Rejuvenation of Gamete Cells; Past, Present and Future

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Boston IVF
Waltham, MA, USA

Conflict of Interest

- I have no conflict of interest related to this presentation.

Eggs and Sperm

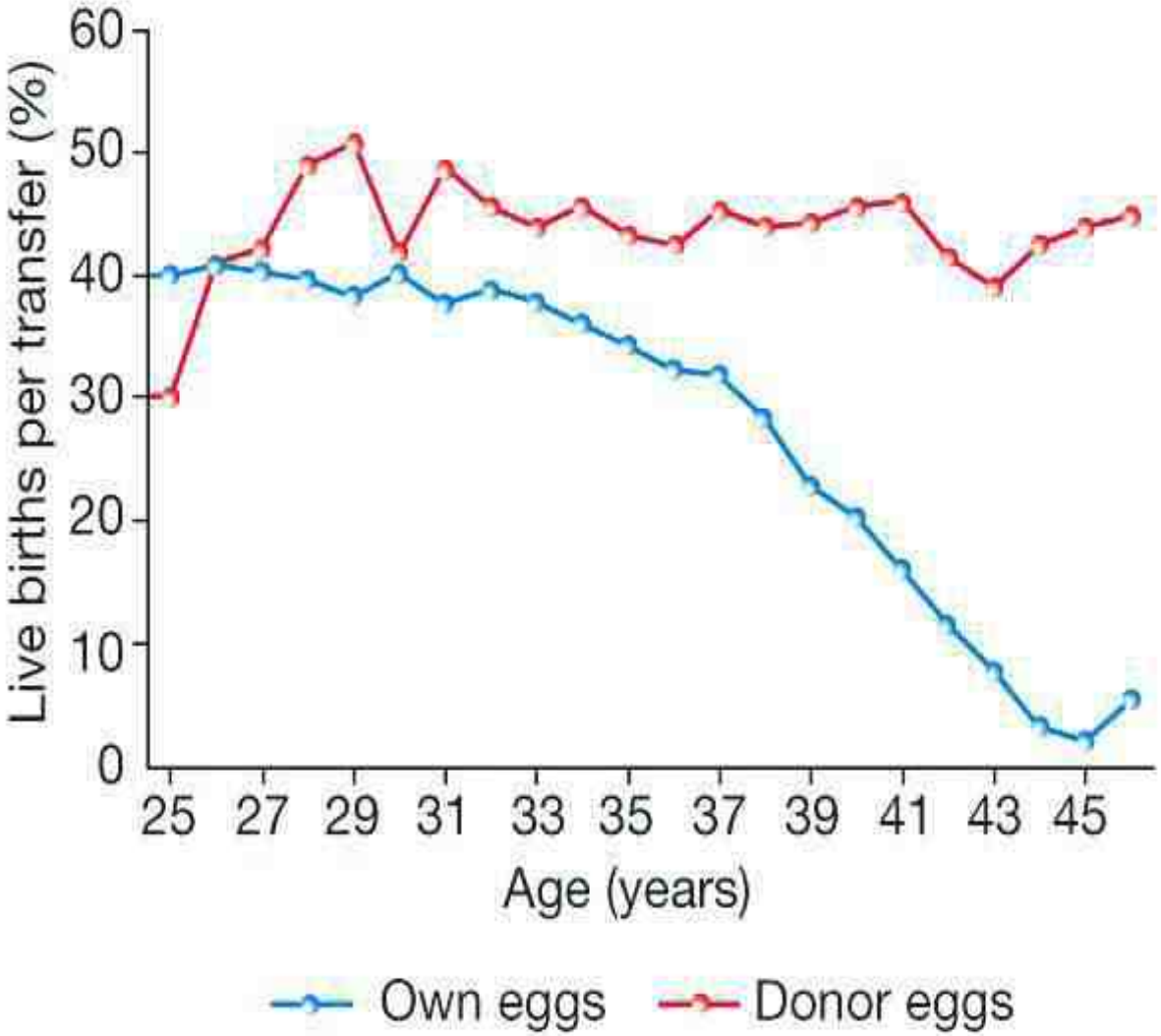
Objectives:

1. Why do we need to improve poor gametes?
2. An update on developing technologies on how to overcome

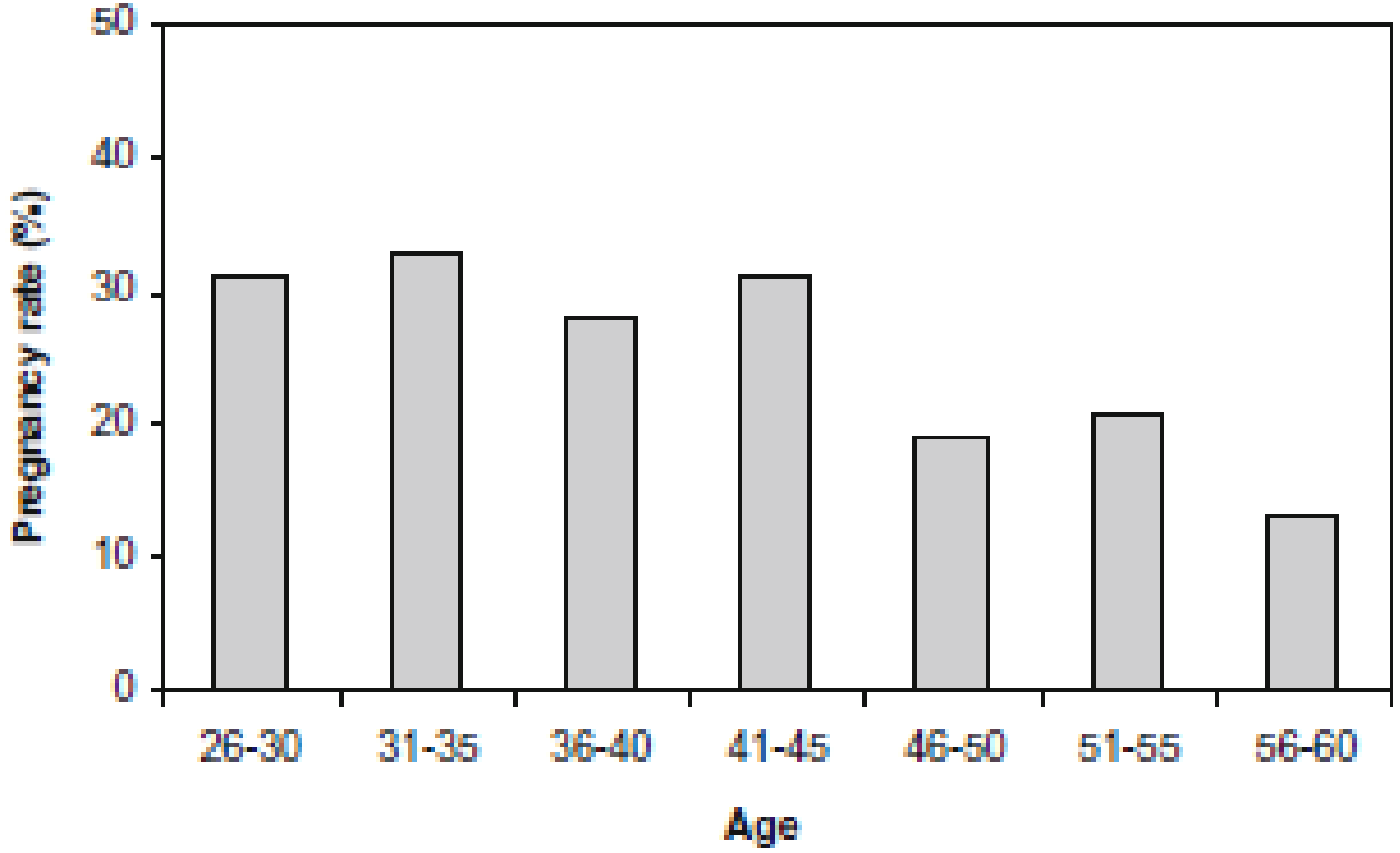
- Age and Reproduction
- Poor quality Gametes
  - Eggs
    - Anovulation
    - Cancer
  - Non-Obstructive Azoospermia

3. Other alternatives

Maternal Influence on Reproductive outcome

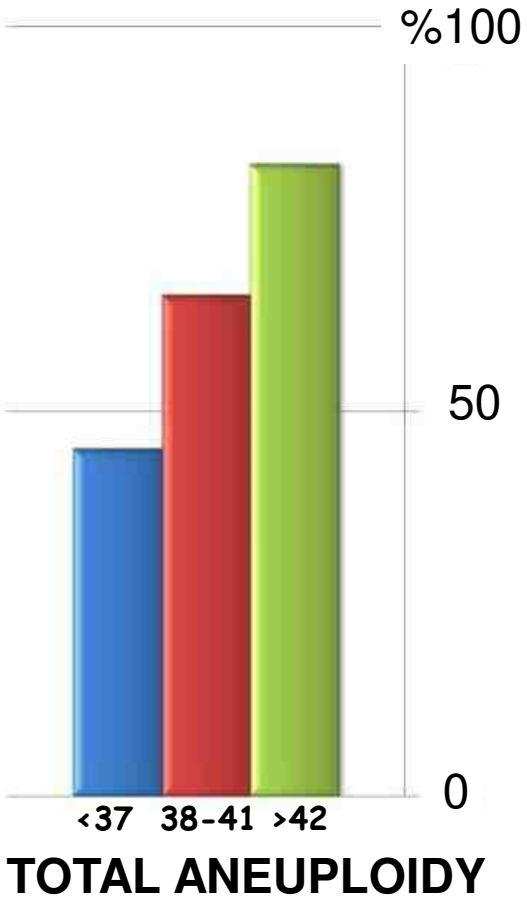


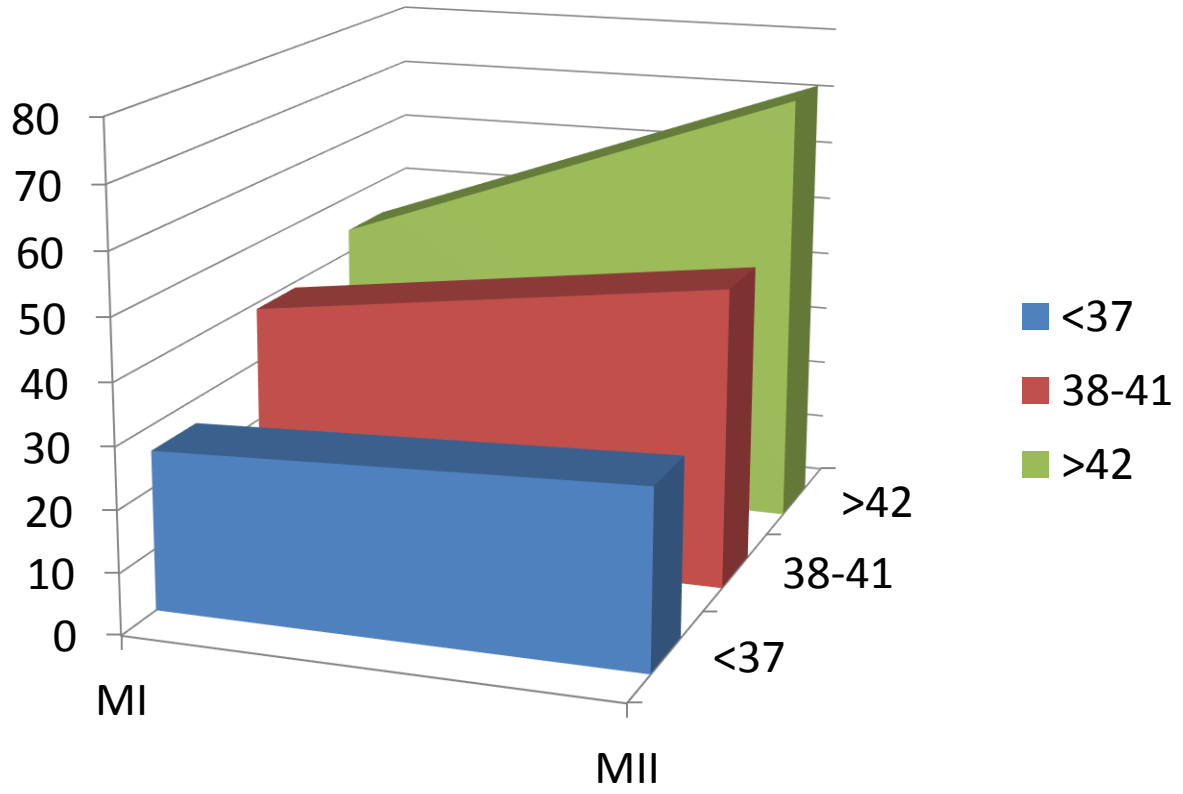
Paternal Age and Fertility Male partners used in an egg donation program (Girsh et al. JARG 2008)



What is the problem in the egg and how to try and cure it?

