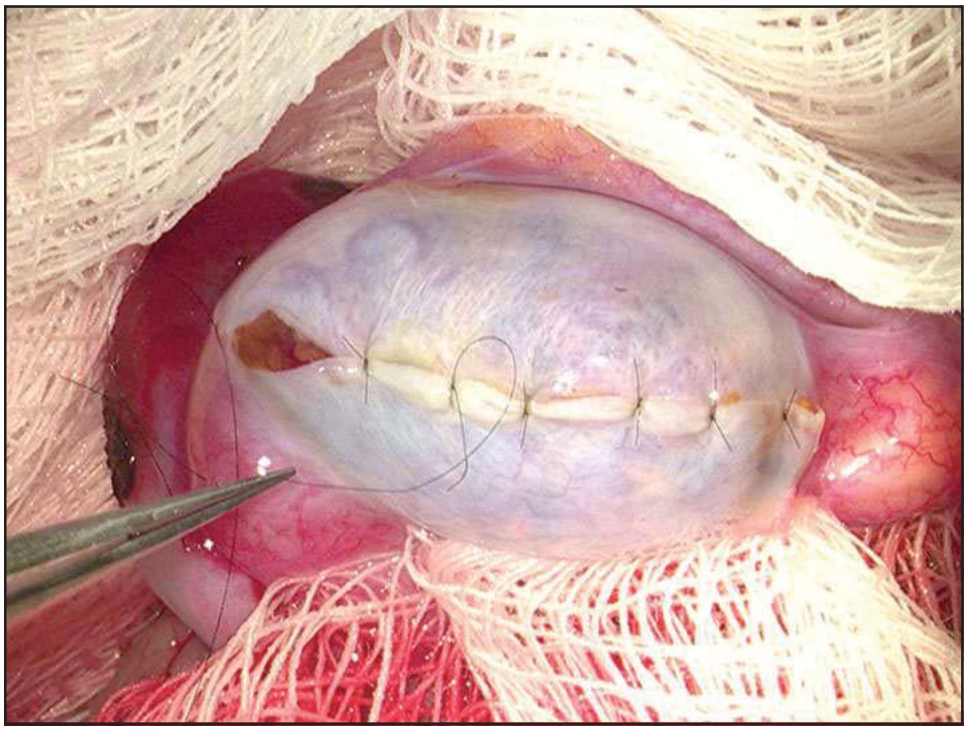
ASRM MEFS AZOOSPERMIA
Oocyte Clip 2

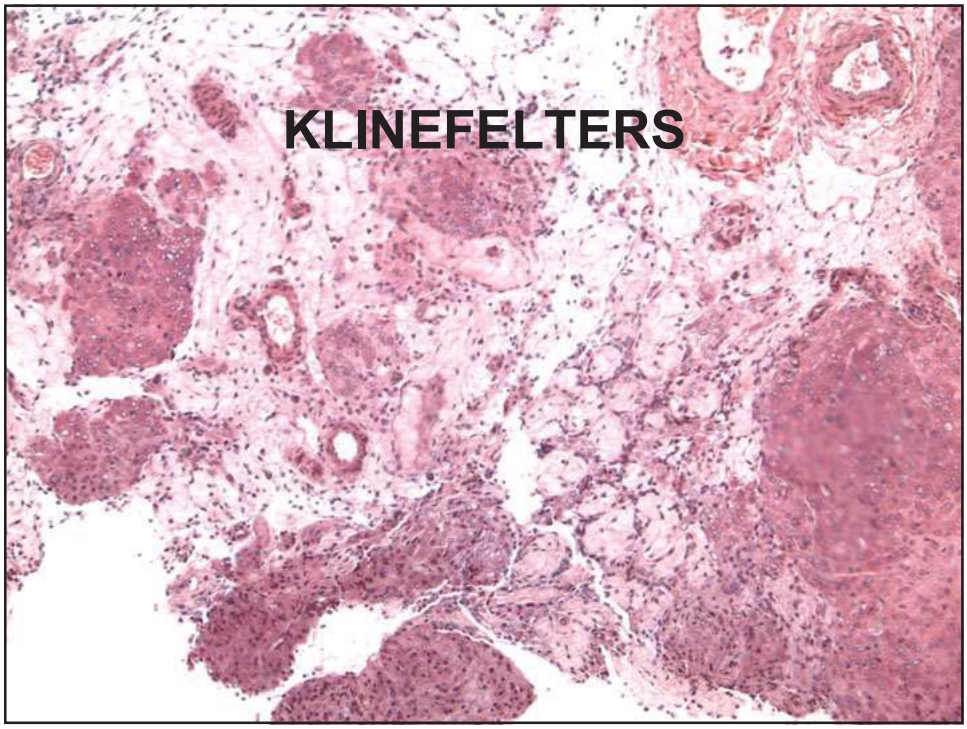
ASRM MEFS AZOOSPERMIA
Oocyte Clip 3

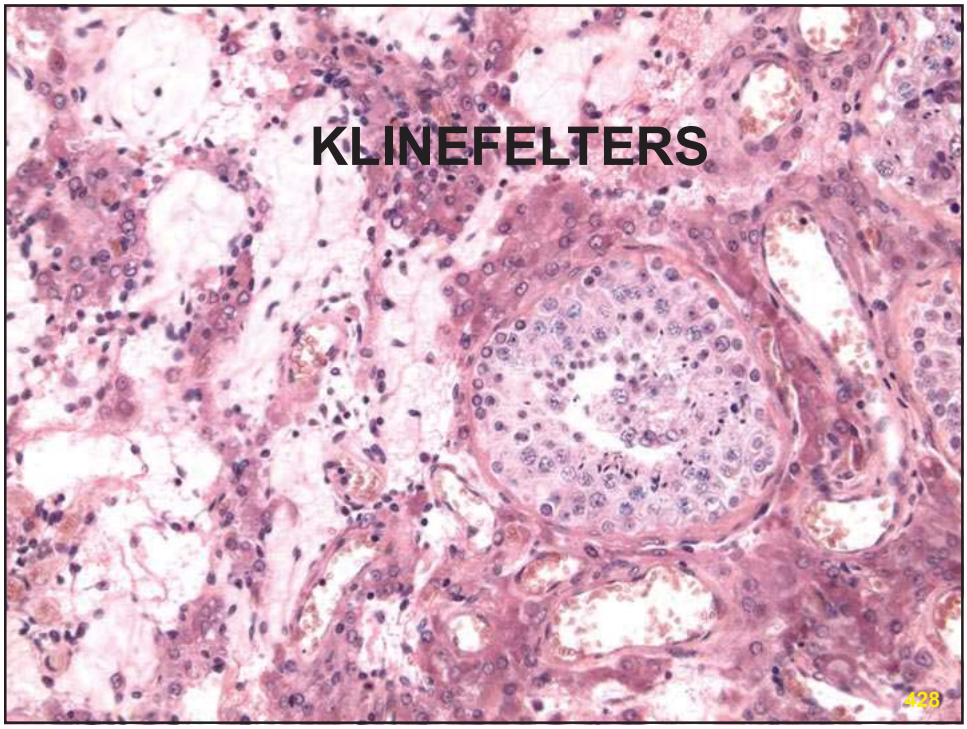
XXY KLINEFELTER



