



反复移植失败实验室策略

徐小明

北京家園醫院生殖中心

2017-12-2



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一、反复移植失败的定义



反复移植失败

- 反复种植失败 (*Recurrent Implantation Failure, RIF*)
- 反复早期流产 (*Recurrent Pregnancy Loss, RPL*)

REVIEW

What exactly do we mean by 'recurrent implantation failure'? A systematic review and opinion



Lukasz T Polanski ^{a,*}, Miriam N Baumgarten ^a, Siobhan Quenby ^b, Jan Brosens ^b, Bruce K Campbell ^a, Nicholas J Raine-Fenning ^a

^a Nurture Fertility, Division of Obstetrics and Gynaecology, School of Clinical Sciences, University of Nottingham, United Kingdom; ^b University of Warwick, Coventry, United Kingdom

* Corresponding author. E-mail address: lukasz.polanski@nottingham.ac.uk (LT Polanski).



Lukasz Polanski obtained his medical degree in 2007 from the Medical University of Lublin, Poland. He completed his foundation training in the East of England Deanery, UK, where he also commenced his core obstetrics and gynaecology specialty training. Currently, he is undertaking a PhD at the University of Nottingham. His interests lie in recurrent reproductive failure and early pregnancy development.

Abstract Recurrent implantation failure (RIF) is an iatrogenic condition, being the result of repetitive unsuccessful cycles of IVF or intracytoplasmic sperm injection (ICSI) treatment. The aim of this review was to assess the definitions of RIF used in literature as well as suggest a uniform definition of this condition. A systematic search of MEDLINE, Embase and Cochrane Library was conducted. The most commonly stated definitions described RIF as 'three or more failed treatment cycles' or 'two or more failed cycles'. Other identified definitions were based solely on the number of embryos transferred in previous cycles or combined the number of previously failed cycles with the number of transferred embryos. Several other definitions were also identified. This review highlights the lack of uniformity of the definition of RIF. Based on the available literature and the expert opinion of the authors, RIF should be defined as the absence of implantation after two consecutive cycles of IVF, ICSI or frozen embryo replacement cycles where the cumulative number of transferred embryos was no less than four for cleavage-stage embryos and no less than two for blastocysts, with all embryos being of good quality and of appropriate developmental stage.

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针对**反复种植失败**目前没有统一标准。

推荐定义：年龄小于40岁，连续两个治疗周期（IVF/ICSI，或者冷冻胚胎移植），累计移植≥4个**优质卵裂期胚胎**，或ET两次≥2个**优质囊胚**仍种植失败。



反复移植失败原因分析

➤1. 母体因素

•子宫结构因素

子宫内**膜**息肉，子宫内**膜**异位症，黏膜下肌瘤，**宫**腔粘连等

•子宫功能因素：

子宫内**膜**容受性

•输卵管积水

•免疫因素

•血栓形成倾向；

•子宫内**膜**蠕动异常

➤2. 胚胎与子宫内**膜**对话

➤3. 配子及胚胎因素

精子DNA碎片（DFI），胚胎染色体异常，卵子**质**量等



二、精子因素

减少氧化**应激**（ROS），**保护**精子DNA完整性

- 精液**处理**方法
- 使用**手术**来源的精子（PESA/TESA）
- 精液**处理**完**毕**后保存在室温下（重度少弱精子）

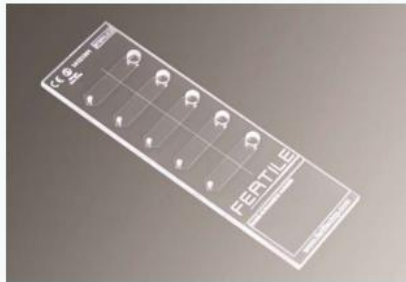
精子非整倍体：

14, 15, 22, X, Y

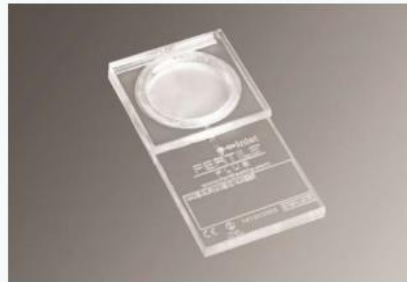
精子中心粒异常：

可引发胚胎**纺锤**体异常，形成**严重**的嵌合体胚胎

优化精液处理方法，降低对精子DNA的损伤



FERTILE (FH0002)



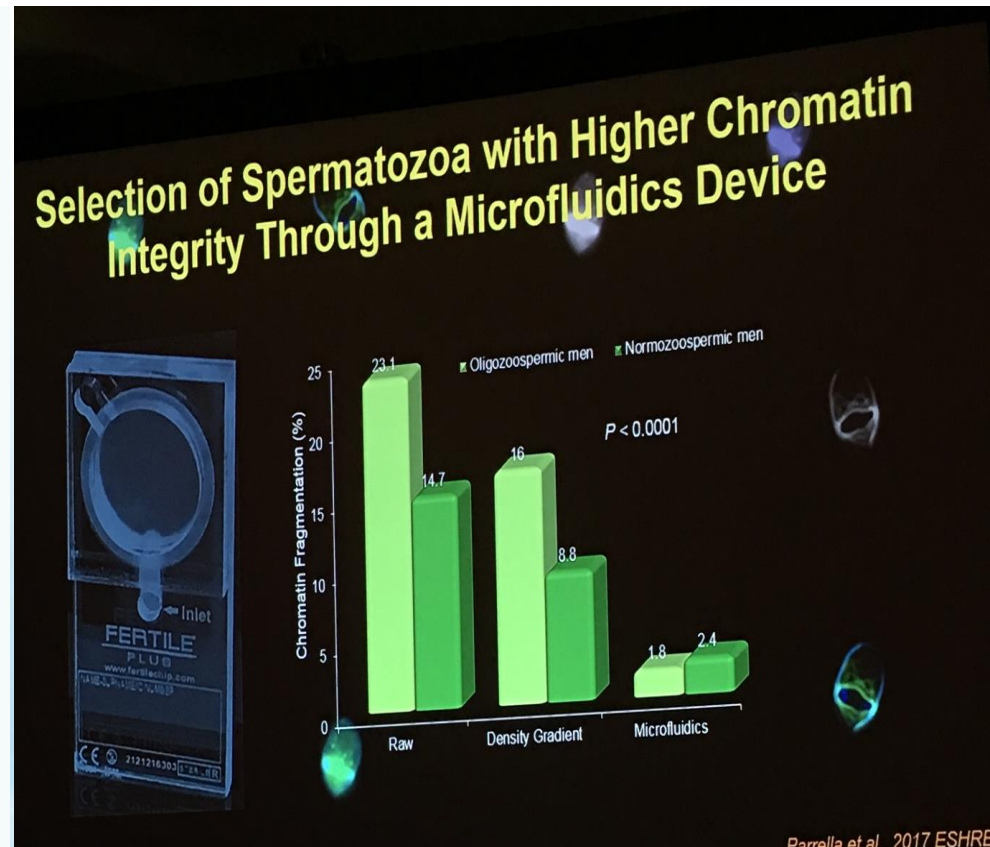
FERTILE PLUS (FPH0850.8)

FERTILE and FERTILE PLUS are flow-free sterile single-use chips for the sorting and separation of healthy normal sperm from the many compromised sperm present in a semen sample.

FERTILE is designed to sort sperm for subsequent use in ICSI (intracytoplasmic sperm injection).

FERTILE PLUS is designed to sort sperm for subsequent use in ICSI, IVF (*in vitro* fertilization) and IUI (intrauterine insemination).

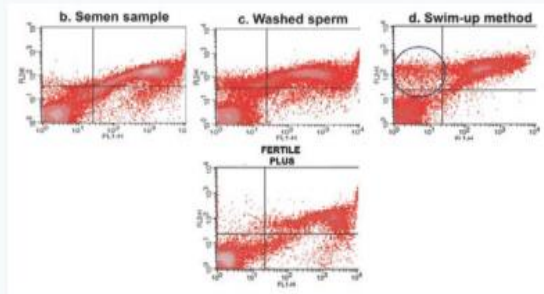
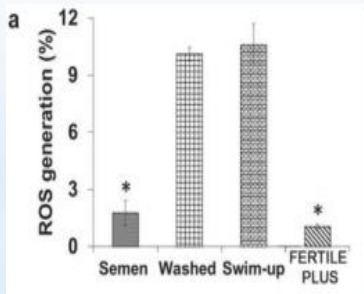
FERTILE and FERTILE PLUS eliminate the use of sperm-damaging procedures such as centrifugation and mixing.