Proportion of meiotic divisions affected by chromosome abnormalities





Fragouli et al. Mol. Hum. Reprod. (2011) 17 (5): 286-295.

BOSTONIVF

Theories of Oocyte Aneuploidy

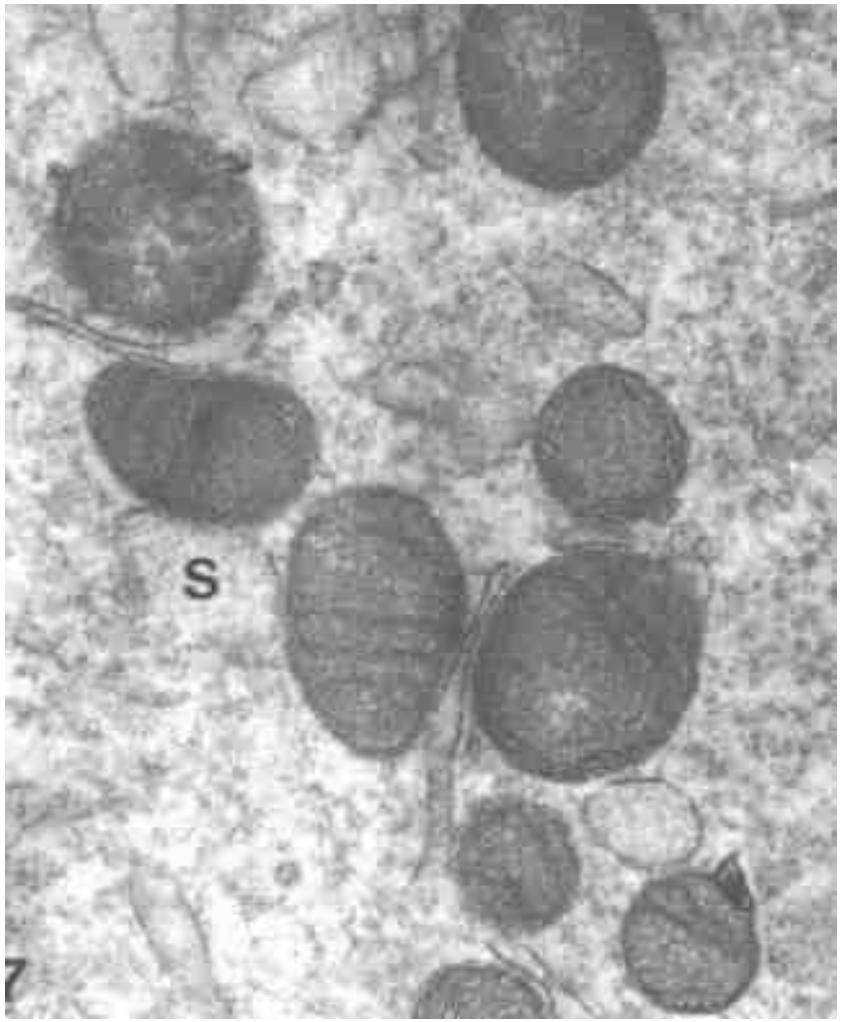
- Telomere shortening
- Mutations in mtDNA

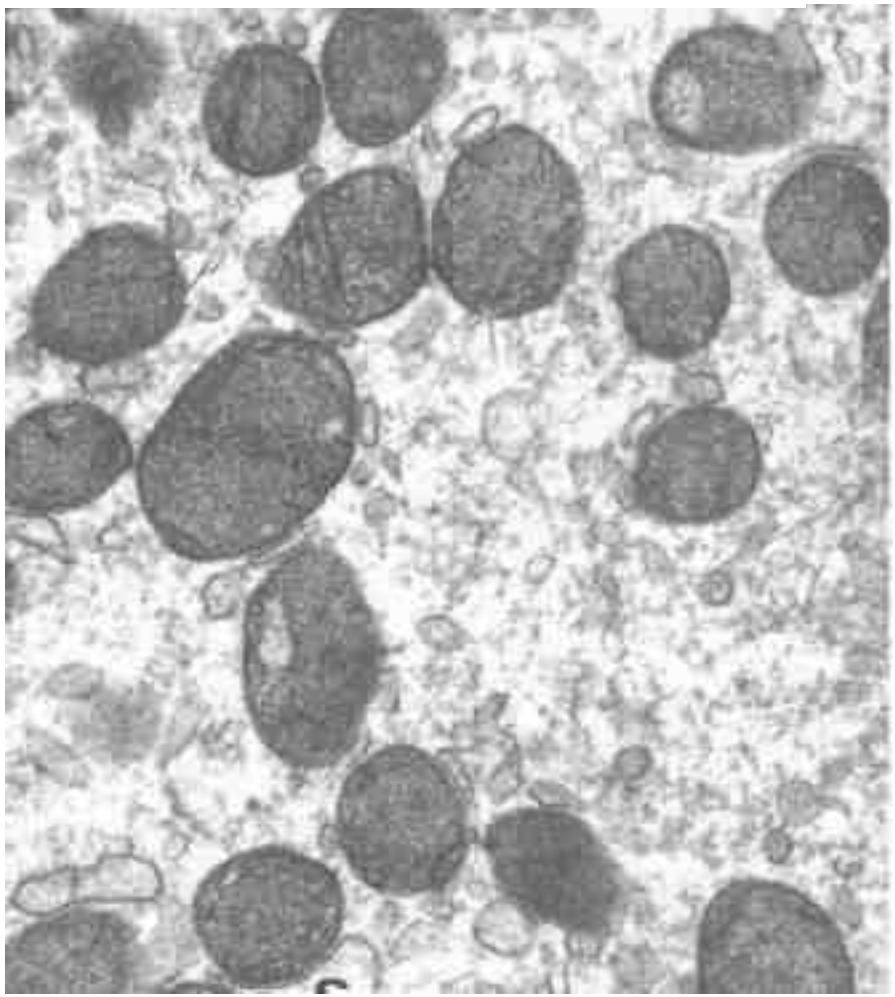
(Both related to Radical Oxygen Species)

Oocyte Mitochondria

- Appear spherical
- Contain a very dense matrix with low number of cristae
- Have one haploid DNA molecule per organelle
- Circular DNA, no histones, no introns, no DNA repair enzymes
- Between 200,000 and 500,000 per oocyte

Mitochondria in an Oocyte

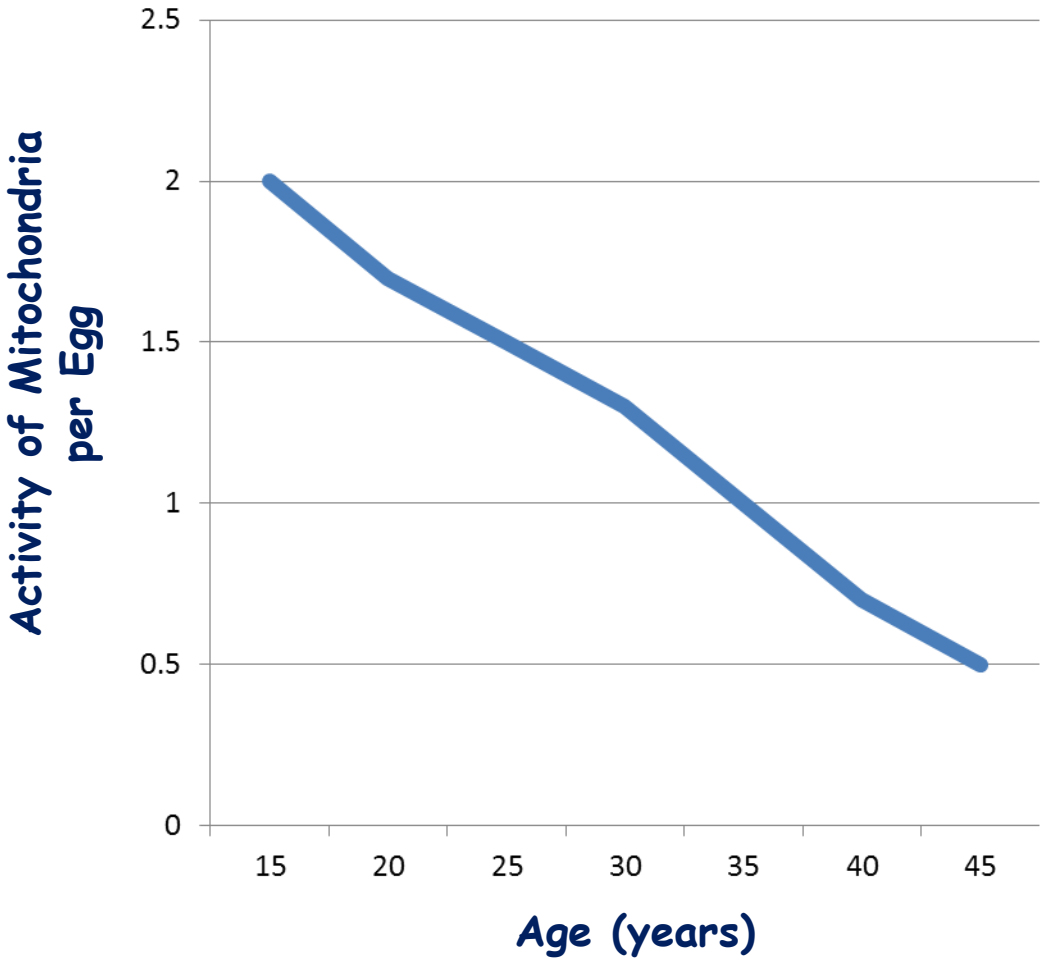




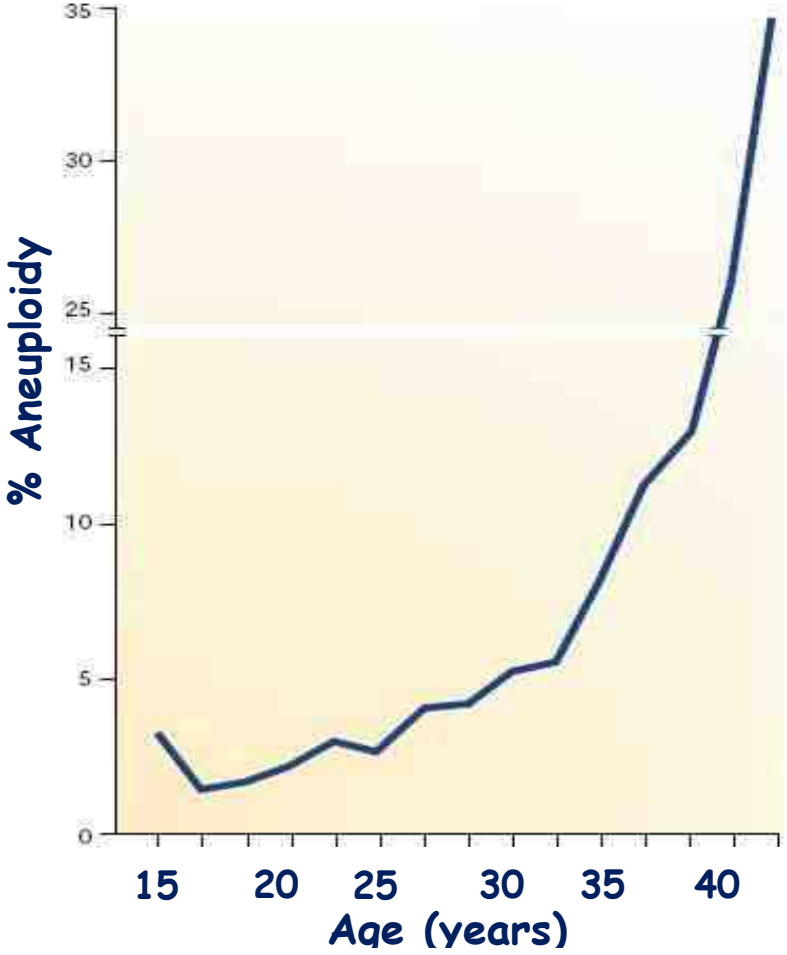
Sathanathan and Trounson, 2000

Energy in the Egg Decreases with Age

Mitochondrial function decreases with age



Aneuploidy increases with age



Wilding, et al., 2001 Human Reproduction
Hassold, et al., 2001 Nature Reviews Genetics