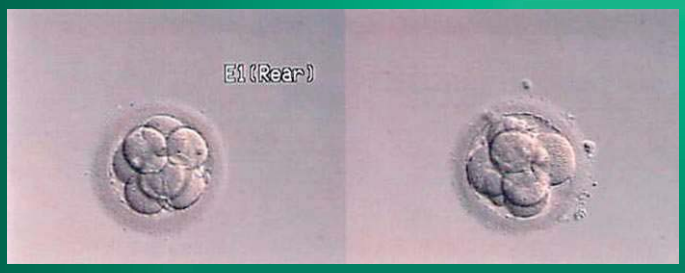




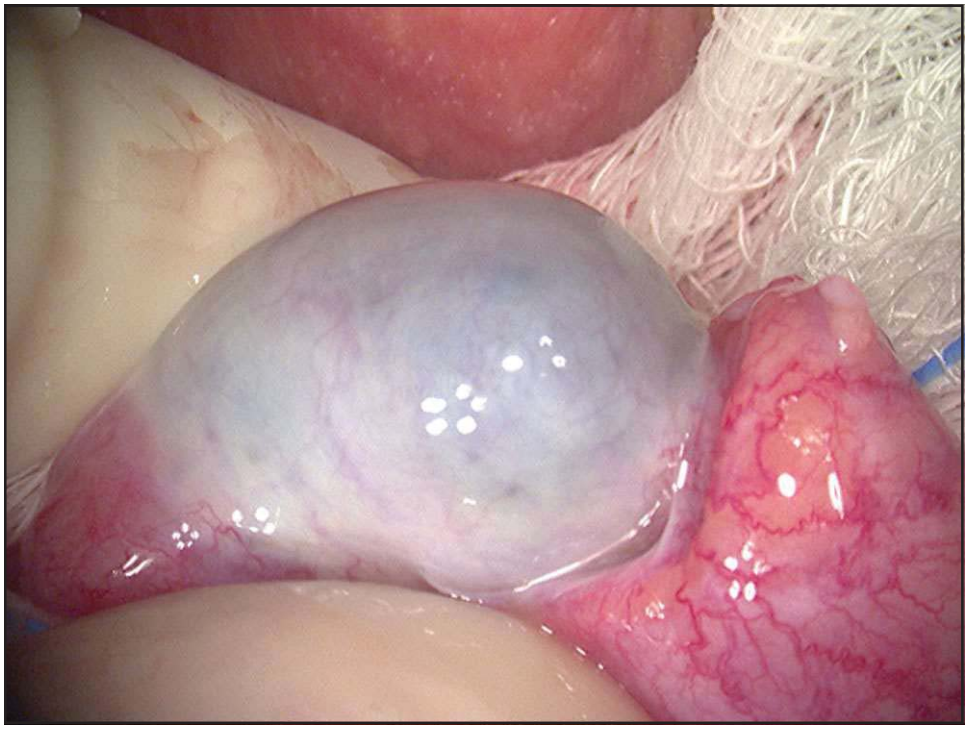

KLINEFELTERS

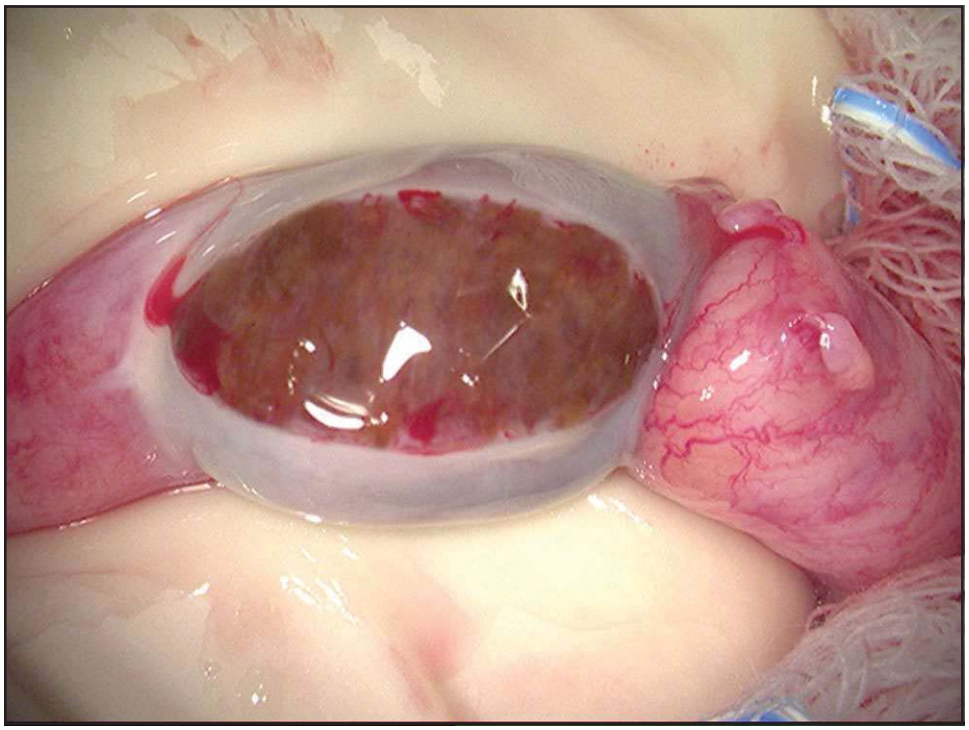

KLINEFELTERS
428

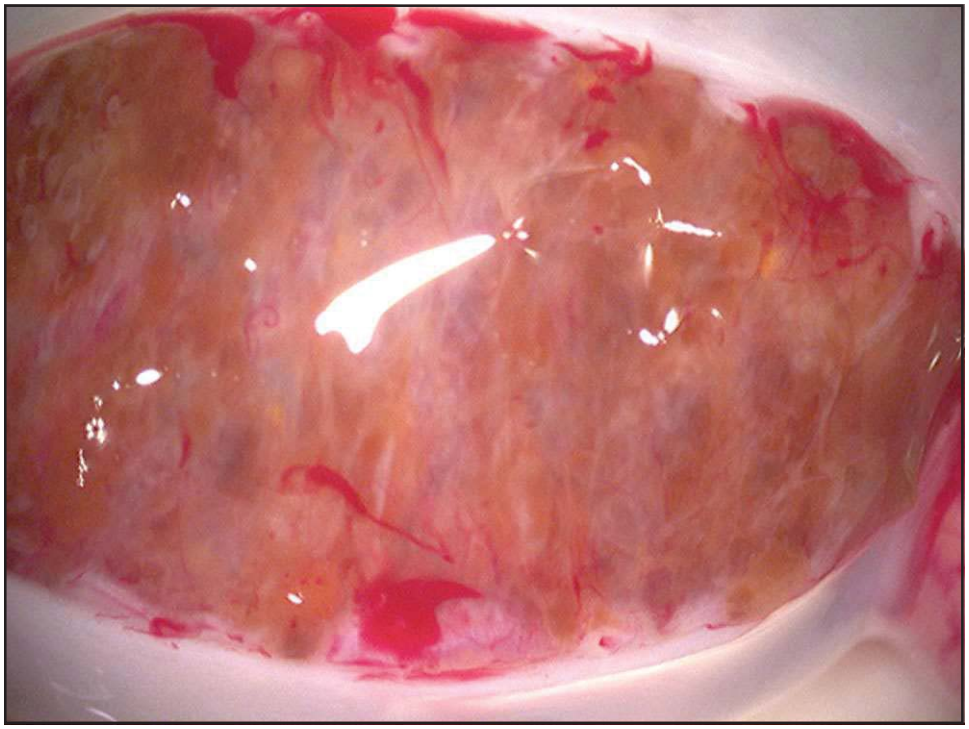
2 GOOD QUALITY EMBRYOS TRANSFERRED AND 3 FROZEN