微流体芯片

优化精液处理方法，降低对精子DNA的损伤

Reactive Oxygen Species

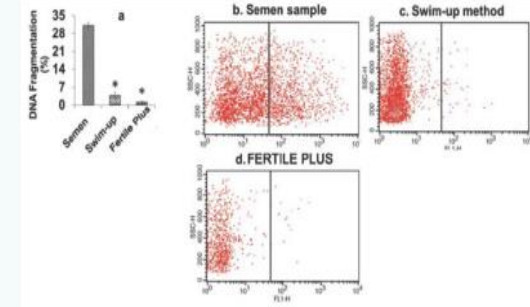
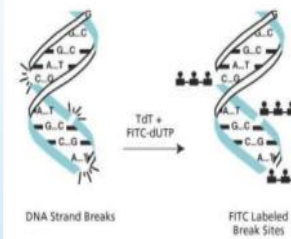


Reactive Oxygen Species (ROS) Analysis – FERTILE PLUS:

Sorting with FERTILE PLUS results in an approximately five-fold reduction in ROS generation.^(3,4,5)

DNA Fragmentation

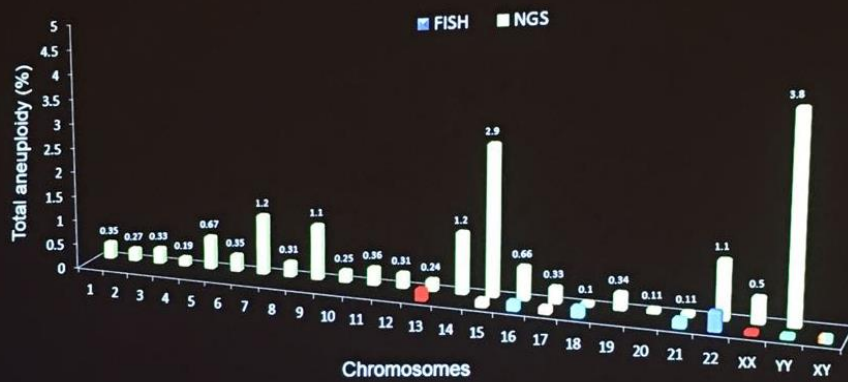
DNA FRAGMENTATION



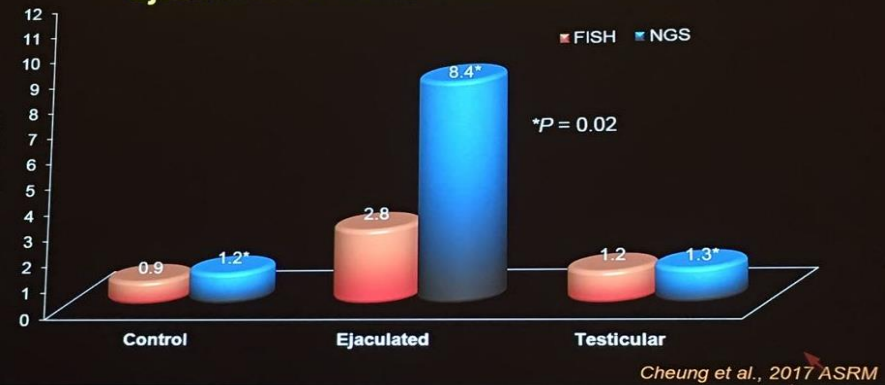
DNA Fragmentation Analysis – FERTILE PLUS:

Sorting with FERTILE PLUS results in an approximately twenty-fold reduction in DNA fragmentation compared to non-sorted semen.^(3,4)

不同精子来源的精子整倍体差异

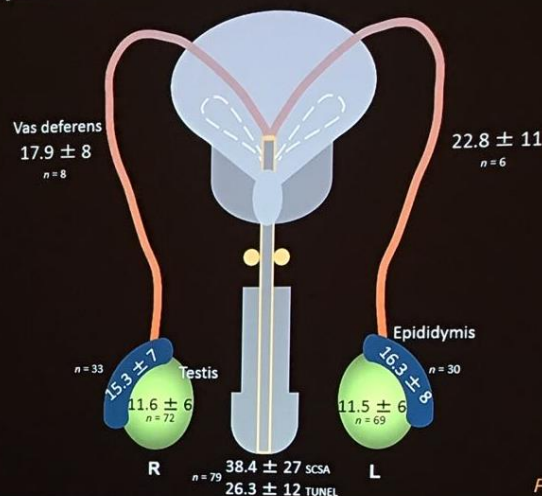


Simultaneous Assessment Ejaculated vs. Surgically Retrieved



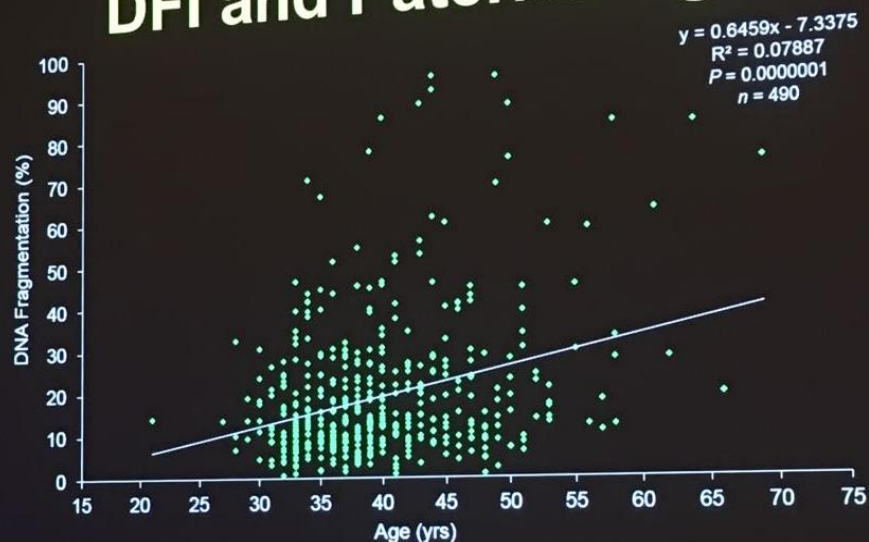
精子DFI与年龄及禁欲时间的关系

Topographic Sperm DFI

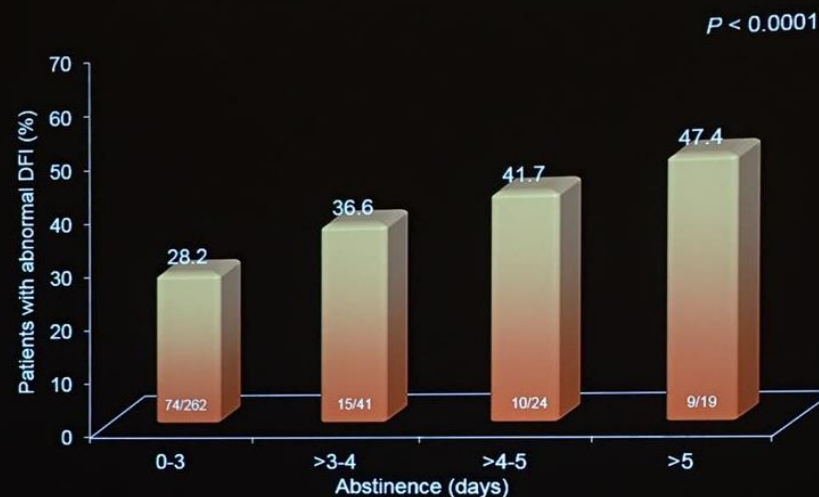


Parrella et al., 2017 ASRM

DFI and Paternal Age



DFI and Abstinence



使用TESA精子可显著提高妊娠结局

严重少弱精患者采用睾丸精子可以获得高的
临床妊娠率

(Mehta et al., 2015 Fertil Steril)

24 severe oligozoospermic ($<5 \times 10^6/\text{mL}$) patients with $>7\%$ DNA damage (TUNEL) and previous failed ≥ 1 ICSI/IVF cycles using ejaculated sperm

	Current ICSI with Testicular sperm	Previous ICSI/IVF with Ejaculate sperm
No of patients	24	24
Sperm DFI	4.6%	24.5%
Clinical pregnancy rate	50%	0%
Live births	12	0

使用TESA精子可显著提高妊娠结局

DFI > 30% 的严重少弱精患者采用睾丸精子可以获得高的临床妊娠率

(Esteves et al., 2015 Fertil Steril)

	Testicular (n=77)	Ejaculate (n=87)
Female age	34.5 ± 4.5	33.4 ± 3.5
No of MII oocytes	9.3 ± 5.4	8.8 ± 4.7
Fert rate (2PN)	56 ± 15	69 ± 17*
Embryos / ET	2.0 ± 0.3	1.9 ± 0.6
Clinical PR	51.9%	40.2%
Miscarriage	10%	34%*
Live birth	46.7%	26.4%*