Numerous current clinical trials are underway to address the problem:

1. Improving Mitochondrial performance (Casper and collaborators, TCART)

2. Adding in new Mitochondria (OvaScience)

Past Mitochondrial Research in IVF

- Cytoplasmic transfer pioneered by Jacque Cohen and showed some benefits
- This was later banned by the FDA because it results in mitochondrial heteroplasmy
- Micro-injection of recombinant mitochondrial proteins was another option investigated

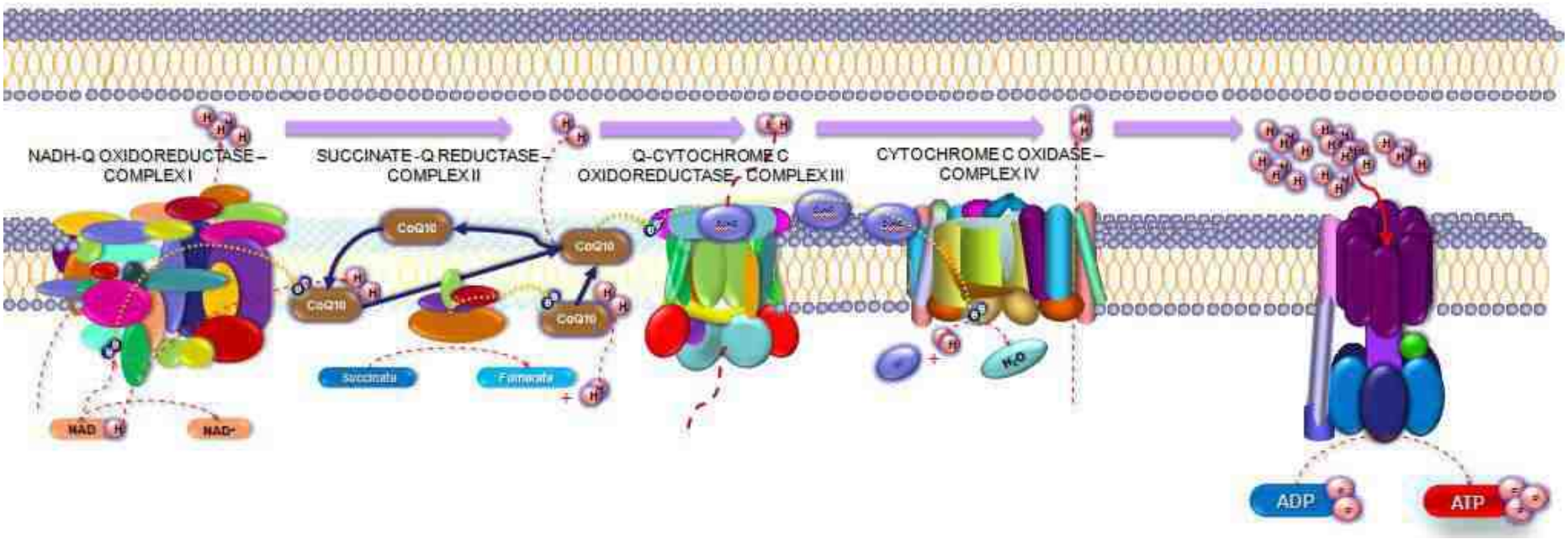
Theories of Oocyte Aneuploidy

- Aging and mitochondrial energy substrate deficiency
  - Pyruvate
  - Coenzyme Q10 (CoQ10)

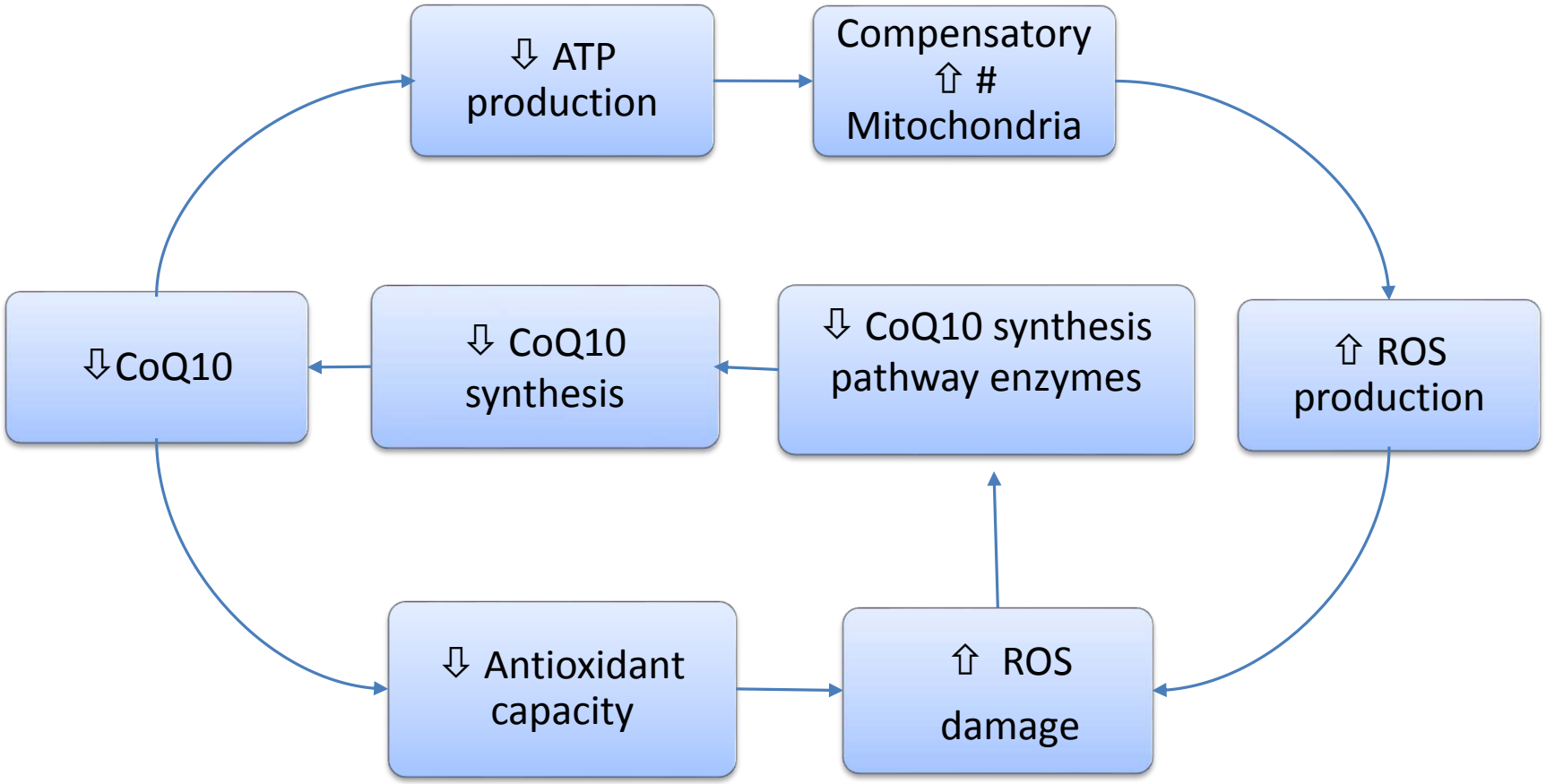
Coenzyme Q10

- Oil-soluble vitamin-like substance present in most cells, primarily in the mitochondria.
- Component of the electron transport chain needed for aerobic cellular respiration to generate energy
- Most potent known antioxidant
- Organs with the highest energy requirements have the highest CoQ10 concentrations

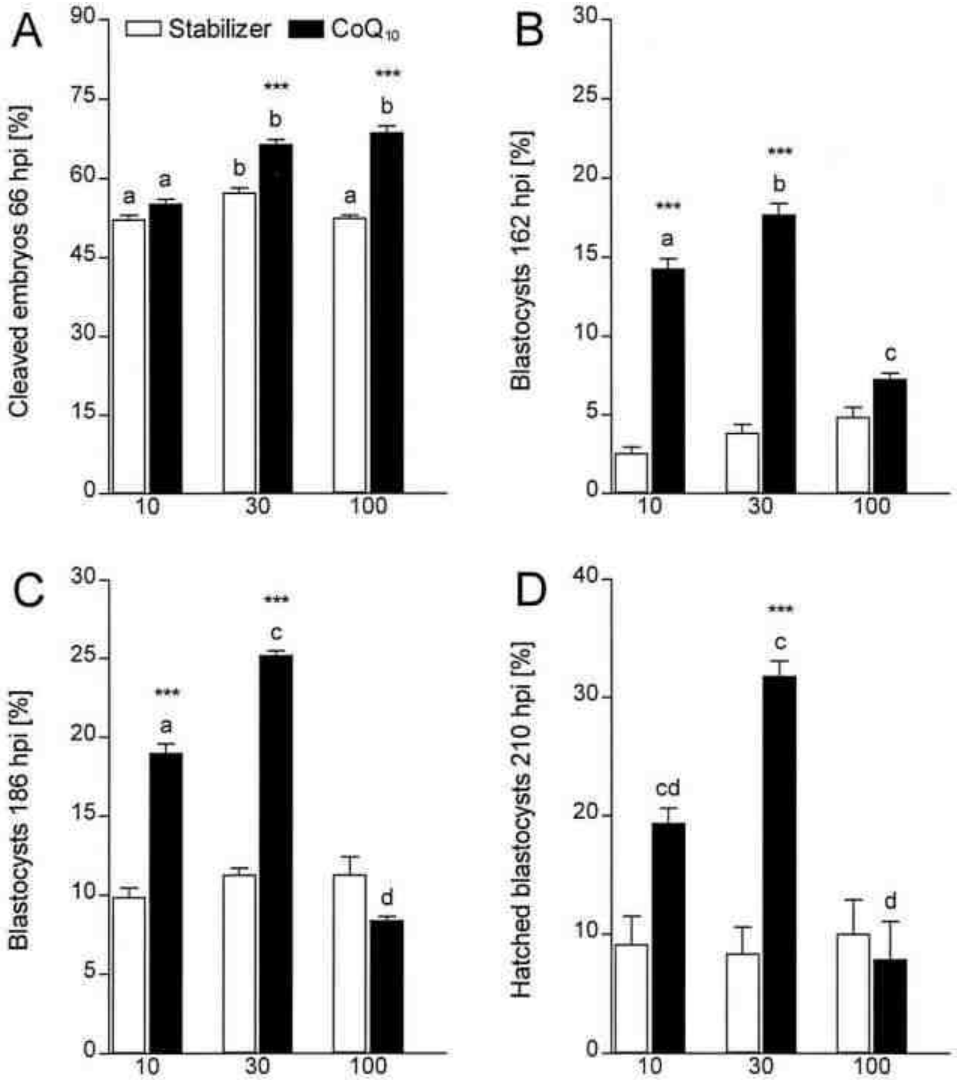
Electron Transport Chain



CoQ10 Decrease leads to Increased ROS production



Effects of CoQ10 and stabilizer on the development of bovine embryos to 5- to 8-cell stages (A), to blastocysts 162 hpi (B) and 186 hpi (C), and to hatched blastocysts 210 hpi (D).