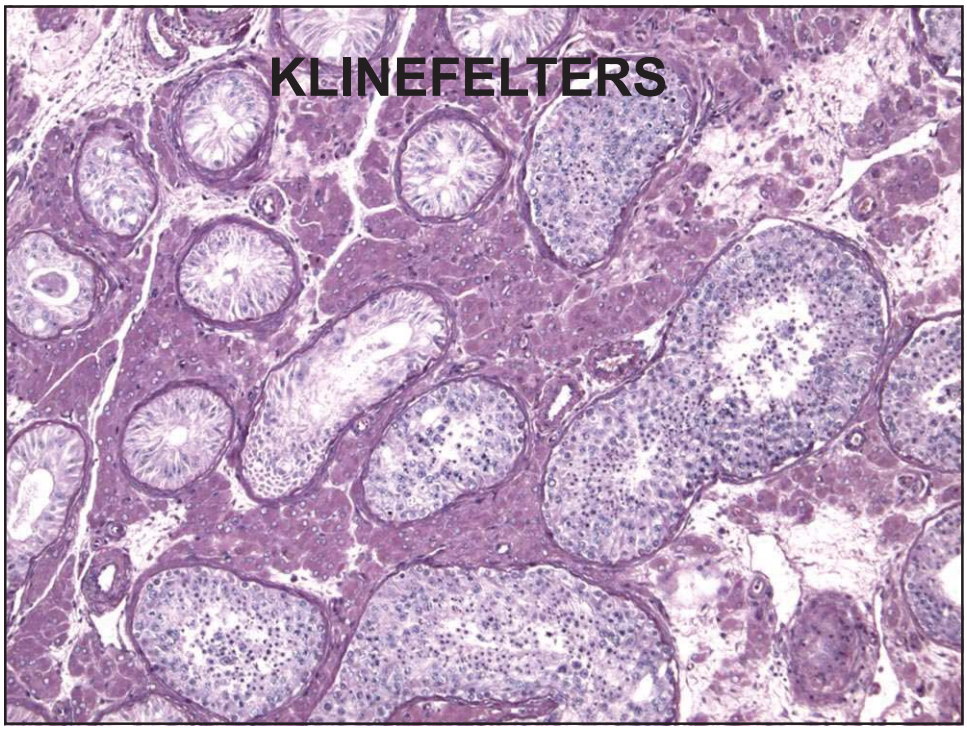


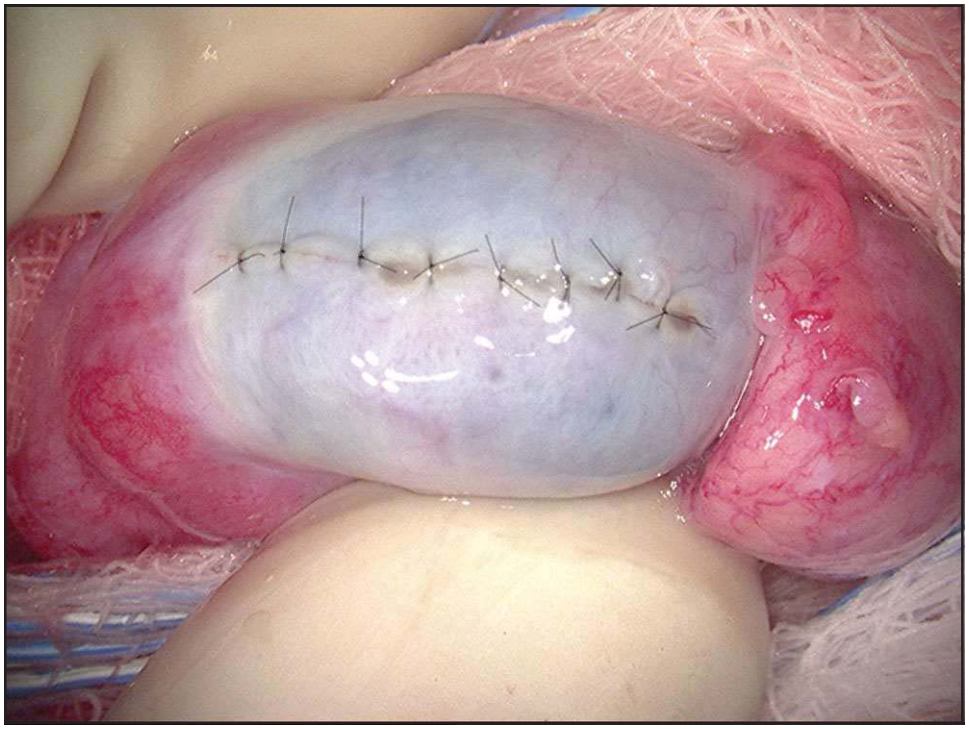
HEALTHY TWINS DELIVERED





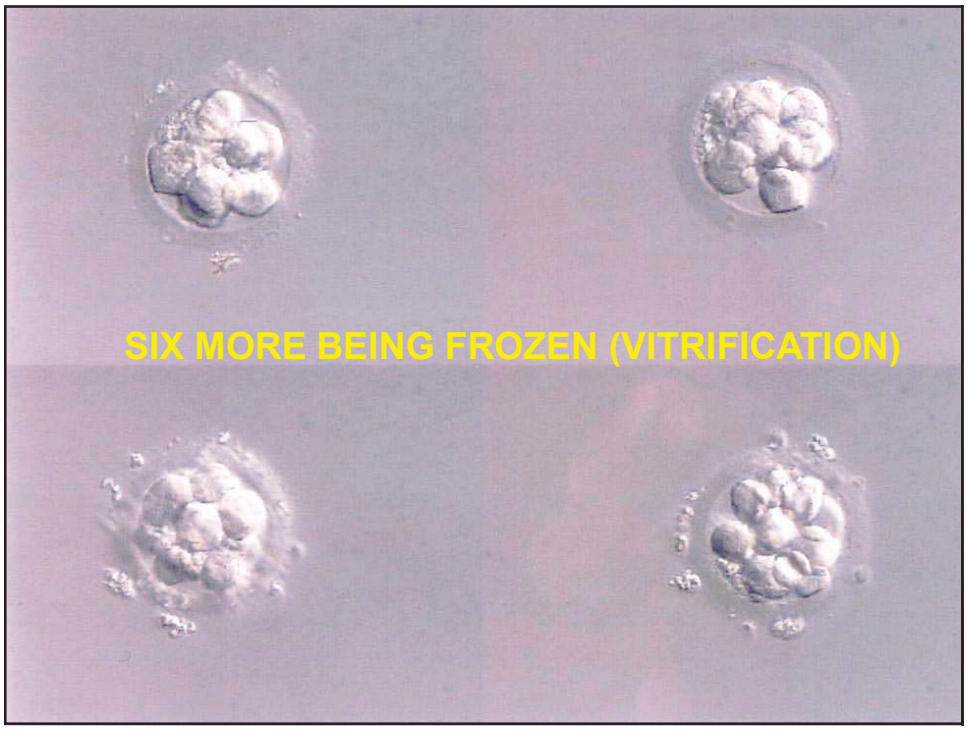



KLINEFELTERS

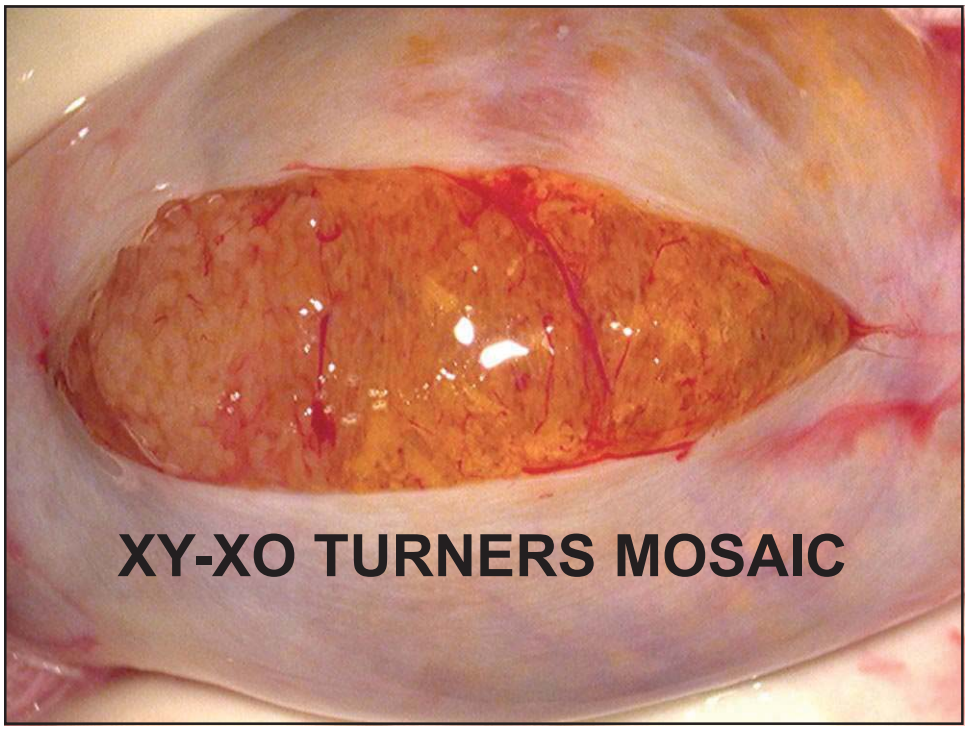


