Welcome to

*Experts in Egg Health: New Advances in Fertility*

**Arthur O. Tzianabos, Ph.D.**

*President and Chief Scientific Officer, OvaScience*
Forward-Looking Statements

Various statements we make in this presentation concerning our future expectations, plans and prospects, including, without limitation, statements about our plans for the AUGMENT treatment and our two fertility treatments in development, as well as statements about our planned introduction of the OvaPrime treatment and expected international expansion, are forward-looking statements. Actual results may differ materially from those indicated by these forward-looking statements as a result of various important factors, including those risks more fully discussed in the “Risk Factors” section of our most recently filed Quarterly Report on Form 10-Q and/or Annual Report on Form 10-K on file with the SEC at sec.gov. In addition, any forward-looking statements represent our views only as of today and should not be relied upon as representing our views as of any subsequent date. We do not assume any obligation to update any forward-looking statement.
## Agenda

<table>
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<th>Topic</th>
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| Welcome and Introduction                                            | Arthur Tzianabos, Ph.D.  
*President and CSO, OvaScience*                                           |
| Innovation in IVF                                                    | Elizabeth Carr  
*First U.S. IVF Baby Born*                                                               |
| Isolation and Characterisation of Ovarian Germline Stem Cells (Egg Precursor Cells) | Prof. Evelyn Telfer, Ph.D.  
*Chair, Reproductive Biology  
University of Edinburgh*                                                               |
| Role of Mitochondria in Reproduction                                | Prof. Yoshiharu Morimoto, M.D., Ph.D.  
*CEO and Chairman, IVF Japan*                                                             |
| The AUGMENT\(^{SM}\) Treatment in Clinical Practice                 | Gabriel Cohn, M.D.  
*VP of Medical Affairs, OvaScience*                                                        |
| The AUGMENT\(^{SM}\) Treatment Using Egg Allocation Approach         | Arthur Tzianabos, Ph.D.  
*President and CSO, OvaScience*                                                            |
| Overview of the OvaPrime\(^{SM}\) Treatment                         | Robert F. Casper, M.D., F.R.C.S.(C)  
*Medical Director,  
TCART Fertility Partners*                                                              |
| Concluding Remarks                                                   | Arthur Tzianabos, Ph.D.  
*President and CSO, OvaScience*                                                            |
Innovation in IVF
Isolation and Characterisation of Putative Ovarian Germline Stem Cells

Prof. Evelyn E Telfer
Institute of Cell Biology
and
Centre for Integrative Physiology
University of Edinburgh
Store of “resting” primordial follicles utilised throughout reproductive life

Decreasing Numbers with Development

Increasing Levels of Apoptosis

Does the ovary have any capacity for regeneration?
2004: Are germline stem cells present in the post-natal ovary?

Germline stem cells and follicular renewal in the postnatal mammalian ovary

Joshua Johnson*, Jacqueline Canning*, Tomoko Kaneko, James K. Pru & Jonathan L. Tilly

Vincent Center for Reproductive Biology, Vincent Obstetrics and Gynecology Service, Massachusetts General Hospital, and Department of Obstetrics, Gynecology and Reproductive Biology, Harvard Medical School, Boston, Massachusetts 02114, USA
Fast developing field of adult stem cells

It is likely that the ovary has stem cells for all ovarian cell components
Publications emerge to support existence of germline stem cells in the ovary

Production of offspring from a germline stem cell line derived from neonatal ovaries
Nature Cell Biology 11, 361 Zou et al 2009 (Ji Wu’s lab)

82% of recipients injected with isolated putative germline stem cells produced offspring
29/108 offspring GFP+ve

Paper criticised for methodology (lack of purity and use of DDX4 to separate cells by MACs)
Oocyte formation by mitotically active germ cells purified from ovaries of reproductive-age women

Yvonne A R White, Dori C Woods, Yasushi Takai, Osamu Ishihara, Hiroyuki Seki & Jonathan L Tilly

Affiliations | Contributions | Corresponding author

Received 26 August 2011 | Accepted 11 January 2011 | Published online 26 February 2012
Putative human oogonial stem cells (OSCs) isolated from frozen-thawed ovarian tissue from adult women

White et al, 2012
Oocyte formation by mitotically-active germ cells purified from ovaries of reproductive-age women
[Nature Medicine; 18; 412-421]
Fluorescent Activated Cell Sorting (FACS) approach

DDX4:BrdU double staining human cultured OSCs:
DDX4=germ cell marker  BrdU=incorporates into proliferating cells
Putative human OSCs form oocyte-like structures