Stojkovic M et al. Biol Reprod 1999;61:541-547

SSR

Hypothesis

- Supplementation of coQ10 will enhance oocyte mitochondrial energy production, increase antioxidant activity, and improve chromosomal disjunction and embryo development

The Effect of Co Enzyme Q10 Together With Fertility Drugs on Pregnancy Outcome of in Vitro Fertilization

Primary Outcome Measures:

- Number and percentage of euploid eggs per retrieval

Secondary Outcome Measures:

- Ovarian response
- Embryo quality
- Cumulative pregnancy rate/retrieval
- Cumulative live birth rate/retrieval
- CoQ10 activity in saliva and follicular fluid

- http://clinicaltrialsfeeds.org/clinical-trials/show/NCT01048385

New Drugs on the Horizon: The Effect of Anti-Apoptosis drugs on oocyte yield and quality

71 BIOLOGY OF REPRODUCTION 82, 000–000 (2010)
Published online before print 27 January 2010.
DOI 10.1095/biolreprod.109.080697

Oocyte Numbers in the Mouse Increase after Treatment with 5-Aminoisoquinolinone: A Potent Inhibitor of Poly(ADP-ribosyl)ation[1]

Hong Qian,[3] Jiasen Xu,[3] Maria D. Lalioti, Kanat Gulle, and Denny Sakkas[2]

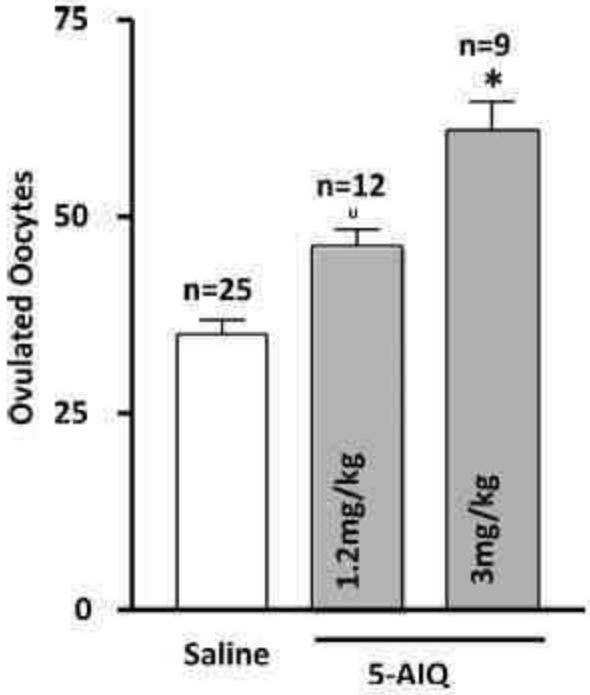
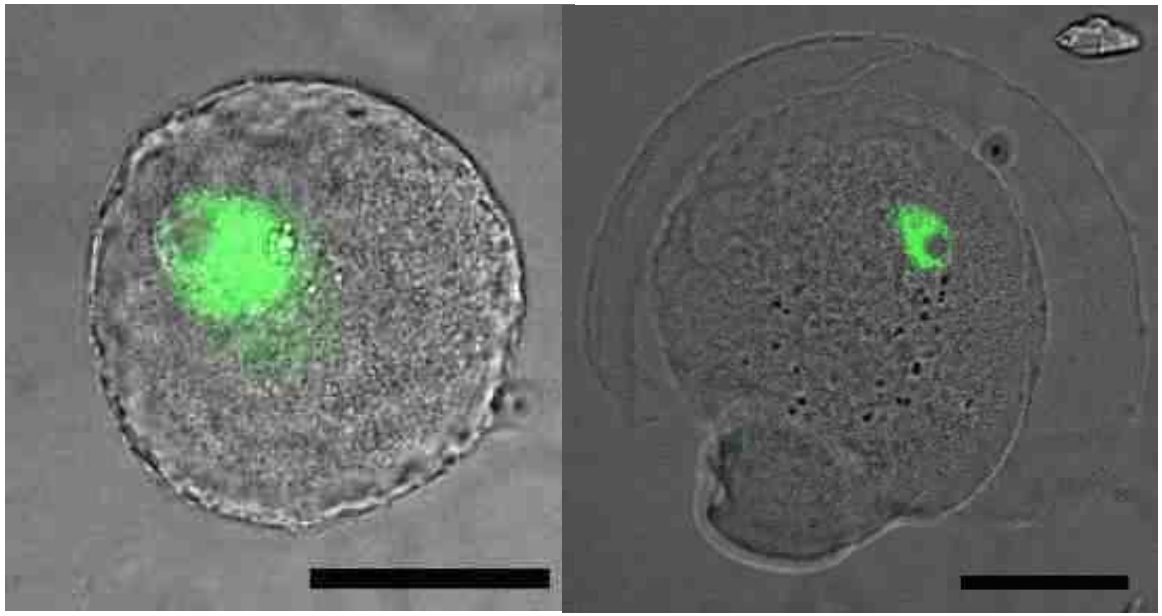


FIG. 2. Effect of 5-AIQ on the number of ovulated oocytes. Oocytes were collected from the saline-treated group and the 5-AIQ plus ovarian stimulation group. The 129S mice were treated with saline and at 1.2 and 3 mg/kg 5-AIQ. Both 5-AIQ groups were significantly different from the saline group. The numbers of mice examined are shown above the columns. (♦$P < 0.002$, *$P < 0.0001$).



Young patient eggs (left) have higher expression of PARP than eggs from older women (right)

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OvaScience℠

Improving Fertility Through Science

- Patented egg precursor cell platform offers potential new infertility treatment options
  - AUGMENT℠ (Autologous Germline Mitochondrial Energy Transfer): potential to improve IVF success
  - OvaTure℠ : potential next-generation IVF

Adding Mitochondria to Human Eggs Shown to Increase IVF Success

Women who have failed multiple IVF cycles, unlikely to achieve success

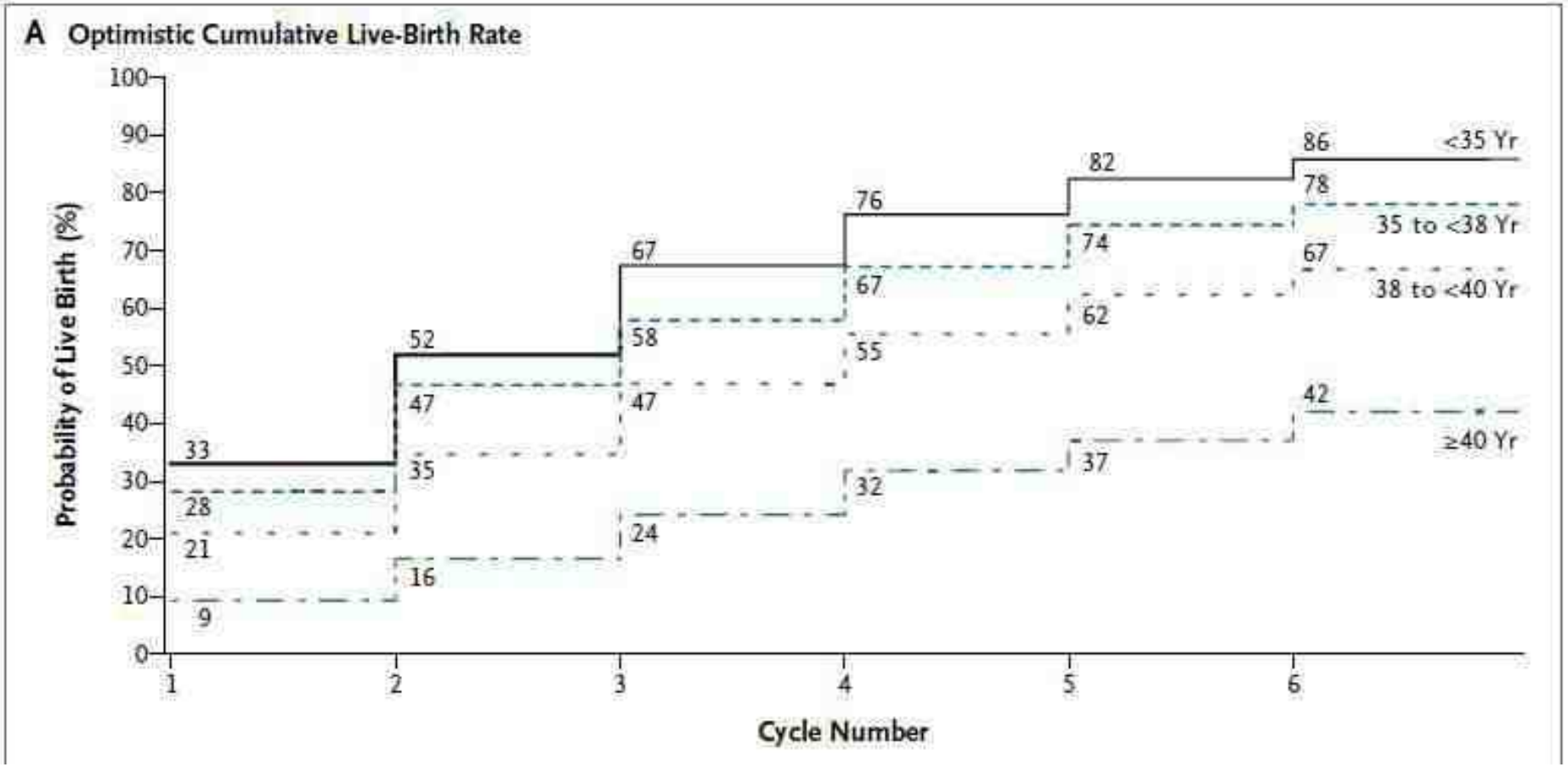
Transfer of cytoplasm/mitochondria from donor eggs	No. of Cycles	Pregnancies	Live Births	Success Rate
Cohen et al., 1997, 1998; Brenner et al., 2000; Barritt et al., 2000, 2001	**30**	**13**	**16**	**43%**
Dale et al., 2001	**1**	**1**	**2**	**100%**
Lanzendorf et al., 1999	**4**	**1**	**2**	**25%**
Tzeng et al., 2004	**71**	**25**	**20**	**35%**
Huang et al., 1999	**9**	**4**	**5**	**44%**
Levron et al	**15**	**5**	**6**	**33%**

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Cumulative Live-Birth Rates after In Vitro Fertilization

Beth A. Malizia, M.D., Michele R. Hacker, Sc.D., M.S.P.H., and Alan S. Penzias, M.D.