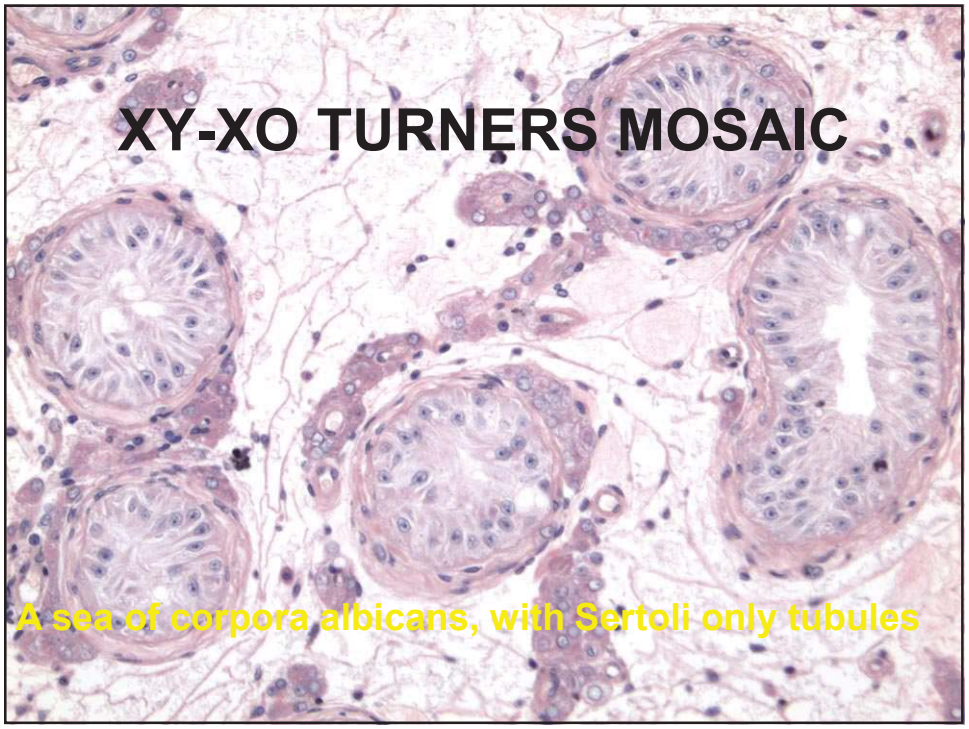
ASRM MEFS AZOOSPERMIA
Klinefelters Patient

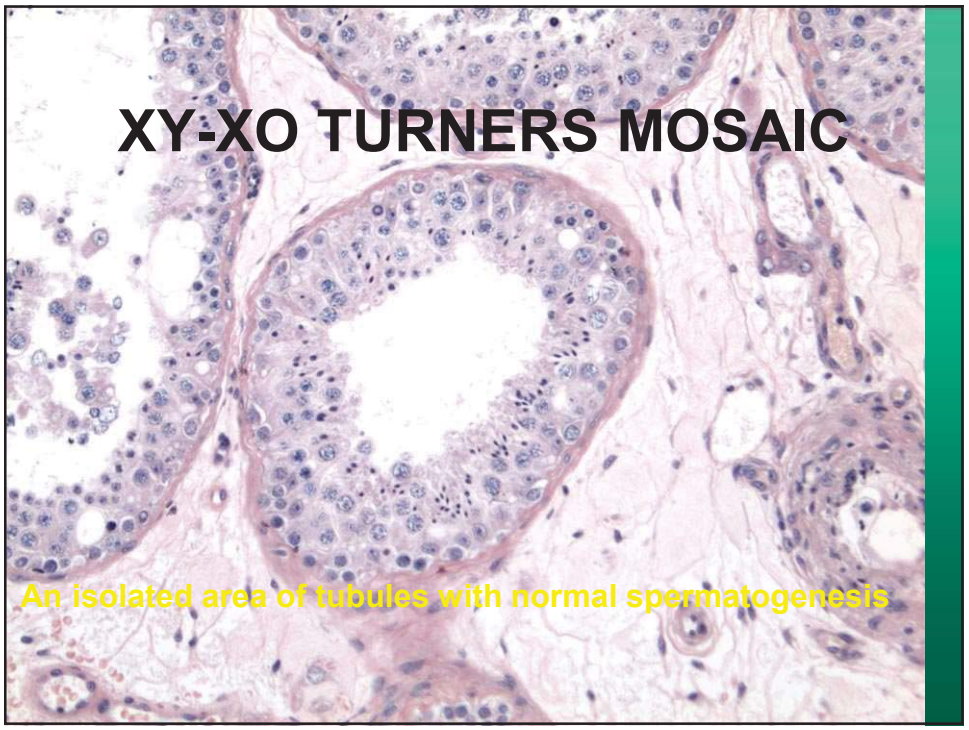

HEALTHY TWINS

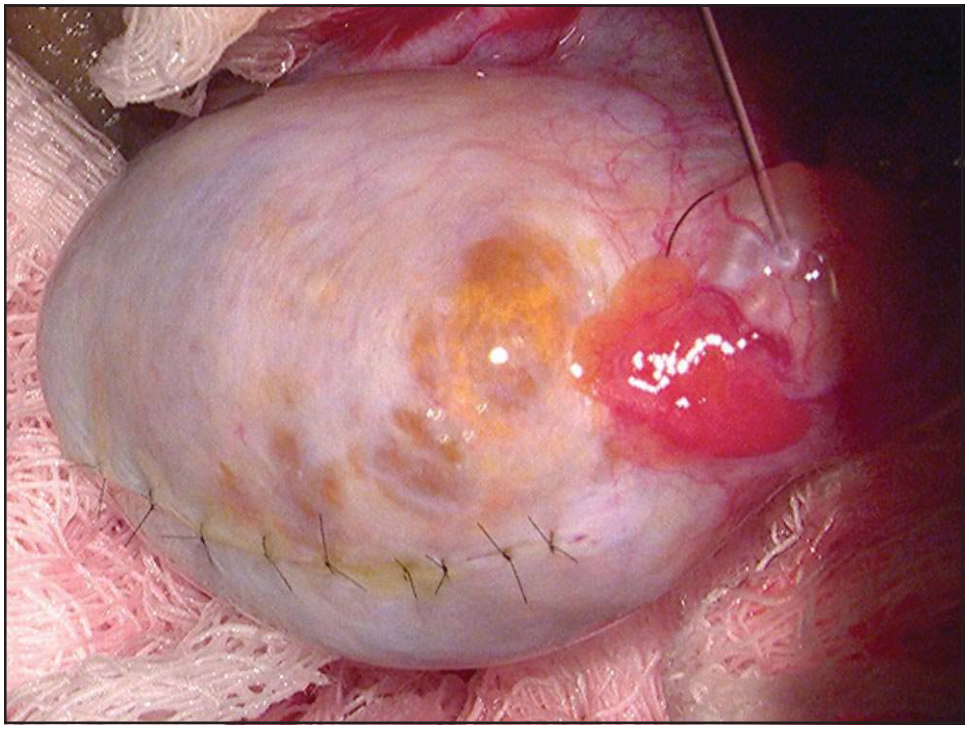

SIX MORE BEING FROZEN (VITRIFICATION)