* P < 0.01

使用TESA精子可显著提高妊娠结局

射出精子DFI>50% 时, 采用睾丸精子可获得高妊娠率

(Arafa et al., Andrology 2017)

	Current ICSI with Testicular sperm	Previous ICSI with Ejaculate sperm
No of patients	36	36
Fertilization rate (2PN)	59.2%	46.4%
High grade embryos	53.4%	47.8%
Clinical pregnancy rate	38.9%	13.8%
No of live births	17	3





精子激活剂

让不动的活精子动起来！

- 严重的少弱精子
- 不动精子或偶见活动精子
- 睾丸源精子

使用精子激活剂（“精子伟哥”）

微量精子冷冻备用

卵子辅助激活 (AOA)

CERM

Human Reproduction, Vol.30, No.8 pp. 1831–1841, 2015
Advanced Access publication on June 16, 2015 doi:10.1093/humrep/dev136

human
reproduction

META-ANALYSIS Embryology

Artificial oocyte activation to improve reproductive outcomes in women with previous fertilization failure: a systematic review and meta-analysis of RCTs

Ioannis A. Sfontouris¹, Carolina O. Nastri², Maria L.S. Lima²,
Eisa Tahmasbpourmarzouni³, Nick Raine-Fenning^{4,5}, and
Wellington P. Martins^{2,*}

¹Eugonia Assisted Reproduction Unit, Athens, Greece ²Ribeirão Preto Medical School, University of São Paulo (FMRP-USP), Ribeirão Preto, Brazil
³Chemical Injury Research Center, Baqiyatallah Medical Science University, Tehran, Iran ⁴Nature Fertility, The East Midlands Fertility Centre, Nottingham, UK ⁵Division of Child Health, Obstetrics & Gynaecology, School of Medicine, University of Nottingham, Nottingham, UK

*Correspondence address: Av. Bandeirantes, 3900-8 andar - HCRP - Campus Universitário, Ribeirão Preto, São Paulo 14048-900, Brazil.
Tel: +55-16-3602-2583; Fax: +55-16-3633-0946; E-mail: wpmartins@gmail.com

Submitted on January 7, 2015; resubmitted on May 12, 2015; accepted on May 19, 2015

STUDY QUESTION: In couples with previous fertilization failure, are reproductive outcomes improved using ICSI followed by artificial oocyte activation (ICSI-AOA) compared with conventional ICSI?

SUMMARY ANSWER: There is insufficient evidence available from RCTs to judge the efficacy and safety of ICSI-AOA for couples with previous fertilization failure.

WHAT IS KNOWN ALREADY: In cases with previous low fertilization rates or total fertilization failure using ICSI due to sperm-related, oocyte activation deficiency, several methods of AOA have been described, which employ mechanical, electrical or chemical stimuli. Reported fertilization and pregnancy rates appear to be improved after ICSI-AOA compared with conventional ICSI; however, the small studies performed to date make it difficult to assess the clinical efficacy or safety of AOA.

STUDY DESIGN, SIZE, AND DURATION: The present systematic review and meta-analysis identified RCTs that compared ICSI-AOA and conventional ICSI. The last electronic search was conducted in August 2014 and there was no limitation regarding language, publication date, or publication status. We included studies that randomized either oocytes or women and included them in two different parts of this review: a women-based review and an oocyte-based review. For the women-based review, the primary outcome of effectiveness was live birth per randomized woman and the primary outcome for safety was congenital anomalies per clinical pregnancy. For the oocyte-based review, the primary outcome was embryo formation per oocyte randomized.

PARTICIPANTS/MATERIALS, SETTING, AND METHODS: Record screening and data extraction were performed independently by two authors and risk of bias was assessed by three authors. The effects of ICSI-AOA compared with conventional ICSI were summarized as risk ratio (RR) and the precision of the estimates was evaluated by the 95% confidence interval (CI).

MAIN RESULTS AND THE ROLE OF CHANCE: A total of 14 articles were assessed for eligibility and 9 included in the meta-analysis: 2 studies comprised the women-based review ($n = 168$ women) and 7 studies the oocyte-based review ($n = 4234$ oocytes). Only four studies evaluated AOA due to fertilization failure after conventional ICSI: these were included in the quantitative analysis. In two studies evaluating couples with a history of fertilization failure in a previous cycle, ICSI-AOA was associated with an increase in the proportion of cleavage stage embryos (RR 5.44, 95% CI 2.98–9.91) and top/high quality cleavage stage embryos (RR 10.02, 95% CI 2.45–40.95). There was no evidence of effect on fertilization rate (RR 2.97, 95% CI 0.84–10.48). In the two studies that evaluated ICSI-AOA as a rescue method for unfertilized oocytes after conventional ICSI, ICSI-AOA was associated with an increase in fertilization (RR 8.26, 95% CI 1.28–53.32, $P = 0.03$) and cleavage

ORIGINAL ARTICLE

<http://dx.doi.org/10.5653/cerm.2015.42.2.45>
pISSN 2233-8233 • eISSN 2233-8241
Clin Exp Reprod Med 2015;42(2):45-50

Artificial oocyte activation in intracytoplasmic sperm injection cycles using testicular sperm in human *in vitro* fertilization

Hee Jung Kang¹, Sun-Hee Lee¹, Yong-Seog Park¹, Chun Kyu Lim¹, Duck Sung Ko¹, Kwang Moon Yang², Dong-Wook Park¹

¹Laboratory of Reproductive Biology and Infertility, ²Department of Obstetrics and Gynecology, Cheil General Hospital and Women's Healthcare Center, Dankook University College of Medicine, Seoul, Korea

Objective: Artificial oocyte activation (AOA) is an effective method to avoid total fertilization failure in human *in vitro* fertilization-embryo transfer (IVF-ET) cycles. AOA performed using a calcium ionophore can induce calcium oscillation in oocytes and initiate the fertilization process. We evaluated the usefulness of AOA with a calcium ionophore in cases of total fertilization failure in previous cycles and in cases of severe male factor infertility patients with non-motile spermatozoa after pentoxifylline (PF) treatment.

Methods: The present study describes 29 intracytoplasmic sperm injection (ICSI)-AOA cycles involving male factor infertility at Cheil General Hospital from January 2006 to June 2013. Patients were divided into two groups (control, $n = 480$; AOA, $n = 29$) depending on whether or not AOA using a calcium ionophore (A23187) was performed after testicular sperm extraction-ICSI (TESE-ICSI). The AOA group was further split into subgroups according to sperm motility after PF treatment: i.e., motile sperm-injected ($n = 12$) and non-motile sperm-injected ($n = 17$) groups (total $n = 29$ cycles).

Results: The good embryo rate (52.3% vs. 66.9%), pregnancy rate (20.7% vs. 52.1%), and delivery rate (10.3% vs. 40.8%) were lower in the PF/AOA group than in the control group. When evaluating the effects of restoration of sperm motility after PF treatment on clinical outcomes there was no difference in fertilization rate (66.6% vs. 64.7% in non-motile and motile sperm, respectively), pregnancy rate (17.6% vs. 33.3%), or delivery rate (5.9% vs. 16.7%) between the two groups.

Conclusion: We suggest that oocyte activation is a useful method to ensure fertilization in TESE-ICSI cycles regardless of restoration of sperm motility after PF treatment. AOA may be useful in selected patients who have a low fertilization rate or total fertilization failure.

Keywords: Artificial oocyte activation; Calcium ionophore; Intracytoplasmic sperm injection; Pentoxifylline; Testicular sperm extraction



三、胚胎因素



胚胎染色体异常

J Assist Reprod Genet. 2017 Oct 23. doi: 10.1007/s10815-017-1060-x. [Epub ahead of print]

Are blastocyst aneuploidy rates different between fertile and infertile populations?

Kort JD¹, McCoy RC², Demko Z³, Lathi RB⁴.

⊕ Author information

Abstract

PURPOSE: This study aimed to determine if patients with infertility or recurrent pregnancy loss have higher rates of embryo aneuploidy than fertile controls.

METHODS: This was a retrospective review of all pre-implantation genetic screening (PGS) cases processed by a single reference lab prior to March 2014 after a blastocyst biopsy. Cases were excluded if no indication for PGS was designated or patients were translocation carriers. The fertile control group consisted of patients undergoing IVF with PGS for sex selection only. The comparison cohorts included those with recurrent pregnancy loss, male factor infertility, unexplained infertility, prior failed IVF, or previous aneuploid conceptions. A quasi-binomial regression model was used to assess the relationship between the dependent variable, aneuploidy rate and the independent variables, maternal age and reason for PGS. A quasi-Poisson regression model was used to evaluate the relationship between similar independent variables and the number of blastocyst biopsies per case.