Egg Precursor Cell (EggPC℠): A New Approach to Infertility

- Long held belief that women are born with a set number of eggs
- Discovery of EggPCs (germline stem cells) that mature into eggs offers potential new fertility treatments

nature

articles

Germline stem cells and follicular renewal in the postnatal mammalian ovary

Jon Lee Johnson*, Jacqueline Gosling*, Tomoko Kamba, James K. Pru & Jonathan L. Tilly

[1]Nature 2004

Egg Precursor Cells: Ideal Source of Mitochondria

Mitochondria Source	Woman's Own	From an Egg	Mutation Free
Young Donor Egg	X	✓	X
Other Body Cell (somatic)	✓	X	X
OvaScience Patented EggPC (germline)	✓	✓	✓[1]

[1]OvaScience data

AUGMENT℠: Ease of Adoption

IVF Cycle

SUITABILITY
• Blood tests, age, past medical history

↓

OVARIAN HYPERSTIMULATION
• Hormone injections

↓

EGG RETRIEVAL

↓

EGG FERTILIZATION by ICSI

↓

EMBRYO CULTURE & TRANSFER

IVF Cycle with AUGMENT℠

SUITABILITY
• Blood tests, age, past medical history

↓

OVARIAN TISSUE BIOPSY

↓

OVARIAN HYPERSTIMULATION
• Hormone injections

↓

EGG RETRIEVAL

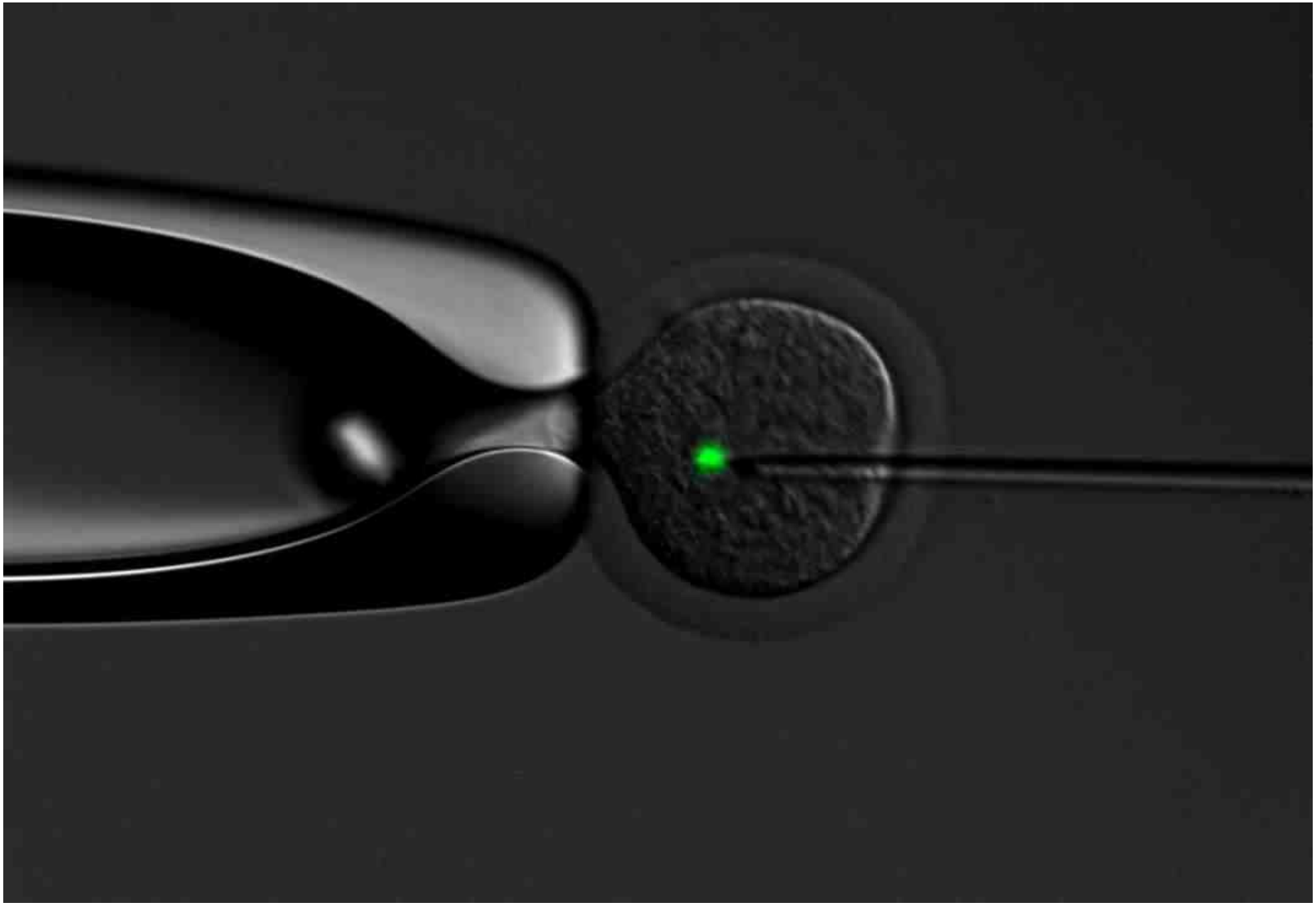
↓

EGG FERTILIZATION by ICSI
+ EggPC MITOCHONDRIA

↓

EMBRYO CULTURE & TRANSFER

AUGMENT: Oocyte Injection



AUGMENT℠ Study Underway

- **Goals:**
  - Gain clinical experience
  - Optimize procedure and process
  - Confirm safety
- **Design:**
  - Women 38-42 years old; 2–5 previous IVF failures
  - 40 women undergoing standard IVF
  - 40 women undergoing IVF with AUGMENT℠ at same clinics
- **Endpoints:**
  - Safety
  - Effectiveness
    - Embryo quality (at transfer)
    - hCG (14 days post retrieval)
    - Ultrasound (6 and 20 weeks)
    - Healthy live births

Journal of Fertilization : In Vitro, IVF-Worldwide, Reproductive Medicine, Genetics & Stem Cell Biology

Fakih et al., JFIV Reprod Med Genet 2015, 3:3
http://dx.doi.org/10.4172/2375-4508.1000154

Research Article

The AUGMENT℠ Treatment: Physician Reported Outcomes of the Initial Global Patient Experience

Michael H Fakih[1*], Mohamad El Shmoury[1], Julia Szeptycki[2], Dennis B dela Cruz[2], Caroline Lux[2], Suleman Verjee[3], Colleen M Burgess[4], Gabriel M Cohn[4] and Robert F Casper[2*]

MBEST at FAKIH IVF

At FAKIH IVF, eggs from a subset of women who underwent successful egg retrieval were allocated to two treatment groups; one group of a patient's eggs underwent the AUGMENT treatment at the time of ICSI while the other group of that woman's eggs underwent conventional ICSI only. The eggs and embryos obtained from both the AUGMENT group and ICSI-only group were maintained under identical culture, environmental, and embryo management conditions. Morphokinetic analysis was performed using the EmbryScope® (VitroLife, formerly Fertilitech) and standard morphology metrics were observed along with the timing of cellular developments from post AUGMENT injection until the time of embryo selection. Embryos were selected from one of these two treatment groups based on standard laboratory, prognostic criteria including embryo morphology and the results of pre-implantation genetic testing. Embryo transfer for a given patient was not performed if none of the embryos from either treatment group met these criteria for transfer. To facilitate further discussion, we have termed this approach Matched, Best Embryo Selection and Transfer (MBEST).

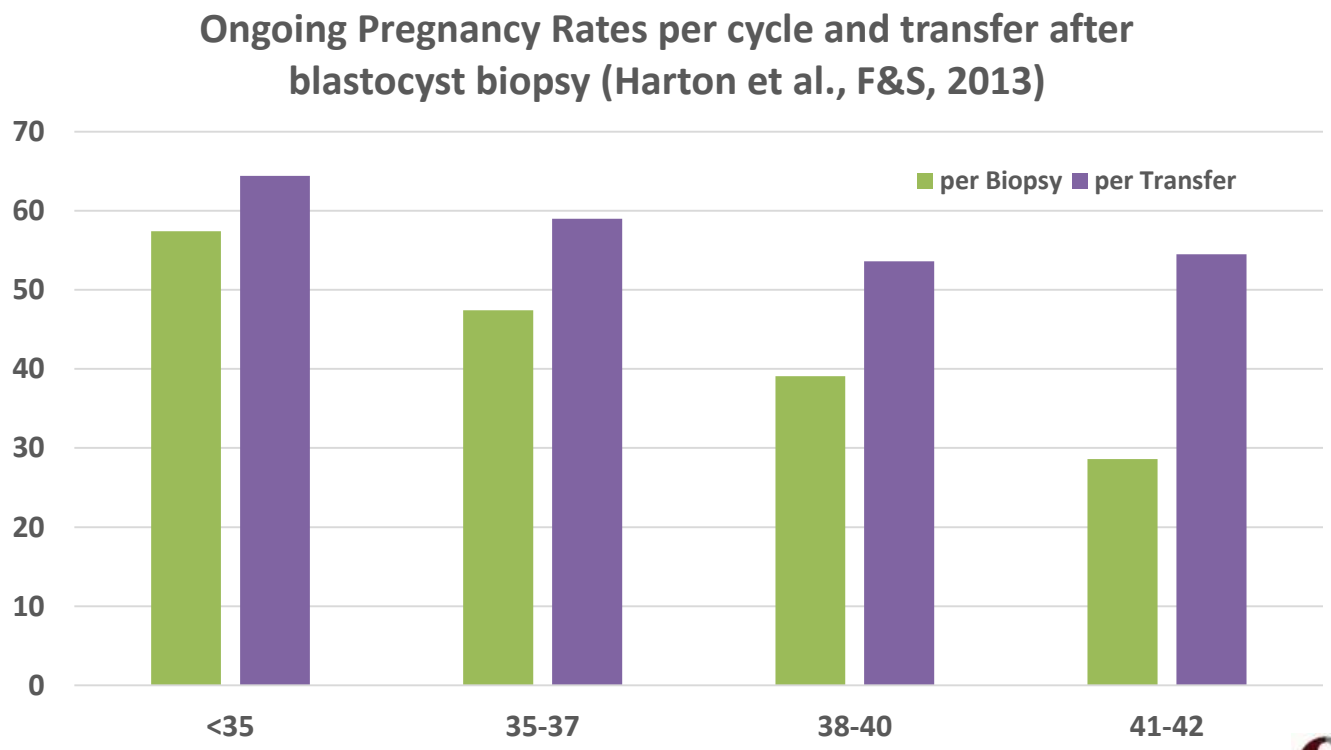
Embryo selection and embryo transfer

Standard clinic selection criteria including morphology, morphokinetic analysis (TCART and FAKIH IVF) and pre-implantation genetic testing (FAKIH IVF only) were utilized in selecting the embryos with the best implantation potential. Morphology evaluations and assessments were consistent with the Society for Assisted Reproductive Technology's (SART) criteria (see supplement). Using clinic standard culture procedures, embryos were cultured to blastocyst stage for embryo transfer selection unless there were selectivity or patient scheduling limitations.

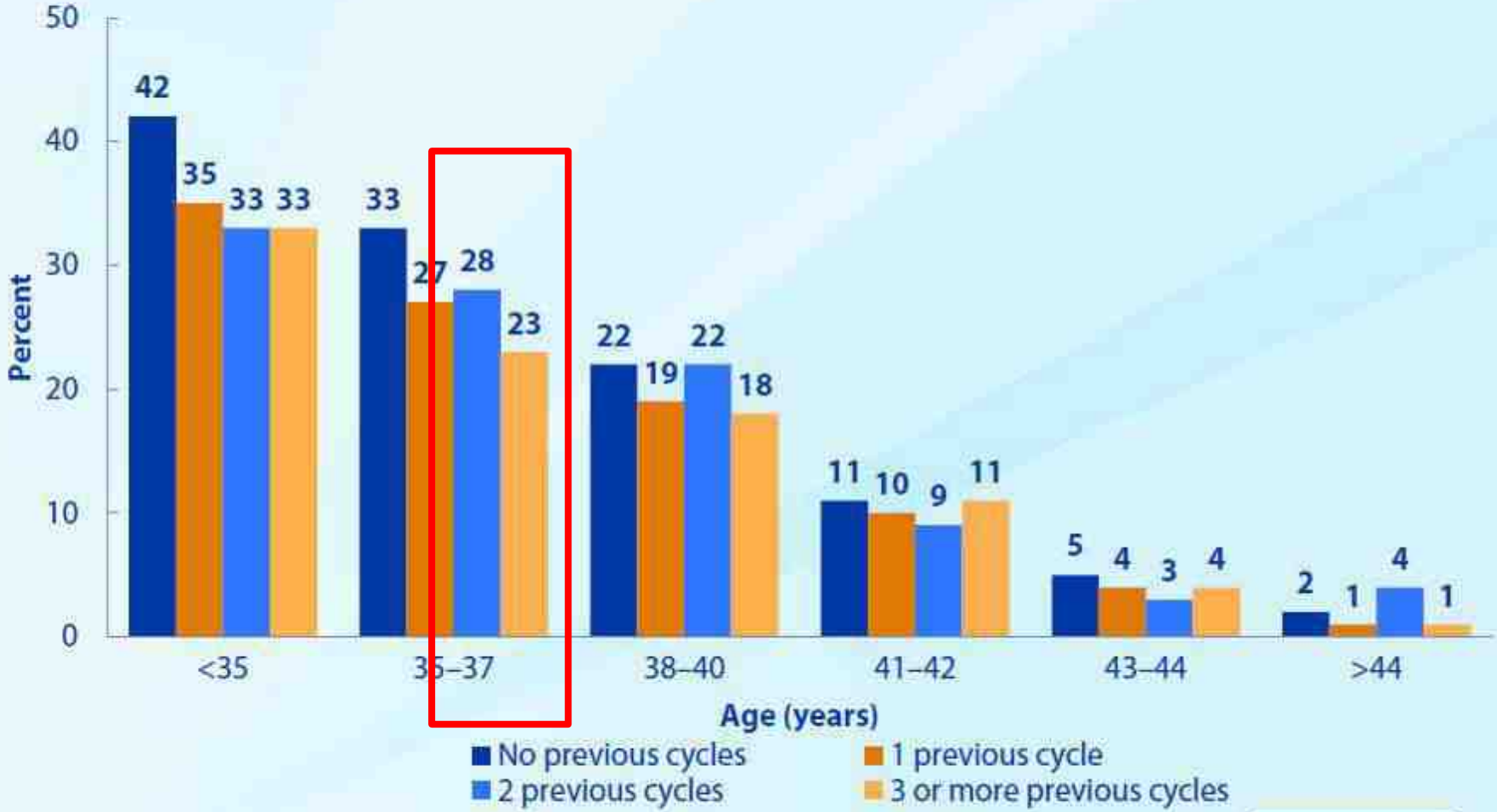
Table 2: Summary of center specific AUGMENT treatment experiences.