XY-XO TURNERS MOSAIC

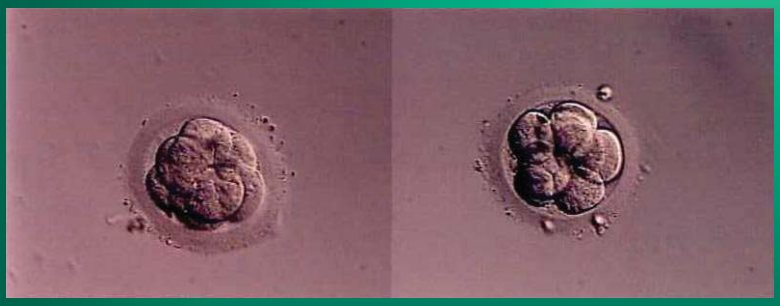



XY-XO TURNERS MOSAIC
A sea of corpora albicans, with Sertoli only tubules


XY-XO TURNERS MOSAIC
An isolated area of tubules with normal spermatogenesis

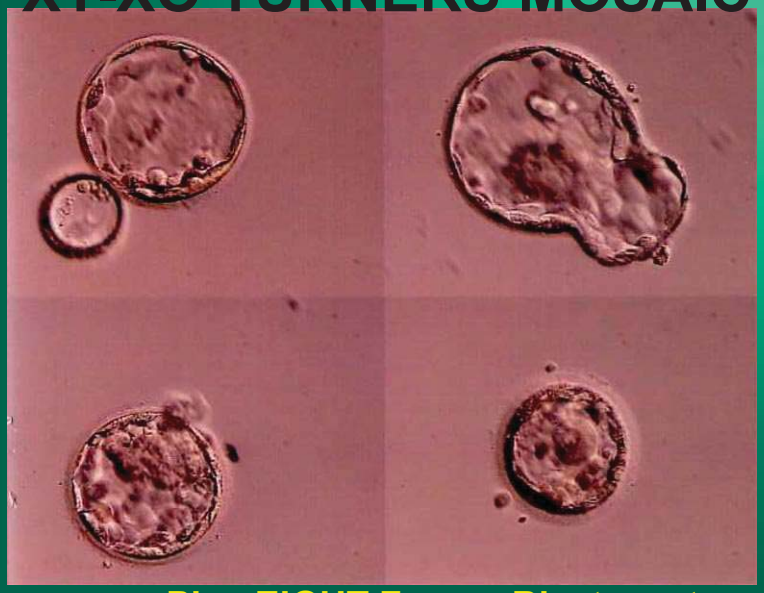


XY-XO TURNERS MOSAIC

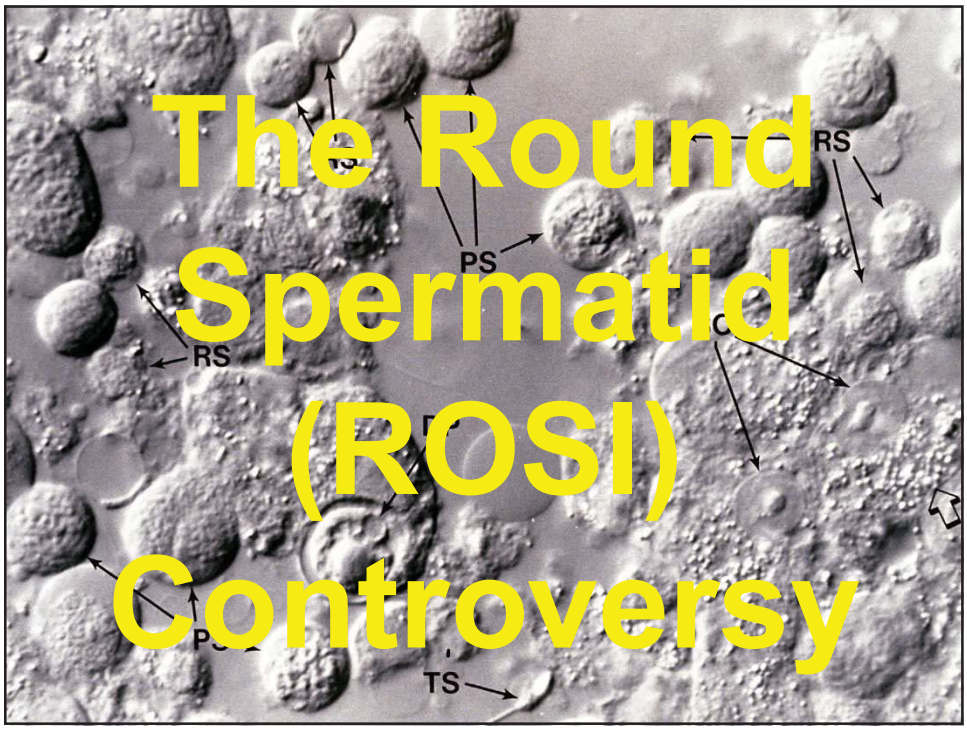


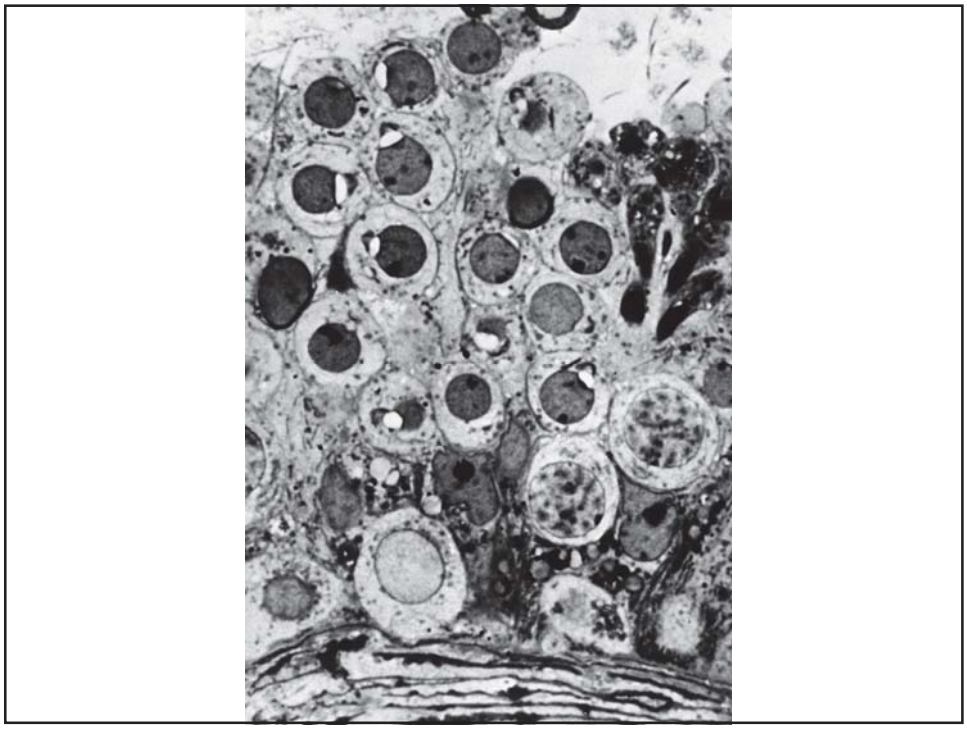
HEALTHY TWINS DELIVERED

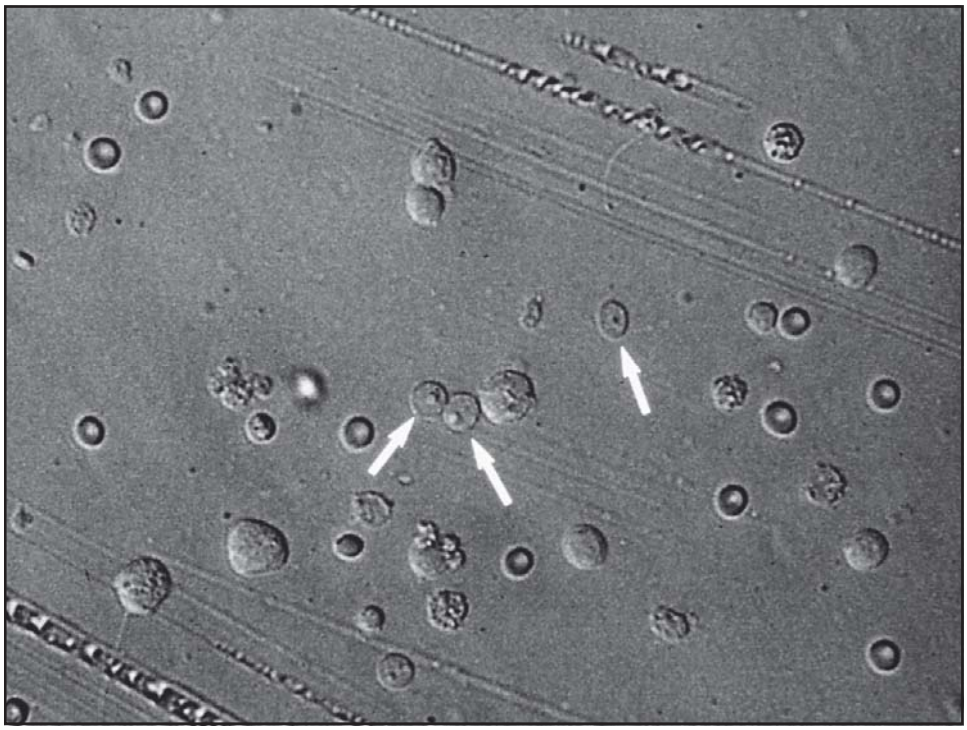
XY-XO TURNERS MOSAIC

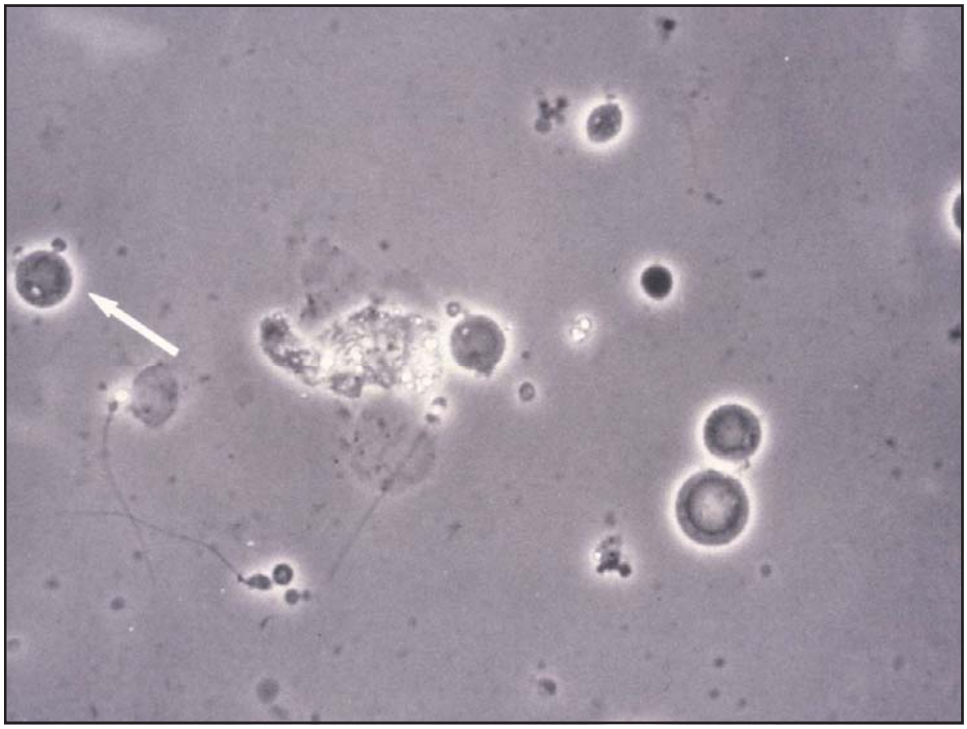


Plus EIGHT Frozen Blastocysts









ICSI Cycle Results with Varying Degrees of Male Factor Infertility

ICSI Live Birth Rates for Obstructive Azoospermia (Testis Vs. Epididymis)

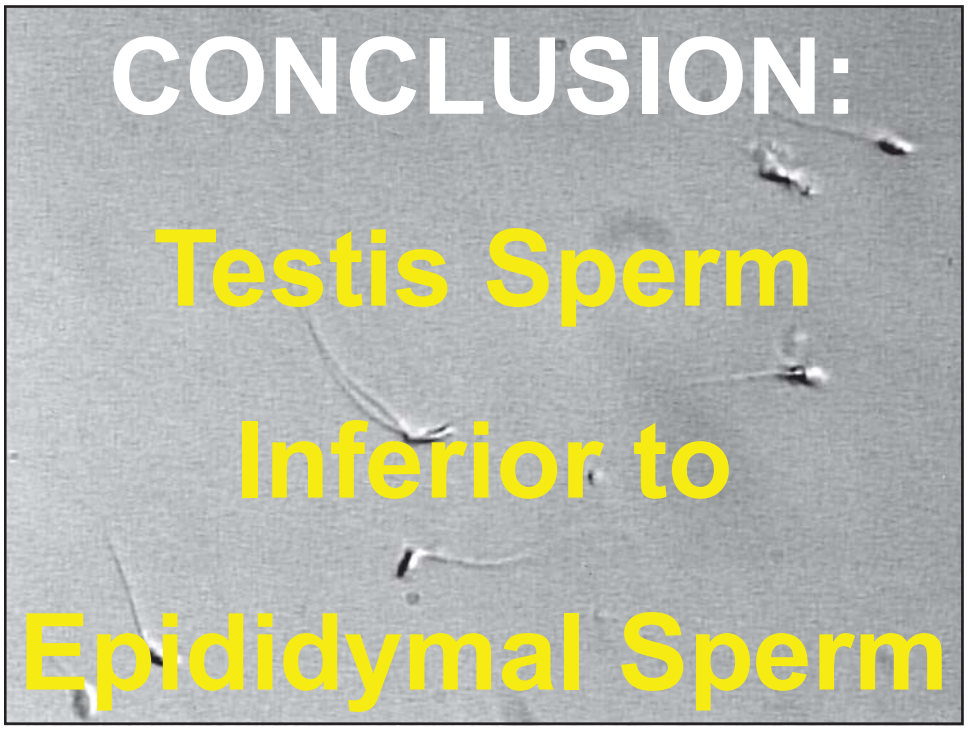
Age	MESA OA Fresh & Frozen		TESE OA Fresh & Frozen		Overall	
≤35	159/377	42%	33/99	33%	192/476	40%
36-40	27/109	25%	5/33	15%	32/142	22%
>40	4/29	14%	0/6	0%	4/35	11%
Overall	190/486	39%	38/132	29%	228/618	37%

ICSI Live Birth Rates for Non-Obstructive Azoospermia (Testis Vs. Epididymis)