RESULTS: The initial study population consisted of 3378 IVF-PGS cycles and 18,387 analyzed trophoctoderm samples. Controlling for maternal age, we observed an increased rate of aneuploidy among patients with recurrent pregnancy loss (OR 1.330, $p < 0.001$), prior aneuploid pregnancy (OR 1.439, $p < 0.001$), or previous failed IVF cycles (OR 1.356, $p = 0.0012$) compared to fertile controls. Patients with unexplained and male factor infertility did not have a significantly different aneuploidy rate than controls ($p > 0.05$). The increase in aneuploidy in patients with RPL and prior IVF failure was driven by both an increase in meiotic (OR 1.488 and 1.508, $p < 0.05$) and mitotic errors (1.269 and 1.393, $p < 0.05$) relative to fertile controls, while patients with prior aneuploid pregnancies had only an increased risk of meiotic error aneuploidies (OR 1.650, $p < 0.05$).

CONCLUSIONS: Patients with recurrent pregnancy loss, previous IVF failures, and prior aneuploid pregnancies have a significantly higher, age-independent, aneuploidy rate compared to patients without infertility.

植入前胚胎发育阻滞有一半左右是胚胎染色体异常所致；

反复早期流产也有一半左右是胚胎染色体异常所致！



挑选最具发育潜能的胚胎进行移植

PGS

Time-lapse

NICS

囊胚培养

代谢组学

蛋白组组学

PGS

Reprod Biomed Online. 2017 Sep 21. pii: S1472-6483(17)30417-0. doi: 10.1016/j.rbmo.2017.09.001. [Epub ahead of print]

Preimplantation genetic screening: results of a worldwide web-based survey.

Weissman A¹, Shoham G², Shoham Z³, Fishel S⁴, Leong M⁵, Yaron Y⁶.

Author information

Abstract

Our objective was to evaluate and characterize the extent and patterns of worldwide usage of preimplantation genetic screening (PGS) among the assisted reproductive technique community. A prospective, web-based questionnaire with questions relating to practices of, and views on, PGS was directed to users and non-users of PGS. A total of 386 IVF units from 70 countries conducting 342,600 IVF cycles annually responded to the survey. A total of 77% of respondents routinely carry out PGS in their clinics for a variety of indications: advanced maternal age (27%), recurrent implantation failure (32%) and recurrent pregnancy loss (31%). Few (6%) offer PGS to all their patients. In most cycles (72%), trophectoderm biopsy is carried out and either array-comparative genomic hybridization (59%) or next-generation sequencing (16%) are used for genetic analysis. Only 30% of respondents regard PGS as clearly evidenced-based, and most (84%) believe that more randomized controlled trials are needed to support the use of PGS. Despite ongoing debate and lack of robust evidence, most respondents support the use of PGS, and believe that it may aid in transferring only euploid embryos, thereby reducing miscarriage rates and multiple pregnancies, increasing live birth rates and reducing the risk of aneuploid pregnancies and births.

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KEYWORDS: Aneuploidy; Chromosomal aberrations; In-vitro fertilization (IVF); Preimplantation genetic screening (PGS)

Gleicher and Orvieto *Journal of Ovarian Research* (2017) 10:21
DOI 10.1186/s13048-017-0318-3

Journal of Ovarian Research

REVIEW

Open Access



Is the hypothesis of preimplantation genetic screening (PGS) still supportable? A review

Norbert Gleicher^{1,2,3,4*} and Raoul Orvieto⁵

Abstract

The hypothesis of preimplantation genetic diagnosis (PGS) was first proposed 20 years ago, suggesting that elimination of aneuploid embryos prior to transfer will improve implantation rates of remaining embryos during in vitro fertilization (IVF), increase pregnancy and live birth rates and reduce miscarriages. The aforementioned improved outcome was based on 5 essential assumptions: (i) Most IVF cycles fail because of aneuploid embryos. (ii) Their elimination prior to embryo transfer will improve IVF outcomes. (iii) A single trophectoderm biopsy (TEB) at blastocyst stage is representative of the whole TE. (iv) TE ploidy reliably represents the inner cell mass (ICM). (v) Ploidy does not change (i.e., self-correct) downstream from blastocyst stage. We aim to offer a review of the aforementioned assumptions and challenge the general hypothesis of PGS. We reviewed 455 publications, which as of January 20, 2017 were listed in PubMed under the search phrase < preimplantation genetic screening (PGS) for aneuploidy>. The literature review was performed by both authors who agreed on the final 55 references. Various reports over the last 18 months have raised significant questions not only about the basic clinical utility of PGS but the biological underpinnings of the hypothesis, the technical ability of a single trophectoderm (TE) biopsy to accurately assess an embryo's ploidy, and suggested that PGS actually negatively affects IVF outcomes while not affecting miscarriage rates. Moreover, due to high rates of false positive diagnoses as a consequence of high mosaicism rates in TE, PGS leads to the discarding of large numbers of normal embryos with potential for normal euploid pregnancies if transferred rather than disposed of. We found all 5 basic assumptions underlying the hypothesis of PGS to be unsupported: (i) The association of embryo aneuploidy with IVF failure has to be reevaluated in view how much more common TE mosaicism is than has until recently been appreciated. (ii) Reliable elimination of presumed aneuploid embryos prior to embryo transfer appears unrealistic. (iii) Mathematical models demonstrate that a single TEB cannot provide reliable information about the whole TE. (iv) TE does not reliably reflect the ICM. (v) Embryos, likely, still have strong innate ability to self-correct downstream from blastocyst stage, with ICM doing so better than TE. The hypothesis of PGS, therefore, no longer appears supportable. With all 5 basic assumptions underlying the hypothesis of PGS demonstrated to have been mistaken, the hypothesis of PGS, itself, appears to be discredited. Clinical use of PGS for the purpose of IVF outcome improvements should, therefore, going forward be restricted to research studies.

Keywords: PGS, IVF, Mosaicism, Blastocyst, Pregnancy rate, Live birth rate, CGH, NGS



PGS存在的问题

- ✓ 有创性的活检有可能会对胚胎质量及后续发育造成潜在影响；
- ✓ 动物实验表明，有创性的活检可能会引起神经退行性病变，表观遗传修饰的异常等不利影响；
- ✓ 有创性的活检对仪器设备要求较高，对操作人员的技术水平和熟练程度要求较高。



非侵入性胚胎评估方法

Time-lapse





胚胎动力学

ARTICLES

**nature
biotechnology**

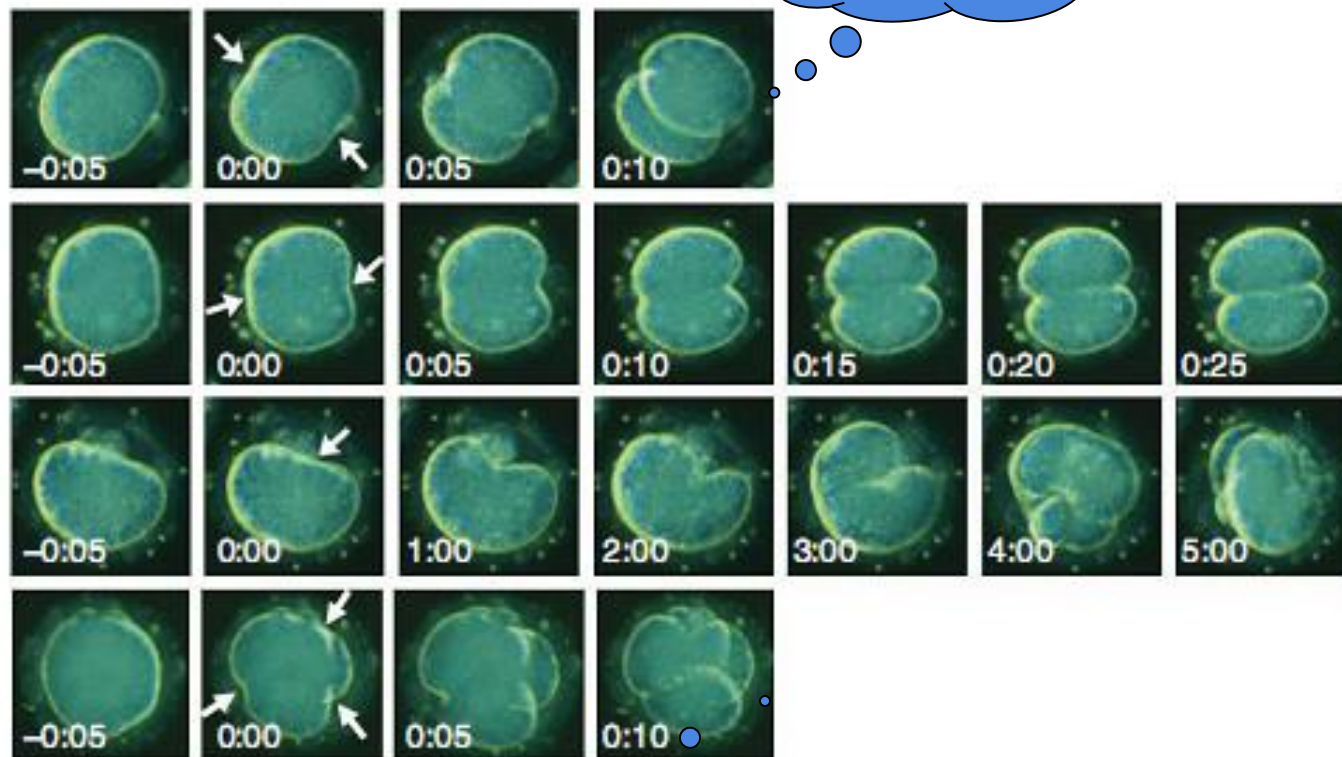
Non-invasive imaging of human embryos before embryonic genome activation predicts development to the blastocyst stage