	Patient History	Previous Clinical Pregnancy Rate per Initiated Cycle	Clinical Pregnancy Rate per Initiated AUGMENT Cycle	Clinical Pregnancy Rate per AUGMENT Embryo Transfer	Previous Ongoing Clinical Pregnancy Rate/ Live Birth Rate per Initiated Cycle	Ongoing Clinical Pregnancy Rate/ Live Birth Rate per Initiated AUGMENT Cycle	Ongoing Clinical Pregnancy/ Live Birth Rate per AUGMENT Embryo Transfer
Canada	• Average age: 36.0 • 1-5 prior IVF cycles	11%	35%*	46%*	1.4%	26%	35%
United Arab Emirates	• Average age: 37.3 • 1-16 prior IVF cycles	4%	22%	38%	2.0%	18%	32%

*9 patients with 23 embryos cryopreserved for future use

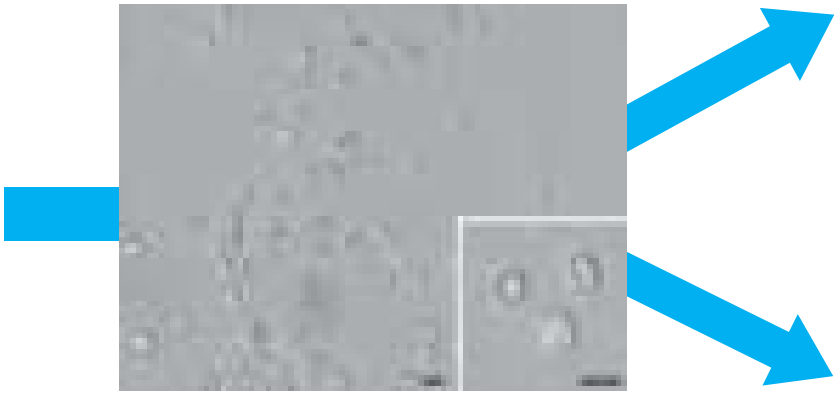


Percentages of ART Cycles Using Fresh Nondonor Eggs or Embryos That Resulted in Live Births, by Age Group and Number of Previous ART Cycles, Among Women with No Previous Live Births, 2012

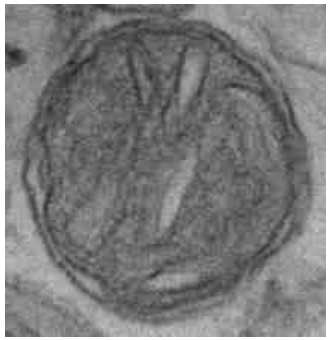


National Center for Chronic Disease Prevention and Health Promotion
Division of Reproductive Health

OvaScience EggPC Platform: Multiple Potential Fertility Treatments



EggPCs



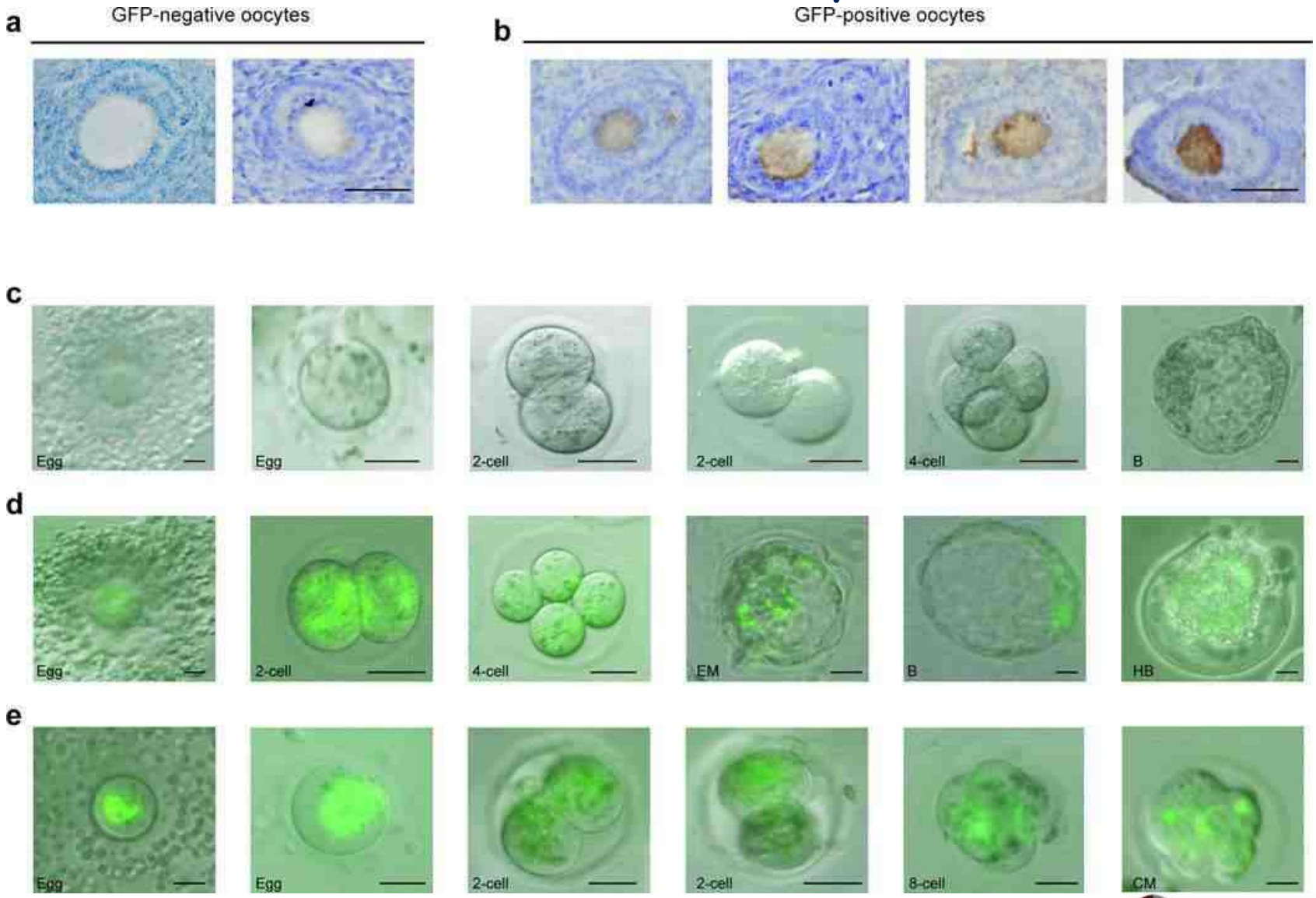
EggPC Mitochondria to rejuvenate eggs

AUGMENT℠

Fresh eggs matured in the lab from EggPCs

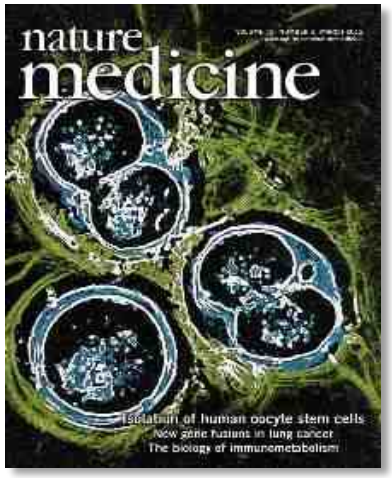
OvaTure℠

Oocyte formation by mitotically active germ cells purified from ovaries of reproductive-age women.
White et al. Nat Med. 2012 February 26; 18(3): 413–421.

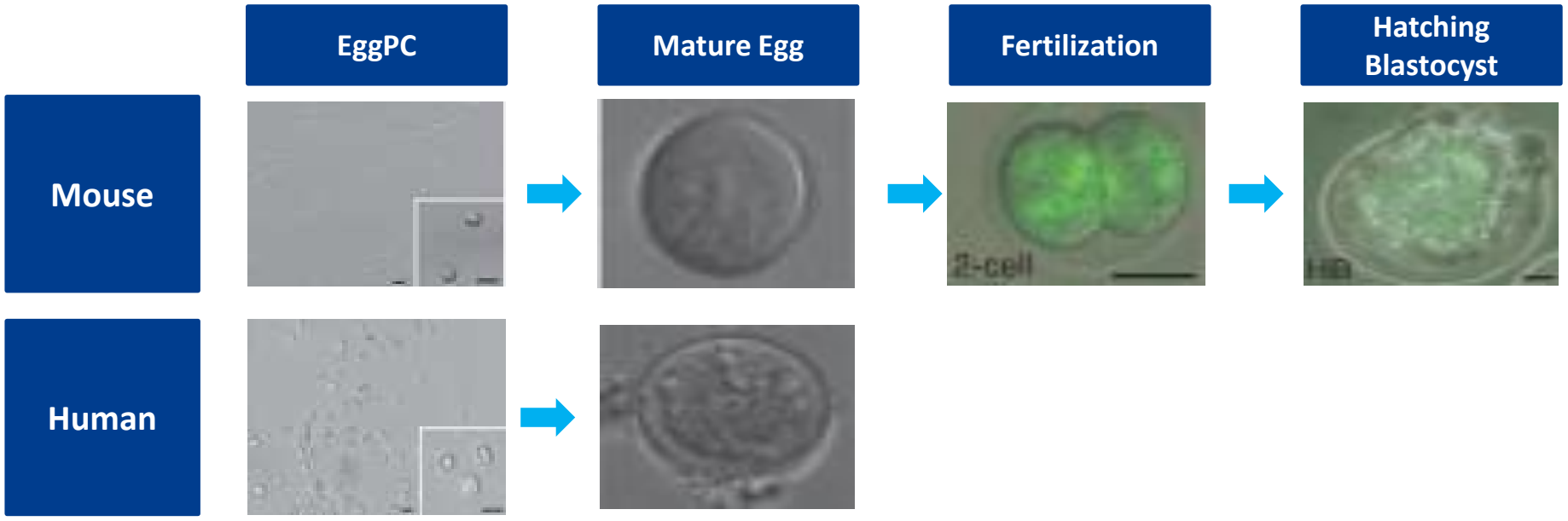


OvaTure℠: Maturation of EggPCs into Fresh, Young, Healthy Eggs

- May eliminate need for hormone hyperstimulation
- Potential to replace standard IVF



March, 2012



OvaTure℠: Maturation of EggPCs into Fresh, Young, Healthy Eggs

- Many questions remain about routine IVF/ICSI and epigenetic inheritance
- Will the technology involving maturation of EggPCs take a long time to prove safety?
- Spermatid injection has a morotorium in many countries and has largely been abandoned
- The first mouse birth from primordial follicles had significant issues even though the methodology has been improved. [O'Brien et al. A revised protocol for in vitro development of mouse oocytes from primordial follicles dramatically improves their developmental competence. Biol Reprod. 2003]
- In Vitro Maturation has not shown significant improvement in treatment of older patients although it will allow a window of opportunity for supplementing the follicle to improve development