Age	MESA OA Fresh & Frozen		TESE OA Fresh & Frozen		TESE NOA Fresh & Frozen		Overall	
≤35	159/377	42%	33/99	33%	52/230	23%	244/706	35%
36-40	27/109	25%	5/33	15%	20/70	29%	52/212	24%
>40	4/29	14%	0/6	0%	0/16	0%	4/51	7%
Overall	190/486	39%	38/132	29%	72/316	23%	300/934	32%

ICSI Live Birth Rates for NOA and OA With Testis Sperm

Age	TESE OA Fresh		TESE NOA Fresh		Overall	
≤35	33/99	33%	52/230	23%	85/329	26%
36-40	5/33	15%	20/70	29%	25/103	24%
>40	0/6	0%	0/16	0%	0/22	0%
Overall	38/132	29%	72/316	23%	110/448	24%



FUTURE OF TESE: Stem Cells

- Retrieve testis tissue prepubertal male cancer patients.
- Culture spermatogonial stem cells in multiple passages to eliminate cancer cells.
- Transfer pure stem cells back to testis.

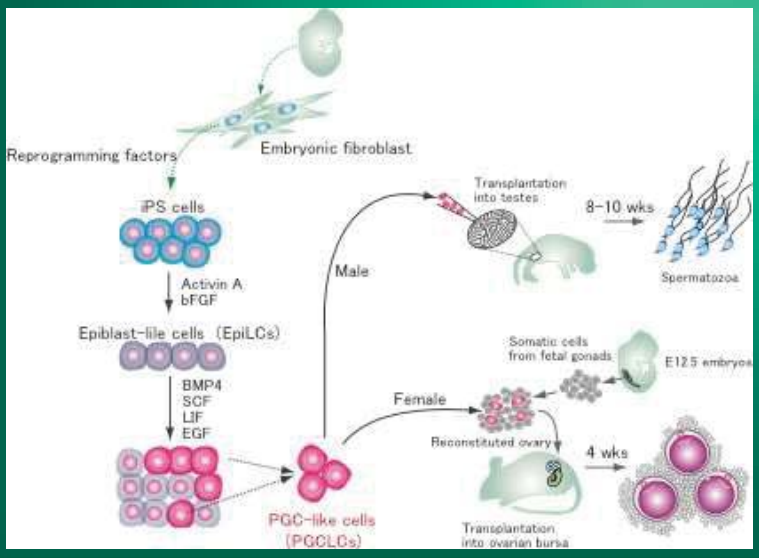
FUTURE OF TESE: Stem Cells

- For severe oligospermic males, retrieve testis tissue and culture spermatogonial stem cells to exponentially increase number.
- Then transfer back to testis via rete testis to increase sperm count.