Connie C Wong^{1,2,7}, Kevin E Loewke^{1-3,6,7}, Nancy L Bossert⁴, Barry Behr², Christopher J De Jonge⁴, Thomas M Baer⁵ & Renee A Reijo Pera^{1,2}

¹Institute for Stem Cell Biology and Regenerative Medicine, School of Medicine, Stanford University, Stanford, California, USA. ²Department of Obstetrics and Gynecology, School of Medicine, Stanford University, Stanford, California, USA. ³Department of Mechanical Engineering, Stanford University, Stanford, California, USA. ⁴Reproductive Medicine Center, University of Minnesota, Minneapolis, Minnesota, USA. ⁵Stanford Photonics Research Center, Department of Applied Physics, Stanford University, Stanford, California, USA. ⁶Present address: Auxogyn, Inc., Menlo Park, California, USA. ⁷These authors contributed equally to this work. Correspondence should be addressed to R.A.R.P. (reneeer@stanford.edu).



碎片可以逆
转！



大部分可以发育至囊胚

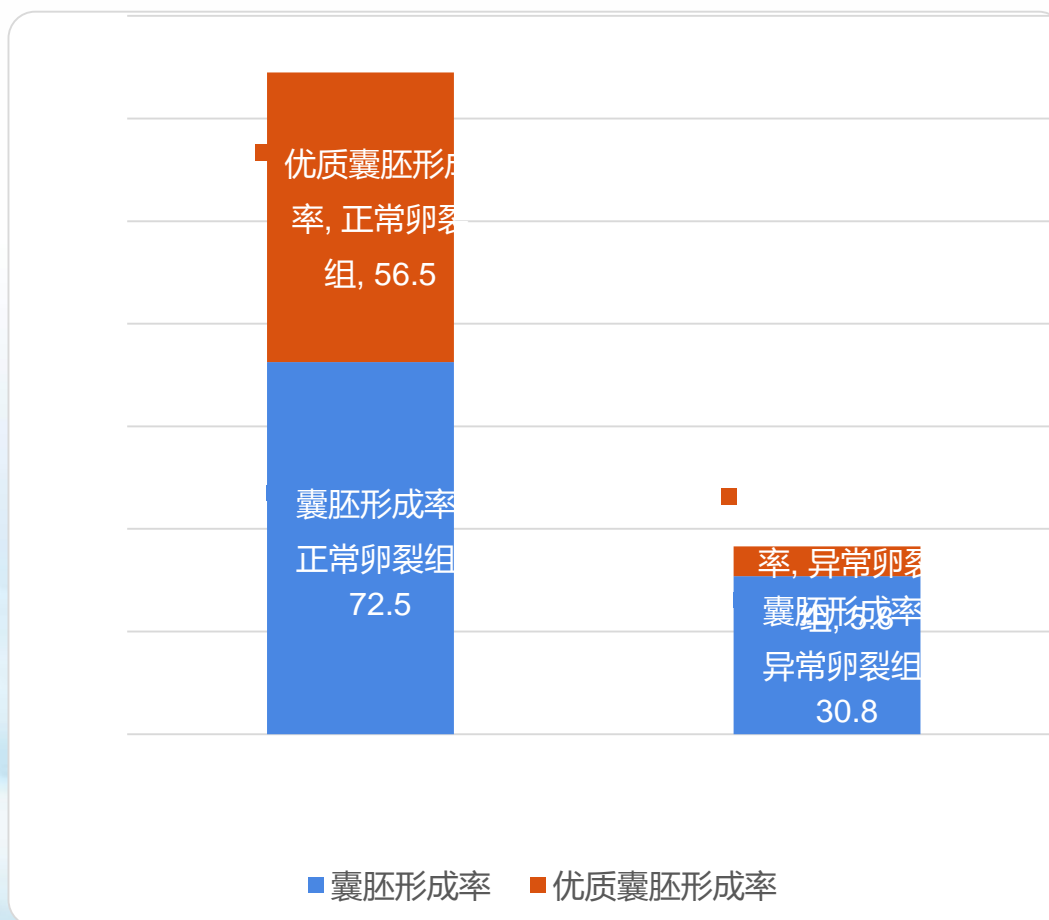
少部分可以发育至囊胚

发育阻滞

发育阻滞

碎片不能逆
转！

卵裂异常



- 1分3, 4**细胞**
- 2分5, 6**细胞**
- 逆分裂

卵裂异常的胚胎可利用囊胚形成率极低 ($p < 0.01$)



胚胎动力学

Reproductive BioMedicine Online (2013) 26, 477–485

ARTICLE

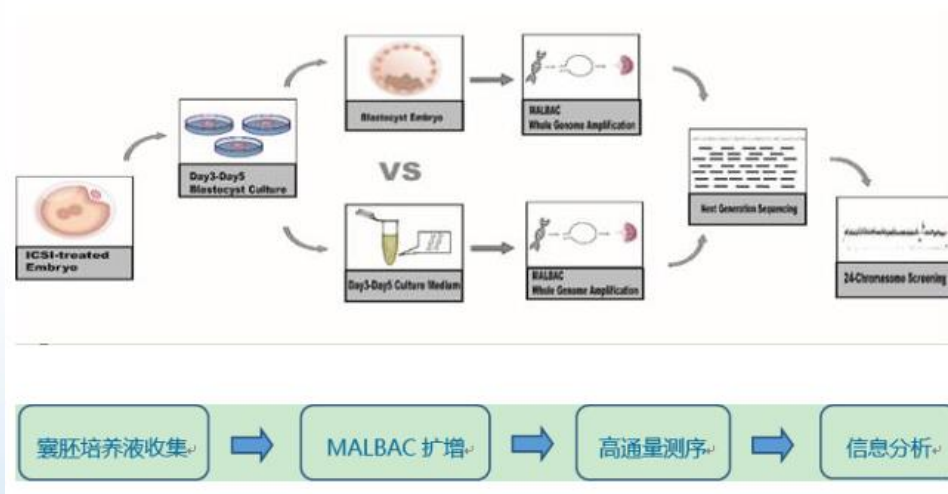
Modelling a risk classification of aneuploidy in human embryos using non-invasive morphokinetics

Alison Campbell ^{a,*}, Simon Fishel ^a, Natalie Bowman ^b, Samantha Duffy ^b, Mark Sedler ^b, Cristina Fontes Lindemann Hickman ^c

^a CARE Fertility, John Webster House, 6 Lawrence Drive, Nottingham Business Park, Nottingham NG8 6PZ, United Kingdom; ^b CARE Fertility, 108–112 Daisy Bank Road, Manchester M14 5QH, United Kingdom; ^c Trinidad and Tobago IVF and Fertility Center, Trinidad and Tobago

* Corresponding author. E-mail address: Alison.campbell@carefertility.com (A Campbell).

无创胚胎染色体筛查 (NICS) 技术



- 使用胚胎培养剩余的培养液进行检测
- 无创
- 技术操作更为简便
- 阴性预测率可达100%
- 阳性预测率达83%
- 胚胎优选

费用较高，患者接受程度？



囊胚培养

- 更加符合自然生理状态（同步性）；
- 高种植率，低多胎率（约1%）；
- 减少胚胎种植前游离在子宫内的时间；
- 胚胎”自我表现”，可以选出更有发育潜能的胚胎进行移植；
 - 发育阻滞（4-8细胞阻滞，胚胎基因组激活前）
 - 异常卵裂
 - 异常受精（0PN, 1PN）
- 减少宫外孕发生率（Fang et al., 2015）；
- 选择性单胚胎移植（eSET）；
- 囊胚较卵裂期胚胎更耐冷冻；

目前囊胚培养是一种经济且相对有效的非侵入性胚胎选择方法！



非侵入性胚胎评估方法

代谢组学

蛋白组学

.....