Derivation of embryonic germ cells and male gametes from embryonic stem cells

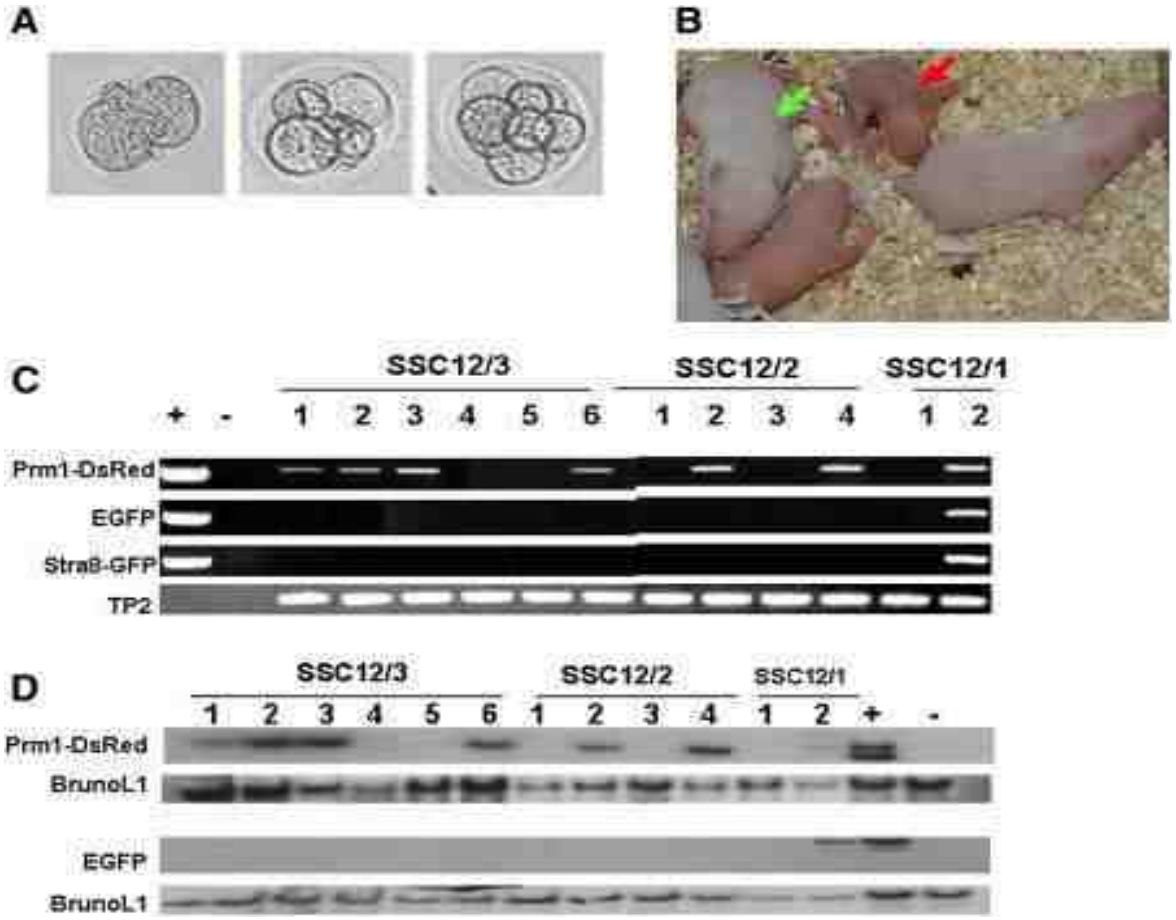
Niels Geijsen[1,2], Melissa Horoschak[1,3], Kitai Kim[1,3], Joost Gribnau[1], Kevin Eggan[4] & George Q. Daley[1,3]

NATURE | VOL 427 | 8 JANUARY 2004 | www.nature.com/nature

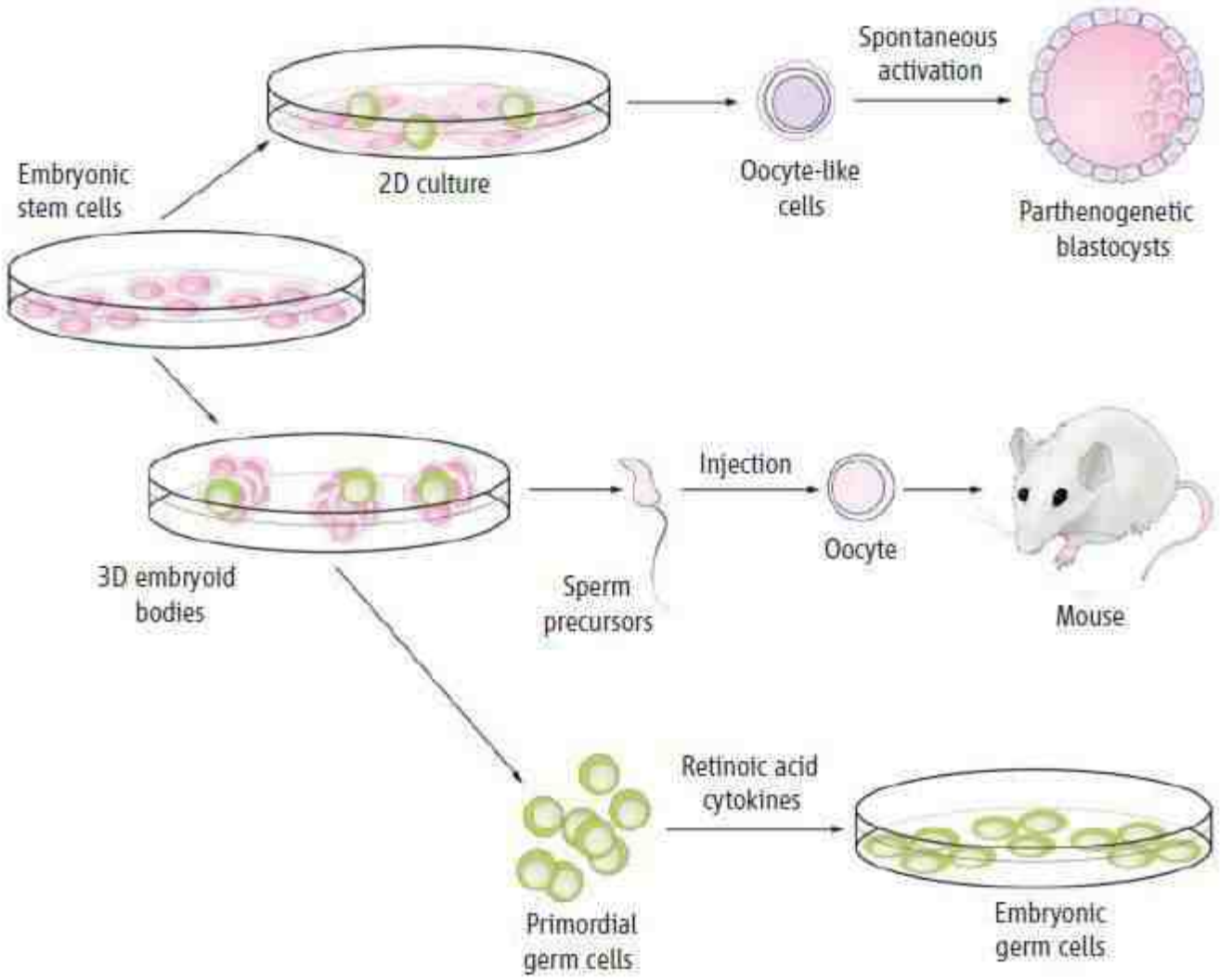
In Vitro-Differentiated Embryonic Stem Cells Give Rise to Male Gametes that Can Generate Offspring Mice.

Nayernia et al. Developmental Cell Volume, 2006

Functionality of ES Cell-Derived Male Gametes (A) Development of preimplantation embryos derived from intracytoplasmic injection (ICSI) of Prm1-DsRed-positive haploid cells into the CD1 or NMRI oocytes. (B) Full-term development of transferred embryos.



Stem Cells to Gametes



Stem Cells to Gametes

REVIEW

Gametes from Embryonic Stem Cells: A Cup Half Empty or Half Full?

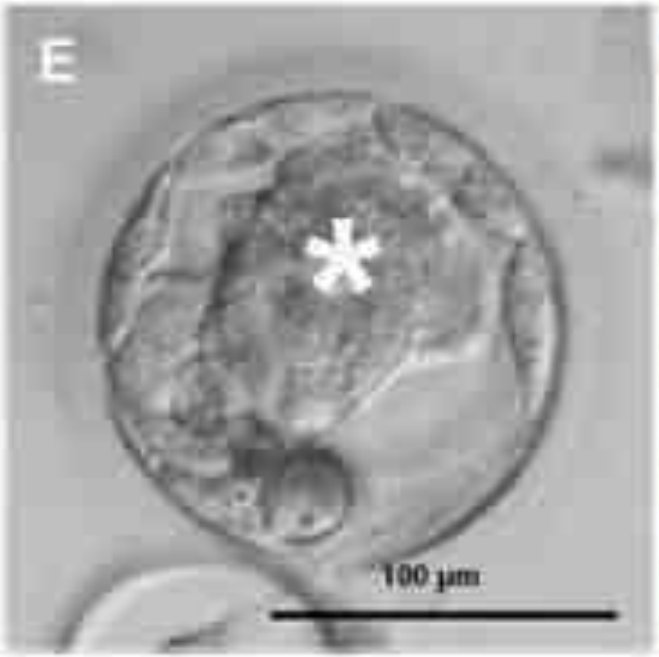
George Q. Daley

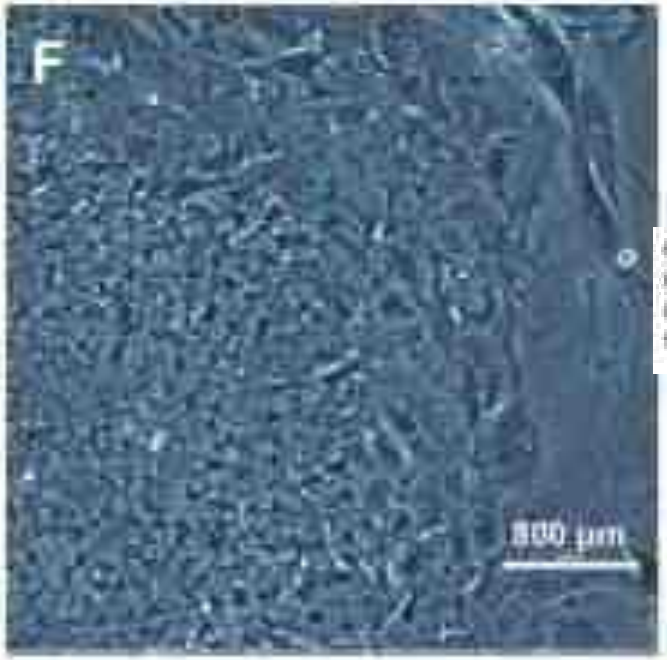
When first reported 4 years ago, gametogenesis from embryonic stem (ES) cells promised an accessible in vitro model to facilitate molecular analysis of the germ lineage. Formation of primordial germ cells is robust, but terminal gametogenesis remains inefficient and doubts about gamete function persist. Although useful for research, clinical use of ES cell–derived gametes appears a distant prospect.