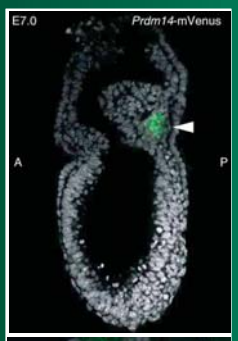
SPERM AND EGGS FROM SKIN CELLS

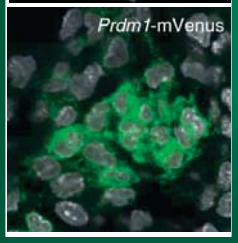
No Need For TESE with NOA

Derivation of artificial gametes from iPS cells in mouse

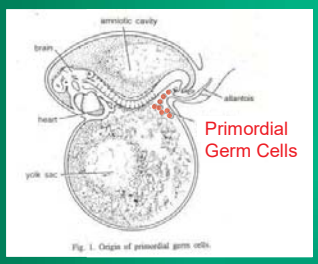


Primordial Germ Cells



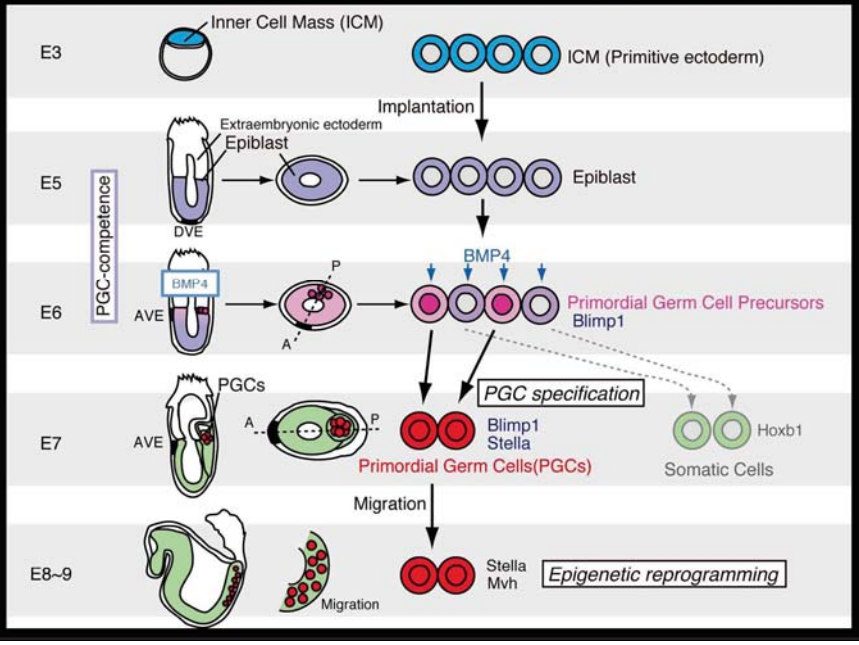


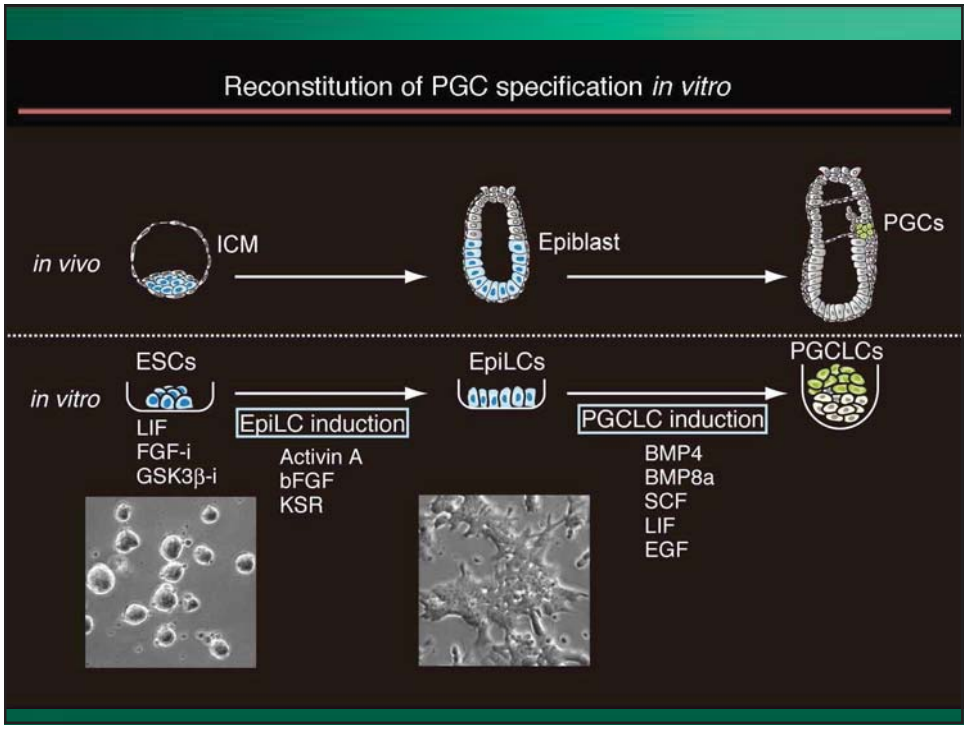
Yamaji et al (2008) Nat.Genet.

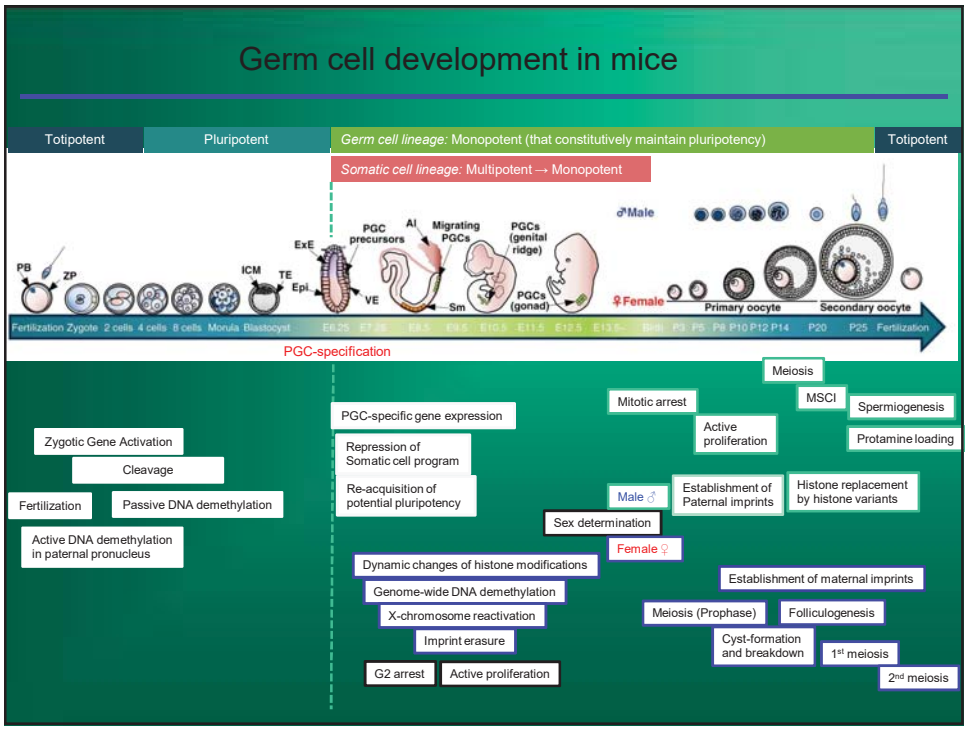


- the origin of all germ cell lineage
- expressing pluripotent genes
- Undergoing genome-wide reprogramming (massive DNA demethylation and conversion of histone modifications)
- **<u>When transferred into testis or ovary, PGCs give rise to functional sperm or oocytes.</u>**

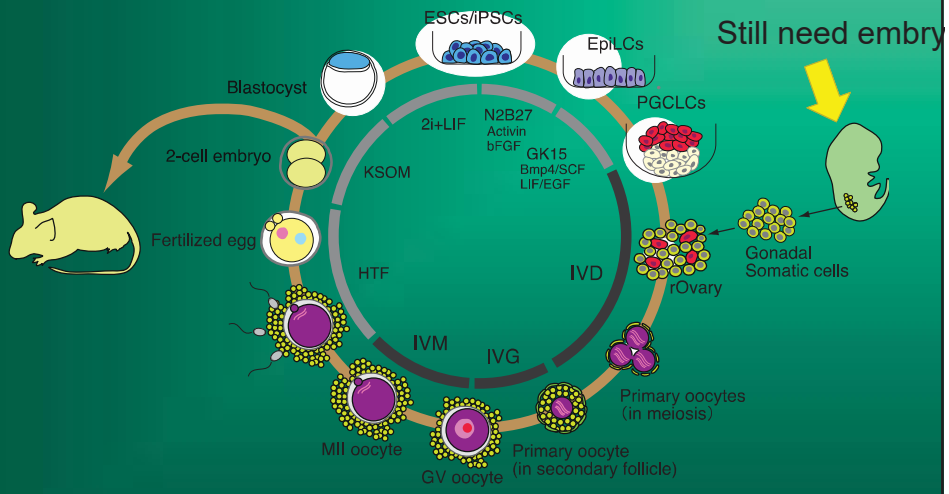
PGC specification followed by epigenetic reprogramming in mice