Isolation of OSCs based on DDX4 protein being extracellular

From Telfer & Albertini 2012
Testing the potential of putative germline stem cells (OSCs) isolated from human ovaries
In vitro growth (IVG) of human oocytes/follicles

- Microcortex before culture
- Cultured microcortex
- Follicles before isolation
- Isolated follicles

Telfer et al., 2008
Antral development from in vitro grown human primordial follicles within 10 days

Telfer et al., 2008: A two step serum free culture system supports development of human oocytes from primordial follicles in the presence of activin. *Human Reproduction* 23: 1151-1158
Third step in human follicle culture

*In vitro* grown follicles (after 2 steps)  Remove oocyte and surrounding cells  Step 3: Culture on membranes

McLaughlin, Anderson, Wallace, Albertini & Telfer (unpublished)
Testing human putative OSCs

Can human ovarian tissue support the formation of follicles from putative OSCs in vitro?
Combining putative human OSCs with a multi-step human ovarian culture system that supports oocyte development

- Step one: Isolated GFP-marked OSCs
- Step two: Oocyte development
- Step three: IVM of isolated oocyte-cumulus complexes and subsequent fertilisation
Injection of GFP labelled human OSCs into micro-cortex

Do these cells form new follicles during culture?
Within 7 days *in vitro* observe larger GFP +ve structures

Fluorescent immunostaining for GFP
(scale bar = 50 microns)
GFP-positive cells in different states

Type I

Type II

Type III

Type IV

Scale bar 25 µm
Only oocyte-like structures are GFP positive

Positive and negative GFP “oocyte-like structures” in same sections - an indication that the injected cells are forming these structures and recruiting somatic cells
Immunohistochemistry of injected cultured tissue after 6 days

GFP +ve “oocytes” and non GFP oocytes in same section
Total number of non-GFP and GFP positive follicles in human ovarian cortical fragments

7 days *in vitro*

New oocytes (green)
Endogenous oocytes (black)
Human OSCs recruit somatic cells

Immunostaining for BrdU (green)

Labelled cells after BrdU added day 1 and 4
Shows some somatic cell proliferation
Human putative OSCs can form follicles within 6 days and these grow and form multilaminar structures within 10-14 days.
Summary

• Cells capable of recruiting somatic cells and forming oocyte-like/follicle structures can be isolated from human ovaries in the Tilly lab.
• Need to independently isolate cells: How reproducible is this?
Isolation of putative OSCs from human ovarian tissue using FACS based sorting in our lab

Marie McLaughlin, Yvonne Clarkson, Paul Skehel, Martin Waterfall, Cheryl Dunlop, Hamish Wallace, Richard Anderson and Evelyn Telfer
Testing cells’ ability to form oocytes and testing function of oocytes, i.e., entry into meiosis
FACS analysis of human tissue using a refined isolation and purification protocol

McLaughlin, Clarkson, Waterfall, Dunlop, Skehel, Anderson, Telfer (unpublished)
DDX4 cells can be observed in human ovaries
DDX4 cells can be observed in human ovaries
DDX4 protein is present in FACS sorted positive cells but not in negative cells

McLaughlin, Clarkson, Waterfall, Dunlop, Skehel, Anderson, Telfer (unpublished)
PCR analysis of human FACS sorted cells

- **a)** Positive control
- **b)** DDX4 positive cells s2
- **c)** DDX4 positive cells s1
- **d)** DDX4 positive cells s3
- **e)** DDX4 negative cells
- **f)** Negative control

**Markers:**

- **GERMLINE MARKERS**
  - DDX4 5’
  - DDX4 3’
  - KIT
  - DAZL
  - PRDM1
  - DPPA3
  - IFITM3

- **STEM CELL MARKERS**
  - NANOG
  - LIN28
  - POU5F1

- **FOLLICLE/OOCYTE MARKERS**
  - CYP19A1
  - HDAC6
  - ZP3

- **HOUSEKEEPING**
  - GAPDH
Testing the potential of putative OSCs isolated in our lab (bovine)

- Chimeric ovary experiments
- Combine putative OSCs (bovine) with murine somatic cells
- Transplant under kidney capsule of SCID mouse

PhD student Kelsey Grieve in collaboration with Williams lab Oxford
Chimeric ovary studies

Isolated somatic cells (mouse) transplanted under kidney capsule of SCID mouse (No follicles observed)
Chimeric ovary studies

Bovine putative OSCs combined with mouse somatic cells. Transplanted to SCID mouse. Oocyte/follicle structures formed.
**Functional oocyte development**

**Growth/meiotic arrest**

**Acquisition of meiotic competence**

**Acquisition of developmental competence**

**Transcription/transcriptional repression**

**Genomic imprinting**
Summary

• Building gametes is **COMPLEX** having much to do with gonadal somatic cells as it does with the germline progenitors!

