IN VITRO FERTILIZATION: FROM REPRODUCTION TO GENETIC REPAIR A CAUTIONARY TALE

PRESENTED BY:
C. MATTHEW PETERSON, MD
DISCLOSURES

• Active IVF physician
• Biased towards "science and intervention"
• No relevant financial/scientific bias but fully aware this can and does happen
OBJECTIVES

• Review biology of early embryo
• Review IVF and how gene editing might be applied
• Review the fields forays into genetic evaluation of early embryonic tissues
• Assess the possibility of gene editing based on a seasoned, candid assessment of our specialty and its history
RELEVANT IVF HISTORY

- 1959 Min Chueh Chang, Rabbit IVF
- 1973 Carl Wood, John Leeton, Alan Trouson, Biochemical pregnancy
- 1976 Patrick Steptoe and Robert Edwards, Ectopic pregnancy
- 1978 Patrick Steptoe and Roberts Edwards, Louise Brown delivered
- 1978 Subash Mukhopadyay, Delivery of Durga
- 1981-2018 Variations in controlled ovarian hysperstimulation
- 1992- Intracytoplastic sperm injection (ICSI)
- 1995-2011 Vitrification
- 1996-2008 Preimplantation Genetic Screening (PB/ Cleaving embryo)
- 2008-2018 Preimplantation Genetic Screening (Blastocyst Trophectoderm)

STEM CELLS

TOTIPOTENT STEM CELLS CAN GIVE RISE TO ALL HUMAN TISSUES INCLUDING THE PLACENTA (FERTILIZED EGG AND EARLY CLEAVAGE STAGES, DAY 1-3)

PLURIPOTENT STEM CELLS CAN GIVE RISE TO ALL HUMAN TISSUES EXCEPT THE PLACENTA. ON DAY 4, THE EMBRYO FORMS THE INNER CELL MASS AND TROPHECTODERM. FROM THIS POINT ON, INNER CELL MASS CELLS ARE CONSIDERED PLURIPOTENT.

MULTIPOTENT STEM CELLS ARE FOUND IN MULTIPLE SITES AND CAN PRODUCE A RESTRICTED DOWNSTREAM LINEAGE. EXAMPLES INCLUDE BONE MARROW, ENDOTHELium, GI TRact, MUSCLE, SKIN
IVF - EMBRYO DEVELOPMENT AND STEMNESS

Early cell division of zygote

- Two-cell stage
- Four-cell stage
- Eight-cell stage

Fertilization

Ovum (Egg)

Corpus luteum

Implanted blastocyst

Morula

Ovarian ligament

Uterus

Cervix

Vagina
Dr Raewyn Teirney
Gynaecologist & Fertility Specialist
Pronuclear Injection of DNA into a One Cell Mouse Embryo
DEFINITIONS

Human Genome - Complete set of genetic material
Human Genome Editing - The process of attempting to make precise additions, deletions or alterations to a human genome
Somatic Cells - All human cells, except the egg and sperm cells and their progenitors (germline)
Somatic Genome Editing - Editing only somatic cells, which affects only the individual treated
Germline Cells - Reproductive cells like sperm and eggs and their progenitors
Heritable (Germline) Editing - Editing a person's reproductive (germline) cells, which is heritable to future generations
CRISPR/Cas9 - A new technique of gene editing that is easier and apparently more efficient
HUBRIS

A quality of extreme or foolish pride or dangerous overconfidence. In its ancient Greek origins, it describes a behavior that defies norms of behavior or challenges the Gods, which in turn brings about the downfall, or nemesis of the perpetrator of hubris.
POLAR BODY AND CLEAVING EMBRYO
Blastocyst-stage embryo

 Inner cell mass
 Ultimate diagnosis target

 Blastocoele

 Trophectoderm
 Source of embryo biopsy

 Evolving structure

 becomes fetus

 becomes placenta

 Unresolved issues with PGS 2.0
 1. Does a single 6-cell TEB reflect the whole TE?
 2. Does the TE chromosomally reflect the ICM?
 3. How much does the ICM self-correct downstream from blastocyst stage?
BLASTOCYST TROPHECTODERM BIOPSY
IVF/PGS: A Cautionary Tale


*Proposed deliverable: improved IVF outcomes and reduce miscarriage*

Mastenbroek S NEJM 2007; 357:359 put and end to polar body and cleavage stage (day 1-3) because of the inaccuracy of PGS due to mosaicism 2008 ASRM states PGS as practiced was ineffective.