Reconstitution of PGC specification *in vitro*
in vivo
ICM
Epiblast
PGCs
in vitro
ESCs
LIF
FGF-i
GSK3β-i
EpiLC induction
Activin A
bFGF
KSR
EpiLCs
PGCLC induction
BMP4
BMP8a
SCF
LIF
EGF
PGCLCs


Germ cell development in mice
Totipotent
Pluripotent
Germ cell lineage: Monopotent (that constitutively maintain pluripotency)
Totipotent
Somatic cell lineage: Multipotent → Monopotent
PB
ZP
ICM
TE
Epi
ExE
VE
PGC precursors
Al
Migrating PGCs
Sm
PGCs (genital ridge)
PGCs (gonad)
♂Male
♀Female
Primary oocyte
Secondary oocyte
Fertilization Zygote 2 cells 4 cells 8 cells Morula Blastocyst
P20
P25 Fertilization
PGC-specification
Zygotic Gene Activation
Cleavage
Fertilization
Passive DNA demethylation
Active DNA demethylation in paternal pronucleus
PGC-specific gene expression
Repression of Somatic cell program
Re-acquisition of potential pluripotency
Dynamic changes of histone modifications
Genome-wide DNA demethylation
X-chromosome reactivation
Imprint erasure
G2 arrest
Active proliferation
Meiosis
MSCI
Mitotic arrest
Spermiogenesis
Active proliferation
Protamine loading
Male ♂
Establishment of Paternal imprints
Histone replacement by histone variants
Sex determination
Female ♀
Establishment of maternal imprints
Meiosis (Prophase)
Folliculogenesis
Cyst-formation and breakdown
1st meiosis
2nd meiosis

Reconstitution in vitro of the entire cycle of the female germline



Bibliography

- Alrabeeah K, Wachter A, Phillips S, Cohen B, Al-Hathal N, Zini A. Sperm retrieval outcomes with microdissection testicular sperm extraction (micro-TESE) in men with cryptozoospermia. Andrology 2015;3:462-6.
- Alrabeeah K, Witmer J, Ruiz S, AlMalki A, Phillips S, Zini A. Mini-incision microdissection testicular sperm extraction: a useful technique for men with cryptozoospermia. Andrology 2016;4:284-9.
- Alvarez C, Cremades N, Blasco N, Bernabeu R. Influence of gonadotrophin-releasing hormone agonist total dose in the ovarian stimulation in the long down-regulation protocol for in-vitro fertilization. Human reproduction 1997;12:2366-9.
- Araujo E, Jr., Tadir Y, Patrizio P, et al. Relative force of human epididymal sperm. Fertility and sterility 1994;62:585-90.
- Cetinkaya M, Onem K, Zorba OU, Ozkara H, Alici B. Evaluation of Microdissection Testicular Sperm Extraction Results in Patients with Non-Obstructive Azoospermia: Independent Predictive Factors and Best Cutoff Values for Sperm Retrieval. Urology journal 2015;12:2436-43.
- Deruyver Y, Vanderschueren D, Van der Aa F. Outcome of microdissection TESE compared with conventional TESE in non-obstructive azoospermia: a systematic review. Andrology 2014;2:20-4.
- Franco G, Scarselli F, Casciani V, et al. A novel stepwise micro-TESE approach in non obstructive azoospermia. BMC urology 2016;16:20.
- Kahraman S, Ozgur S, Alatas C, et al. Fertility with testicular sperm extraction and intracytoplasmic sperm injection in non-obstructive azoospermic men. Human reproduction 1996;11:756-60.
- Kalsi JS, Shah P, Thum Y, Muneer A, Ralph DJ, Minhas S. Salvage micro-dissection testicular sperm extraction; outcome in men with non-obstructive azoospermia with previous failed sperm retrievals. BJU international 2015;116:460-5.
- Karacan M, Ulug M, Arvas A, Cebi Z, Erkan S, Camlibel T. Live birth rate with repeat microdissection TESE and intracytoplasmic sperm injection after a conventional testicular biopsy in men with nonobstructive azoospermia. European journal of obstetrics, gynecology, and reproductive biology 2014;183:174-7.
- Modarresi T, Sabbaghian M, Shahverdi A, Hosseinifar H, Akhlaghi AA, Sadighi Gilani MA. Enzymatic digestion improves testicular sperm retrieval in non-obstructive azoospermic patients. Iranian journal of reproductive medicine 2013;11:447-52.
- Niederberger C. Re: Salvage Micro-Dissection Testicular Sperm Extraction; Outcome in Men with Non-Obstructive Azoospermia with Previous Failed Sperm Retrievals. The Journal of urology 2016;195:1565.

Bibliography Continued

•Omurtag K, Cooper A, Bullock A, et al. Sperm recovery and IVF after testicular sperm extraction (TESE): effect of male diagnosis and use of off-site surgical centers on sperm recovery and IVF. PloS one 2013;8:e69838.

•Sacca A, Pastore AL, Roscigno M, et al. Conventional testicular sperm extraction (TESE) and non-obstructive azoospermia: is there still a chance in the era of microdissection TESE? Results from a single non-academic community hospital. Andrology 2016;4:425-9.

•Safarinejad MR. Re: Evaluation of Microdissection Testicular Sperm Extraction Results in Patients with Non-Obstructive Azoospermia: Independent Predictive Factors and Best Cutoff Values for Sperm Retrieval. Urology journal 2015;12:2444-6.

•Silber SJ, Nagy ZP, Liu J, Godoy H, Devroey P, Van Steirteghem AC. Conventional in-vitro fertilization versus intracytoplasmic sperm injection for patients requiring microsurgical sperm aspiration. Human reproduction 1994;9:1705-9.

•Silber SJ, Nagy Z, Liu J, et al. The use of epididymal and testicular spermatozoa for intracytoplasmic sperm injection: the genetic implications for male infertility. Human reproduction 1995;10:2031-43.

•Silber SJ, Van Steirteghem AC, Liu J, Nagy Z, Tournaye H, Devroey P. High fertilization and pregnancy rate after intracytoplasmic sperm injection with spermatozoa obtained from testicle biopsy. Human reproduction 1995;10:148-52.

•Silber SJ, van Steirteghem A, Nagy Z, Liu J, Tournaye H, Devroey P. Normal pregnancies resulting from testicular sperm extraction and intracytoplasmic sperm injection for azoospermia due to maturation arrest. Fertility and sterility 1996;66:110-7.

•Silber SJ, Nagy Z, Devroey P, Tournaye H, Van Steirteghem AC. Distribution of spermatogenesis in the testicles of azoospermic men: the presence or absence of spermatids in the testes of men with germinal failure. Human reproduction 1997;12:2422-8.

•Silber SJ, Nagy Z, Devroey P, Camus M, Van Steirteghem AC. The effect of female age and ovarian reserve on pregnancy rate in male infertility: treatment of azoospermia with sperm retrieval and intracytoplasmic sperm injection. Human reproduction 1997;12:2693-700.

•Tournaye H. How to predict fatherhood for men with non-obstructive azoospermia opting for TESE-ICSI? Human reproduction 2011;26:3213-4.

•Vloeberghs V, Verheyen G, Haentjens P, Goossens A, Polyzos NP, Tournaye H. How successful is TESE-ICSI in couples with non-obstructive azoospermia? Human reproduction 2015;30:1790-6.

•Working PK. Male reproductive toxicology: comparison of the human to animal models. Environmental health perspectives 1988;77:37-44.

•Yamaji M, Seki Y, Kurimoto K, et al. Critical function of Prdm14 for the establishment of the germ cell lineage in mice. Nature genetics 2008;40:1016-22.

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