• Stem cells can make germ cells in mice **IF** the somatic environment is synchronised with germ cell state.

• We are isolating and characterising a population of ovarian stem cells that seem capable of making follicles *in vitro*.

• We are now testing somatic cell support for our human cells.
What does this mean?

• The menopause happens!
• But cells with germ and stem cell markers can be isolated from ovaries of women and prepubertal girls
• Useful model of human germ cell development/maturation
• Potential clinical applications: Fertility preservation/restoration. *In vivo* and *in vitro*
Can new oocytes be formed \textit{in vivo}?
The future

• The field of germline stem cells is new
• Concentration of effort has been on doubting their existence
• OSCs have now been identified in a range of species (bats, cows, pigs, rats, rhesus monkey, Lemur)
• Time to concentrate on defining the populations of stem cells that reside within the mature ovary
• Applying cell-based strategies to infertility treatment and fertility preservation
Acknowledgements

Marie McLaughlin
Yvonne Clarkson
Cheryl Dunlop
Kelsey Grieve
Paul Skehel
Martin Waterfall
John Binnie
Joan Creiger

Richard Anderson
Hamish Wallace
David Albertini (IVG work)
Jon Tilly (Boston)
Suzanne Williams (Oxford)

All the patients who kindly donate their ovarian tissue and the clinical teams that support this
Role of Mitochondria in Reproduction

CEO, IVF JAPAN
Yoshiharu Morimoto
Infertility = Mitochondrial-related insufficiency

New Concept

Asthenozoospermia

Cleavage arrest

Low energy production
Low energy utilization

Implantation disorder

Chromosome abnormality
Mitochondria

Cellular Power Plant
- Produce the energy currency
- Heat production
- Storage of calcium
- Regulation of the membrane potential
- Steroid synthesis

Wikipedia
Mitochondrial network

Human fibroblast
Immunofluorescence staining

5 Fusion & Fission cycles / hr
Autophagy for quality maintenance
(Twig 2008)
Mitochondrial migration during oocyte maturation (human)
Mitochondrial potential

GV

MI

MII
Critical threshold of mtDNA copy number

Subthreshold (50,000) oocyte: abortion
E6.0 mDNA replication resumes

Wai et al. Biol Reprod, 2010
A normal oocyte has ~100,000–200,000 mitochondria.

If the egg is deficient in functional mitochondria, it may not provide the energy necessary for fertilization and embryogenesis.

There is evidence that poor oocyte quality, caused by aging and other factors, is due to a deficiency in the number of functioning mitochondria.

Liver
Muscle
Blood Cells
Cumulus cells
ESC/ iPS/ Oogonial SC
Mitochondrial transfer
Allograft (Donation)
Transferred mitochondrial migration and microfilament

Control

Cytochalasin B

Central

Periphery

Porcine

Yamochi et al. Zygote in press
## The benefit of mitochondrial supplementation to egg quality (animal)

<table>
<thead>
<tr>
<th>Species</th>
<th>Cytoplasmic (Cyto) or Mitochondrial (Mito) Transfer</th>
<th>Safe for Oocyte</th>
<th>Increased Fertilization Rate</th>
<th>Viable Blastocyst</th>
<th>Healthy Live Births</th>
<th>Reference</th>
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<td>Partheno</td>
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In Multiple Animal Species, the Addition of Mitochondria to Eggs Improves Egg Quality and Embryogenesis
Mitochondria transfer (porcine)

Materials: Porcine oocytes
Methods:
BCB (Brilliant cresyl blue)
Indicator: G6PD (Glucose-6-phosphate dehydrogenase)
Mt transfer  BCB+oocyte  to BCB−oocyte
139200 copy number

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<tr>
<th>Treatment</th>
<th>IVF Fertilization rate(%)</th>
<th>ICSI Fertilization rate</th>
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<tr>
<td>BCB+</td>
<td>37.5</td>
<td>40.4</td>
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<tr>
<td>BCB−</td>
<td>17.6</td>
<td>19.8</td>
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<tr>
<td>BCB− MT</td>
<td>31.0</td>
<td>34.0</td>
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<tr>
<td>BCB− sham injection</td>
<td>17.0</td>
<td>10.0</td>
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</tbody>
</table>

Shourbagy et al., Reproduction 2006
Mitochondrial transfer between oocytes: potential applications of mitochondrial donation and the issue of heteroplasmy.