??



四、辅助孵化



辅助孵化

Zygote 24 (October), pp. 742-747. © Cambridge University Press 2016
doi:10.1017/S0967199416000058 First Published Online 9 March 2016

The impact of laser-assisted hatching on the outcome of frozen human embryo transfer cycles

Katalin Kanyo², Jozsef Zeke², Rita Kriston², Zoltan Szücs², Sandor Cseh^{1,2}, Bence Somoskői³
and Janos Konc²

Infertility and IVF Center of Buda, Szent Janos Hospital, Budapest, Hungary; and Szent Istvan University Faculty of Veterinary Science, Budapest, Hungary

Date submitted: 10.03.2015. Date revised: 08.10.2015. Date accepted: 14.01.2016

Summary

Biochemical modifications of zona pellucida (ZP) result in zona hardening. Zona hardening (ZH) is induced by several factors such as advancing maternal age, *in vitro* culture conditions and cryopreservation and adversely effects implantation. The objective of the clinical study was to determine whether or not laser-assisted hatching (LAH) applied on day 3 frozen embryos improves the outcome of frozen embryo transfer (FET) cycles in patients with recurrent implantation failure and/or advanced female age. In total, 413 patients of different ages with recurrent implantation failure (maximum three cycles) were involved into the study. Patients were allocated randomly into LAH and control groups. On the day of FET, after thawing and just before FET, the ZP was thinned using a laser system. In the control group no treatment was applied on frozen embryo before transfer. The main outcome measures were clinical pregnancy rate. Overall, the results indicate a tendency that LAH increased ($P = 0.08$) clinical pregnancy. However, for patients older than 37 years, LAH increased pregnancy rates significantly ($P = 0.03$). In the LAH and control groups, the age of patients and the number of transferred embryos influenced pregnancy rates ($P = 0.01$). For patients older than 37 years, no effect of number of transferred embryos was detected ($P = 0.14$). The incidence of multiple pregnancies also increased in the LAH group ($P = 0.01$). In conclusion, in older woman, to overcome the negative effect of zona hardening, LAH could be performed on frozen embryos as a routine strategy before FET in frozen cycles in order to increase the possibility of pregnancy formation.

Keywords: Age of patients, Clinical outcome of laser-assisted hatching, Frozen embryo transfer cycle, Laser-assisted hatching, Slow freezing of embryos



(十一) 辅助孵出

辅助孵出应用于新鲜周期移植的价值一直存在争议,目前的证据不推荐对所有移植的胚胎进行辅助孵出。而对于那些临床预后较差的患者,比如反复植入失败、胚胎质量较差、胚胎透明带异常的胚胎以及高龄的患者(>38岁),辅助孵出可能改善种植率^[45]。通过时差成像技术观察,发现复苏胚胎,尤其是复苏囊胚,辅助孵出后可以利于胚胎的后续孵出,但对临床结局是否存在改善尚不明确。目前可以考虑采用激光辅助孵出设备进行胚胎的辅助孵出。



辅助孵化

Reproductive BioMedicine Online (2014) 29, 692–698



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ARTICLE

No adverse effects were identified on the perinatal outcomes after laser-assisted hatching treatment



Hanying Zhou ^{a,1}, Wanqiu Zao ^{b,1}, Wei Zhang ^a, Juanzi Shi ^{a,*}, Wenhao Shi ^a

^a Assisted Reproduction Center, Maternal & Child Health Care Hospital of Shaanxi Province, Xi'an Houzai Gate 73#, Xi'an 710003, China; ^b Respiratory Internal Medicine, Shaanxi Provincial People's Hospital, YouYiXilu 236#, Xi'an 710068, China

* Corresponding author. E-mail address: shijuanzi123@126.com (J Shi). ¹ H. Z. and W. Z. contributed equally to this work.



Dr Juanzi Shi has been the Director of the Assisted Reproduction Center since, Maternal and Child Health Care Hospital of Shaanxi Province since 2006. She received her PhD in Pathology in 2003 from the Fourth Military Medical University. She is Professor of Physicians in IVF unit and has 5 years experience in IVF Laboratory work. Her primary area of research is embryology and assisted reproductive technology. She has published several articles and book chapters about the vitrification for human embryos.

Table 3 Incidence of major and minor malformations.

Malformations	Laser-assisted hatching group	Control group
Miscarriages	2	0
	Umbilical deformity 1	
	Tracheoesophageal fistula 1	
Live births		
Major	4	1
	Spina bifida 1	Lower limb malformation 1
	Congenital heart disease 3	
Minor	0	2
		Lingual hernia 1
		Polydactyly 1
Total	6	3



五、卵子因素

1、胞浆置换

- ✓ GV移植
- ✓ 纺锤体 (Spindle) 移植
- ✓ 极体 (PB) 移植
- ✓ 原核 (PN) 移植

2、线粒体移植

- ✓ 卵母细胞线粒体移植
- ✓ 自体间充质干细胞线粒体移植

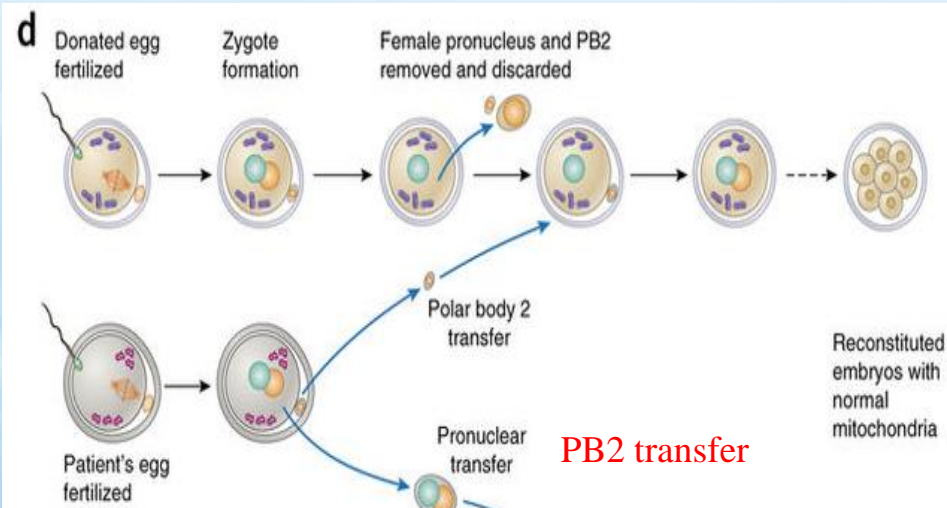
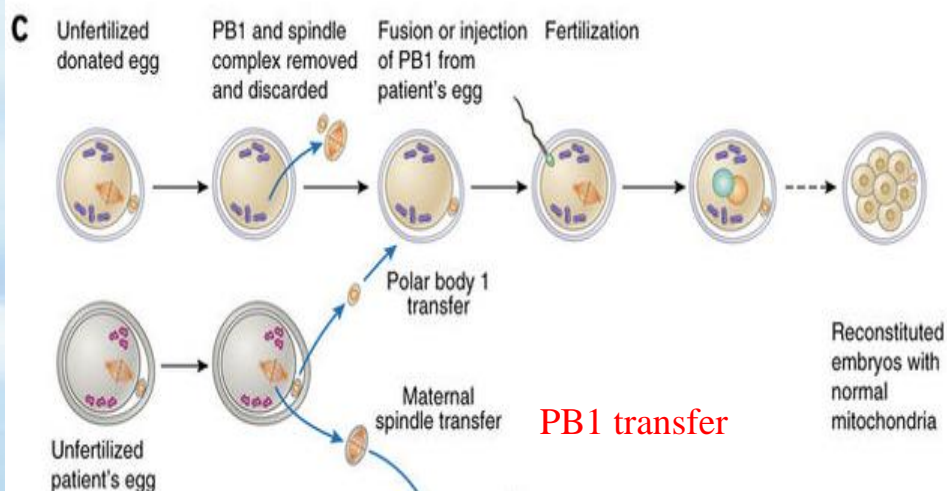
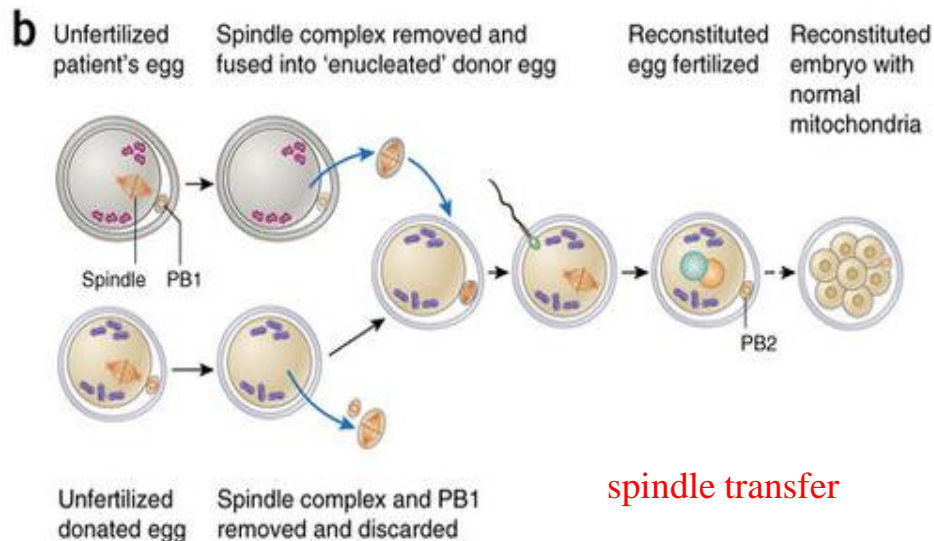
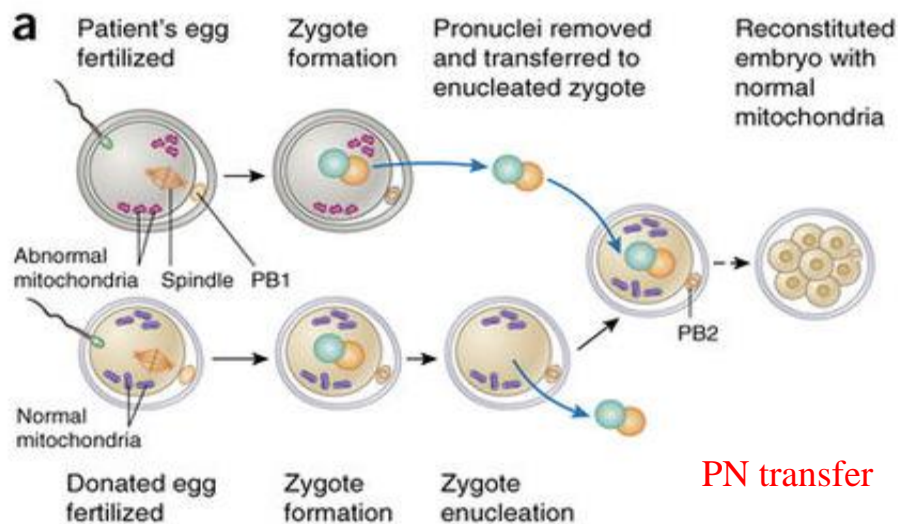
3、定制培养液