2008-2018 Preimplantation Genetic Screening (Trophectoderm biopsy (TE) at Blastocyst stage (Day 5-6) Dokras A Human Repro 1990;5:821; Schoolcraft WB Fertil Steril 2010; 94:1700

*Proposed deliverable: improved IVF outcomes and reduce miscarriage*

Gleicher N J Ovarian Res 2017; 10:21 Questions raised regarding the hypothesis of PGS as a supportable position 2018 Insufficient evidence to recommend routine use of PGS
Approximately 111,000 embryos are screened and using PGS (array CGH) nearly 40% (44,640) may be labeled as abnormal. Transferred embryos are routinely "normal", however, groups are now transferring "abnormal-aneuploid" embryos with the following results:

Munne (58) (Fertil Steril 2017:108:62-71) "One hundred healthy babies have been born thus far"
58/143 successful pregnancies (41%)

Florentino (6) NEJM 2017; 21:2089
18 women received transfers and 6/18 (33%) achieved and all delivered healthy babies

5/8 Gleicher (5) Verbal
Eight women transferred abnormal embryos and 5/8 (62.5%) became pregnant and delivered healthy normal babies.
Mouse embryos have the capacity to eliminate mosaicism. Embryos "marbled with up to 67% abnormal cells have the potential to self correct and deliver normal mice.


Comments and criticisms are pending regarding non targeted mutations, indels, suggestion that coinjection of sperm and CRISPR-Cas 9 components reduces mosaicism, sampling size error, using embryo development as indicator wellbeing, etc etc
SURVEY OF IVF PROVIDERS - APPLICATION OF TECHNOLOGY

• Knowledge of CRISPR/Cas9
  – None -18%, A little - 60%, A lot - 22%
• Should gene editing be allowed?
  – Yes - 50%, No -15%, Don't know - 35%
• If available, would you offer?
  – Yes - 55%
• If available, what percentage of patients would use it?
  – <25% - 18%, 25-50% - 21%, 50-75% - 23%, >75% - 38%
• Should research be allowed in human embryos?
  – Yes - 70%
• Should human gene editing be allowed?
  – Yes - 55%, No - 15%, I don't know - 30%

n =189, ASRM
Palter, Caplan, Amato, Egli, Lu
28 Sept 2017
Genetic Journal Club

http://events.genndi.com/r/3/0/replay/169105139238436922/c03c7289be/0/10909011
RECOMMENDATIONS OF NAS/NAM:

• NO ALTERNATIVES

• RESTRICTED TO: SERIOUS DISEASE OR CONDITION, EDITING OF GENES CONVINCINGLY ASSOCIATED WITH THE DISEASE OR CONDITION, CONVERTING SUCH GENES TO VERSIONS PREVALENT IN THE POPULATION AND HAVE NO KNOWN ADVERSE EFFECTS.

• AVAILABILITY OF CREDIBLE PRE-CLINICAL OR CLINICAL DATA

• RIGOROUS OVERSIGHT AND LONG TERM, MULTIGENERATIONAL FOLLOWUP

• MAXIMUM TRANSPARENCY CONSISTENT WITH PATIENT PRIVACY

• CONTINUAL ASSESSMENT OF HEALTH AND SAFETY WITH BROAD PUBLIC INPUT

• RELIABLE OVERSIGHT TO PREVENT UNAPPROVED USE
SUMMARY

Our purpose in life is to help others. And, if you can't help them, at least don't hurt them.

The Dalai Lama

Wisdom is the daughter of experience.

Leonardo Da Vinci

Real knowledge is to know the extent of one's ignorance.

Confucius

Be not wise in thine own eyes: fear the Lord and depart from evil. It shall be health to thy navel, and marrow to thy bones.

Proverbs 3:7-8

Sufficient for us is Allah, and He is the best disposer of affairs.

Quran 3:173