Cell

Human Embryonic Stem Cells Derived by Somatic Cell Nuclear Transfer

Masahito Tachibana,[1] Paula Amato,[2] Michelle Sparman,[1] Nuria Marti Gutierrez,[1] Rebecca Tippner-Hedges,[1] Hong Ma,[1] Eunju Kang,[1] Alimujiang Fulati,[1] Hyo-Sang Lee,[1,6] Hathaitip Sritanaudomchai,[3] Keith Masterson,[2] Janine Larson,[2] Deborah Eaton,[2] Karen Sadler-Fredd,[2] David Battaglia,[2] David Lee,[2] Diana Wu,[2] Jeffrey Jensen,[1,4] Phillip Patton,[2] Sumita Gokhale,[5] Richard L. Stouffer,[1,2] Don Wolf,[1] and Shoukhrat Mitalipov[1,2,*]





(E) Human SCNT blastocyst with prominent ICM (asterisk) produced after caffeine treatment. (F) NT-ESC colony with typical morphology derived from a caffeine-treated SCNT human blastocyst.

Table II Outcome of ICSI procedure according to the different methods with complete asthenozoospermia.

Study	Patients (*n*)	Sperm origin	Technique applied	Viable spermatozoa (%)	Fertilization rate (2PN) (%)	Clinical pregnancies (*n*)
Casper *et al.* (1996)	8 patients	Epidymal (4 cases)	HOST	31.1 ± 5.8	43	3
Liu *et al.* (1997)	3 patients	Ejaculated and F-TESE	HOST	NM	76.4	1
Vandervorst *et al.* (1997)	11 patients	Ejaculated	Eosin Y stain[a]	0–34	12.4	0
Barros *et al.* (1997)	6 cycles	Ejaculated	HOST	5	41.9	2 (1 twin)
Nagy *et al.* (1998)	14 cycles	F-TESE	HOST	NM	46	4
Ron-El *et al.* (1998)	3 initial cycles 6 repeated cycles	Ejaculated Ejaculated	Eosin Y stain[a] Eosin Y stain[a]	41 ± 7.4 71 ± 6.9	3 48	1 (twin)
Okada *et al.* (1999)	16 patients	Ejaculated	Eosin Y stain[a] HOST	15–80	38.6	0
Shulman *et al.* (1999)	19 cycles	TESE	NM	NM	51	3
Terriou *et al.* (2000)	20 cycles	MESA, F-TESE, Fr-Th TESE, Fr. Ep	PTX	6–60	45.2	6
El-Nour *et al.* (2001)	4 patients 10 patients 1 patient	Ejaculated TESE Electroejaculated	HOST HOST HOST	15–46 20–100 18	47 43 60	2 4 (1 twin) 1 (twin)
Sallam *et al.* (2001)	15 patients 12 patients	Ejaculated F-TESE	HOST mod. HOST mod.	NM NM	42.7 30.1	2 2
Soares *et al.* (2003)	10 cycles	F-TESE, TESA, PESA	MTT	NM	30.3	1
Aktan *et al.* (2004)	10 patients 21 patients 24 patients	Ejaculated, F-TESE F-TESE Ejaculated	HOST versus LAISS LAISS LAISS	21.5 versus 22 NM NM	 45.4 64.2	 5 8
Marques de Oliveira *et al.* (2004)	6 patients 10 patients	Fr-Th TESE F-TESE	MTT MTT	NM NM	65.7 73.4	2 3
Sallam *et al.* (2005)	25 patients 19 patients	F-TESE Fr-Th TESE	HOST HOST	NM NM	44 42.7	7 5
Kovacic *et al.* (2006)	47 cycles	TESA, TESE	PTX	NM	66	18

Fr-Ep, frozen epididymal; F-TESE, FRESH TESE; Fr-Th TESE, frozen-thawed TESE; HOST, Hypo-osmotic swelling test; HOST mod, modified hypo-osmotic swelling test; LAISS, laser-assisted immotile sperm selection; MESA, microsurgical epididymal sperm aspiration; MTT, mechanical touch technique; PESA, percutaneous epididymal sperm aspiration ; PTX, pentoxifylline; TESA, testicular sperm aspiration; TESE, testicular sperm extraction; NM, not mentioned.

[a]To detect viable spermatozoa.

Pentoxifylline treatment of non-motile spermatozoa

- Previous 18 months at Boston IVF
- 33 cases (9 cryo all and 24 transfers)
- 24 cases (11 Testicular, 8 Epididymal, 5 Ejaculate)
- Mean age of females 35.6

- 10 ongoing pregnancies (41.7%)

Other alternatives for patients

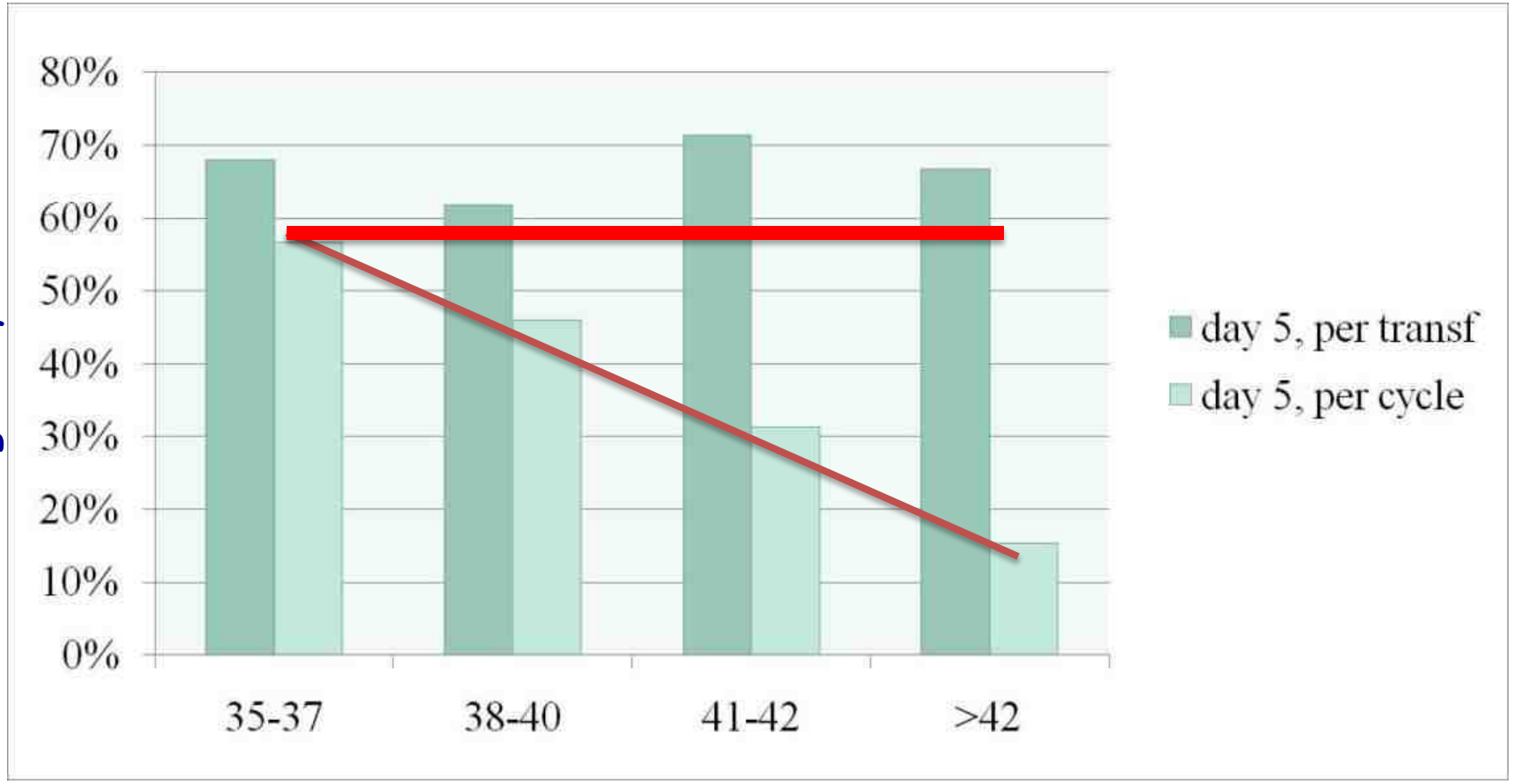
- Female
  - Aneuploidy screening of embryos
  - Donor oocytes [Fresh and Vitrified/Frozen]
- Male
  - Aneuploidy screening of embryos
  - Donor sperm

- COST

Blastocyst Biopsy

BOSTONIVF

Blastocyst Biopsy and aCGH does appear to limit the effect of age when performed on Day 5



Harton et al., Fertil. Steril. 2013

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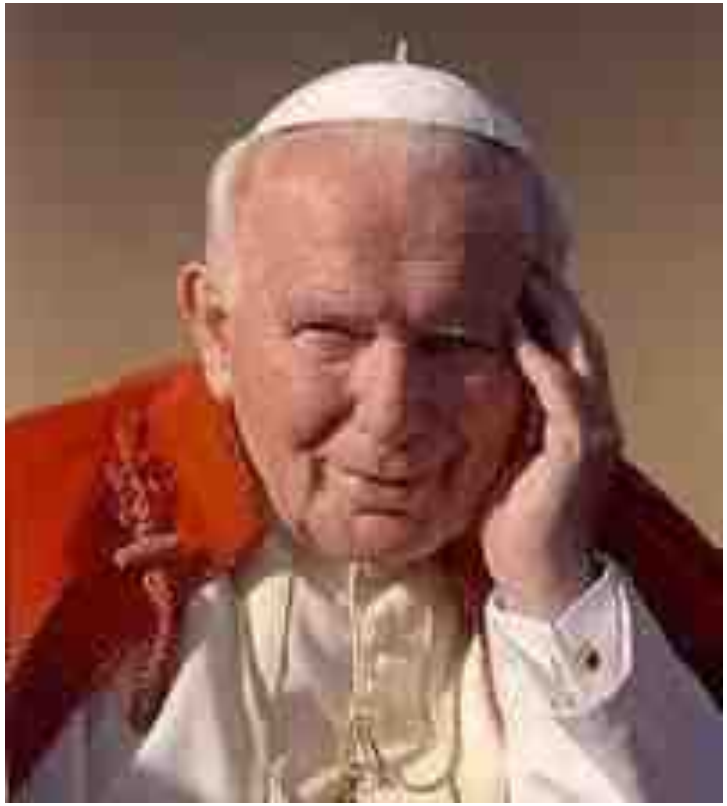
Oocyte Vitrification

- On the 10th March 2004, no more than three oocytes were allowed to be fertilized at one time during an IVF treatment in Italy, by application of a new law regulating assisted reproduction technology
- The law has since changed



Donor Egg Bank Thaw Cycles

	January 2009-2011 at one center	Multi-Site Experience Through 2012
# Oocytes Banked	1311	2258
# Oocytes Thawed	745	691
# Oocytes Survived	**658 (88%)**	**609 (88%)**
# Oocytes Injected	655	606
# 2PN embryos	512 (78%)	444 (73%)
# of thaws/# oocytes	**118 (6.39)**	**99 (6.17)**
# of Embryo Transfers	116	96
# Day 3 ET /# Embryos	61 (53%)/1.96	47 (49%)/1.85
# Day 5/6 ET /# Embryos	55 (47%) /1.76	49 (51%)/1.61
D3 Preg % / ET	33.90%	40.42%
D5 Preg % / ET	60.71%	55.10%
# of Cycles with Vit Blasts	33	50
Blast Utilization	172/512 (34%)	175/444 (39%)
# Cancelled cycle	2	3
Clinical Pregnancy	62 (53%)	46 (48%)
SAB	9	1
Ongoing	**55 (47%)**	**45 (47%)**

Summary New Techniques

In the human

- Technologies exist to improve poor gametes and are currently being tested in clinical trials
- Technologies also exists to create new gametes from various types of precursor cells
- Whether these treatments induce an epigenetic trans-generational inheritance of diseases will be the greatest concern

Summary of Alternatives

In the human

- The success of oocyte Vitrification will allow storage of "younger" oocytes by women prior to choosing to create a family
- Donor Vitrification oocytes may allow easier access to gamete donation by lowering costs

Cost and Desire

- All these technologies will ultimately rely on the patients DESIRE / NEED to propagate their own genes in relation to accepting donor gametes
- In addition, the COST / STRESS of the new technologies versus the alternatives will also drive patient acceptance

Thank You

- Boston IVF
- Prof Bob Casper
- Ovascience