- ✓ GM-CSF



需要捐卵

胞浆置换



纺锤体 (spindle) 移植



ELSEVIER

Article

Live birth derived from oocyte spindle transfer to prevent mitochondrial disease



**John Zhang^{a,b,*}, Hui Liu^b, Shiyu Luo^c, Zhuo Lu^b,
Alejandro Chávez-Badiola^a, Zitao Liu^b, Mingxue Yang^b, Zaher Merhi^d,
Sherman J Silber^e, Santiago Munné^f, Michalis Konstantinidis^f,
Dagan Wells^f, Jian J Tang^g, Taosheng Huang^{c,*}**

^a New Hope Fertility Center, Punto Sao Paulo, Lobby Corporativo, Américas 1545 Providencia, Guadalajara, Mexico

^b New Hope Fertility Center, 4 Columbus Circle, New York, NY 10019, USA

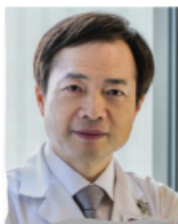
^c Division of Human Genetics, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH 45229, USA

^d Department of Obstetrics and Gynecology, Division of Reproductive Biology, NYU School of Medicine, 180 Varick Street, New York, NY 10014, USA

^e Infertility Center of St Louis, St Luke's Hospital, St Louis, MO 63017, USA

^f Reprogenetics, 3 Regent Street, Livingston, NJ 07078, USA

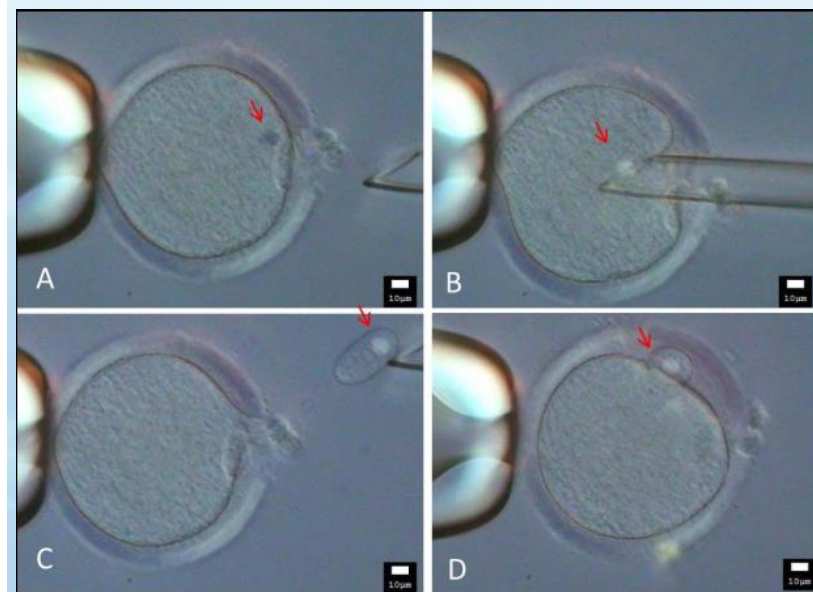
^g Department of Obstetrics and Gynecology, The Mount Sinai Hospital, E 101st Street, New York, NY 10029, USA



Dr John Zhang completed his medical degree at Zhejiang University School of Medicine in China, and subsequently received his Master's Degree at Birmingham University in the UK. In 1991, Dr Zhang earned his PhD in IVF, and, after researching the biology of mammalian reproduction and human embryology for nearly 10 years he completed his fellowship training in Reproductive Endocrinology and Infertility at New York University's School of Medicine in 2001. Dr. Zhang continues his clinical research in minimal stimulation IVF, non-embryonic stem cell, long-term oocyte cryopreservation, and oocyte reconstruction by nuclear transfer.

KEY MESSAGE

We report a live birth after oocyte spindle transfer to prevent transmission of the mitochondrial disease, Leigh syndrome.



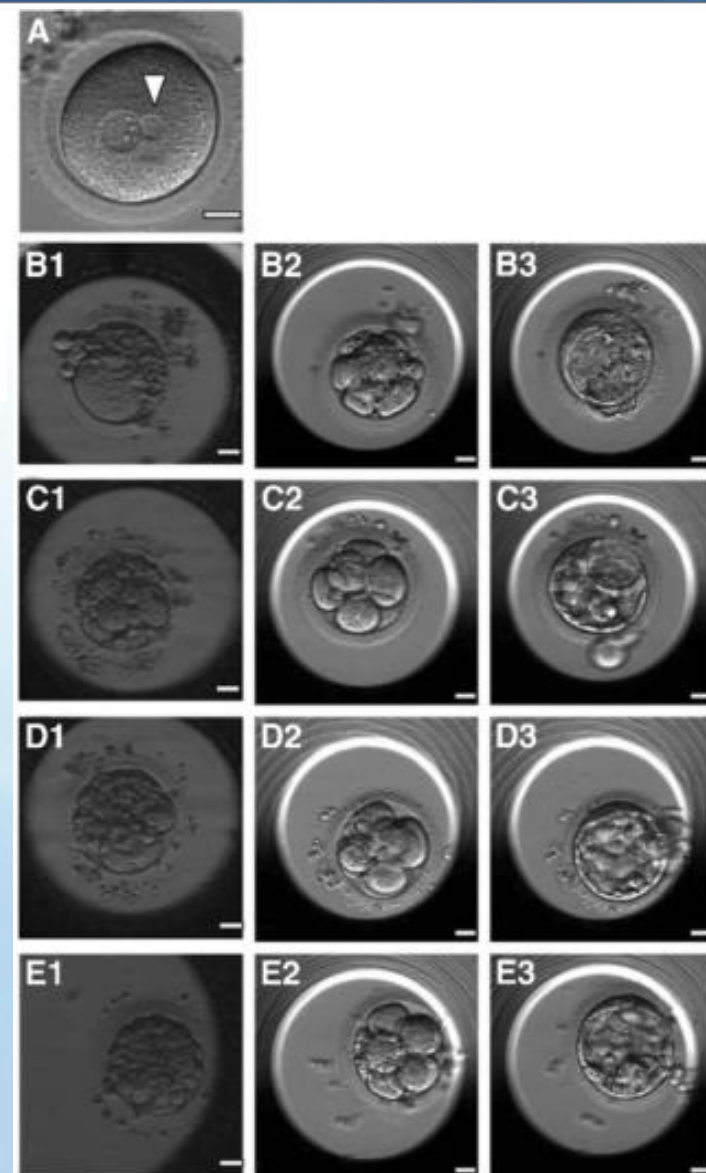
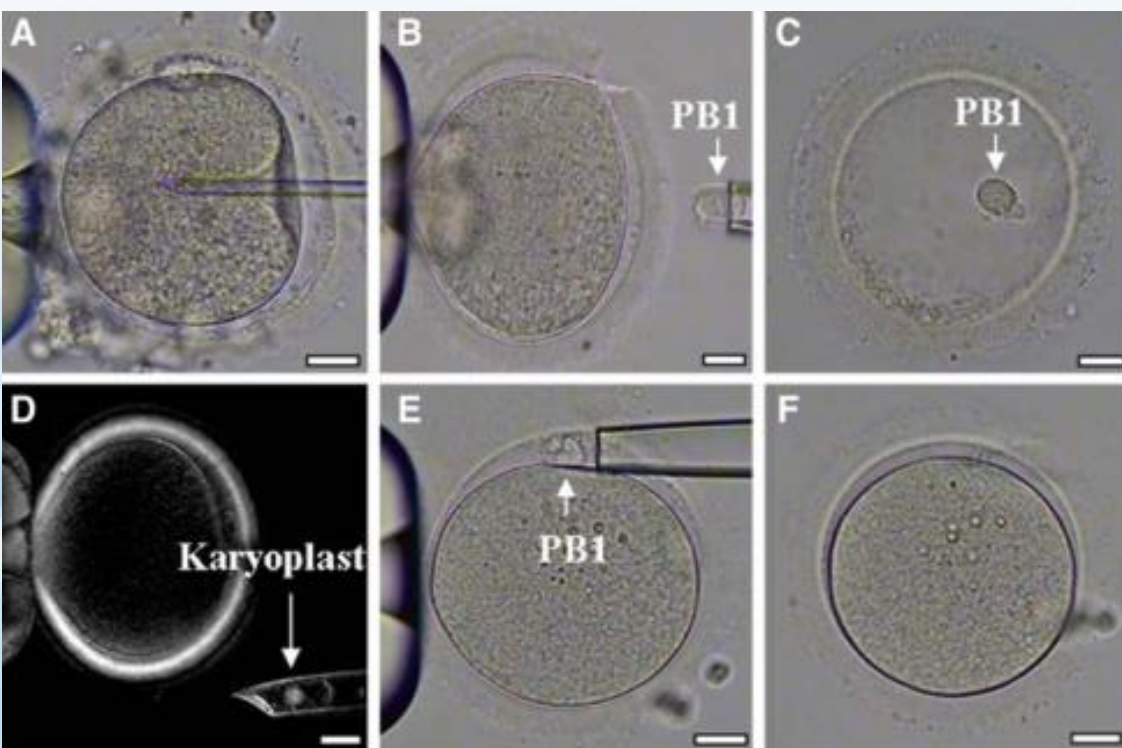
极体 (PB) 移植

J Assist Reprod Genet. 2017 May;34(5):563-571. doi: 10.1007/s10815-017-0881-y. Epub 2017 Feb 11.

Polar body transfer restores the developmental potential of oocytes to blastocyst stage in a case of repeated embryo fragmentation.

Zhang SP^{1,2}, Lu CF^{1,2,3}, Gong F^{1,2,3}, Xie PY^{1,4}, Hu L^{1,2,3,4}, Zhang SJ², Lu GX^{1,2,3,4}, Lin G^{5,6,7,8}.

✚ Author information



原核 (PN) 移植

Pregnancy derived from human zygote pronuclear transfer in a patient who had arrested embryos after IVF




John Zhang^{a,b,*}, Guanglun Zhuang^c, Yong Zeng^c, Jamie Grifo^d,
Carlo Acosta^c, Yimin Shu^c, Hui Liu^{a,b}

^a Reproductive Endocrinology and Infertility, Beijing, China; ^b New Hope Fertility Center, New York, NY, USA; ^c Sun Yat-Sen University of Medical Science, Guangzhou, China; ^d New York University School of Medicine, Division of Reproductive Endocrinology and Infertility, New York, NY, USA

* Corresponding author. E-mail address: johnzhang211@gmail.com (J Zhang).



Dr Zhang completed his medical degree in at the Zhejiang University School of Medicine, and subsequently received his Master's Degree at Birmingham University in the UK. In 1991, Dr Zhang earned his PhD in IVF, and, after studying and researching the biology of mammalian reproduction and human embryology for nearly 10 years, became the first Fellow in the Division of Reproductive Endocrinology and Infertility of New York University's School of Medicine in 2001. Dr. Zhang continues his research in minimal stimulation IVF, non-embryonic stem cell research, long-term cryopreservation of oocytes, and oocyte reconstruction by nuclear transfer.

Abstract Nuclear transfer of an oocyte into the cytoplasm of another enucleated oocyte has shown that embryogenesis and implantation are influenced by cytoplasmic factors. We report a case of a 30-year-old nulligravida woman who had two failed IVF cycles characterized by all her embryos arresting at the two-cell stage and ultimately had pronuclear transfer using donor oocytes. After her third IVF cycle, eight out of 12 patient oocytes and 12 out of 15 donor oocytes were fertilized. The patient's pronuclei were transferred subzonally into an enucleated donor cytoplasm resulting in seven reconstructed zygotes. Five viable reconstructed embryos were transferred into the patient's uterus resulting in a triplet pregnancy with fetal heartbeats, normal karyotypes and nuclear genetic fingerprinting matching the mother's genetic fingerprinting. Fetal mitochondrial DNA profiles were identical to those from donor cytoplasm with no detection of patient's mitochondrial DNA. This report suggests that a potentially viable pregnancy with normal karyotype can be achieved through pronuclear transfer. Ongoing work to establish the efficacy and safety of pronuclear transfer will result in its use as an aid for human reproduction. 



自体间充质干细胞线粒体移植

世界首例！中山大学梁晓燕团队成功实施世界首例“卵母细胞内注射自体骨髓细胞线粒体”



2017年11月07日11:53 中山大

“试管婴儿”这个名词，想必大家已经不陌生，可是你知道吗？除了精子质量、年龄、子宫内膜环境等之外，胚胎质量是试管婴儿成功率的一个非常重要的因素，正常而言，胚胎质量越高，成功率越高！

然而，长期以来，临床上有相当一部分患者反复IVF失败的原因就是胚胎因素，在附属六院生殖医学中心乃至全国，这个比例大约是10%，即使多个周期都不能获得可用于移植或者冷冻的胚胎，特别是反复大量碎片的患者以及高龄患者。

目前，附属六院生殖医学中心在改善胚胎发育质量研究中获得了重大的突破！梁晓燕教授团队在世界上首次利用“卵母细胞内注射自体骨髓细胞线粒体”技术为一患者改善了胚胎质量，行宫腔内胚胎移植成功妊娠并且B超下证实有胎心搏动。梁晓燕教授期待在后续的临床治疗中，能将这部分患者的妊娠率从0提升到20%，力争在该领域获得突破性进展并将这一治疗技术向全世界推广。



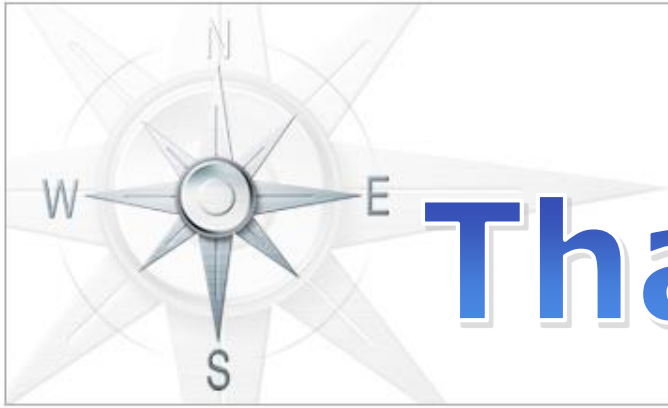


存在的问题

- 卵子捐献
- 伦理与法规
- 有效性
- 安全性

小结

- 减少ROS，保护精子DNA完整性；
- 一定情况下，手术来源（PESA/TESA）的精子可显著提高妊娠结局；
- 精子激活剂和卵子辅助激活（AOA）；
- 非侵入性的胚胎优选方法（囊胚培养，PGS，Time-lapse, NICS技术）；
- 辅助孵化技术；
- 慎用胞浆置换及自体间充质干细胞线粒体移植技术



Thank You !