Jonathan Van Blerkom et al., Hum Reprod 13, 2857-2868, 1998

Oocyte to oocyte transplant
Production of mitochondria-rich cytoplast

Viable mitochondria
ATP production increased
The benefit of mitochondrial supplementation to oocyte quality (human)

<table>
<thead>
<tr>
<th>Cytoplasmic/Mitochondria Studies</th>
<th>No. of Cycles</th>
<th>Pregnancies</th>
<th>Rate</th>
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<tr>
<td>Huang¹</td>
<td>9</td>
<td>4</td>
<td>44%</td>
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<tr>
<td>Cohen²*</td>
<td>30</td>
<td>13</td>
<td>43%</td>
</tr>
<tr>
<td>Lanzendorf³*</td>
<td>4</td>
<td>1</td>
<td>25%</td>
</tr>
<tr>
<td>Tzeng⁴</td>
<td>71</td>
<td>25</td>
<td>35%</td>
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</table>

Women Who Failed 2 or More IVF Cycles (IVF Success = 0)

- The delivery of ~15–20% of the total mitochondria from the normal egg of a younger donor resulted in an increase in the rate of healthy live births.
- The birth of about 50 babies
- Tzeng : Autologous granulosa cell

* Studies conducted in the U.S.
Mitochondrial activities are critical for oocyte maturation, fertilization, embryogenesis and implantation.

Mitochondria migrate actively in ooplasm during oocyte maturation.

Microfilaments make an important role for the mechanism of mitochondrial migration.

Low mitochondrial function in oocytes cause poor oogenesis, failure of fertilization, embryogenesis and implantation failure.

Mitochondrial supplementation of oocytes across several species has been shown to improve oocyte quality and to increase IVF success rates.
Thank you for your kind attention.
The AUGMENT\textsuperscript{SM} Treatment is Not Available in the United States
The AUGMENT™ Treatment

Ovary

Ovarian Cortex

Isolated EggPC cells

EggPC™ cells

Mitochondria extracted from woman’s own EggPC cells

EggPC-derived mitochondria injected into woman’s own egg during ICSI*

*ICSI = Intracytoplasmic Sperm Injection
Approaches to Evaluating New Fertility Technologies

Control Group

**Historical**
- Case Control
  - IVF vs. Different Patient Group using AUGMENT

**Prospective**
- RCT
  - IVF vs. Different Patient Group using AUGMENT

Patient as Own Control

**Historical Comparison**
- Patient IVF History vs. IVF + AUGMENT

**Egg Allocation**
- IVF vs. IVF + AUGMENT
Fakih et al: An Observational Report
The AUGMENT℠ Treatment Initial Global Patient Experience

**Prospectively undertaken; retrospective sub-group analysis**
No clinical trials of the AUGMENT treatment were undertaken
Patient as Own Control: Patient IVF History vs AUGMENT℠ Treatment

- Evaluating AUGMENT treatment outcomes in two IVF clinics
  - TCART Fertility Partners, Canada (n = 34 patients)
  - Fakih IVF, United Arab Emirates (n = 59 patients)
  - Late 2014 through mid 2015

- Center-specific analyses
  - Current age
  - Historical vs. AUGMENT treatment
    - Prior number of IVF cycles vs. AUGMENT cycles
    - Clinical pregnancy rate and ongoing clinical pregnancy rate/live birth rate
  - Descriptive analyses
    - Clinical pregnancy rate (determined by transvaginal ultrasound to detect gestational sac, fetal heartbeat approx. 3-4 weeks post-transfer)
    - Ongoing clinical pregnancy rate/live birth rate (at least one documented fetal heartbeat and at least 12 weeks of gestation)

Patient as Own Control: Patient IVF History vs AUGMENT SM Treatment

Canada

- Patient IVF History: Live birth rate per initiated cycle (without AUGMENT)
- AUGMENT Treatment: Ongoing clinical pregnancy rate/live birth rate per 1 AUGMENT cycle initiated

- (n=34)
- Average age: 36.0
- 1-5 prior IVF cycles
- 71 total prior IVF cycles

UAE

- (n=59)
- Average age: 37.3
- 1-16 prior IVF cycles
- 257 total prior IVF cycles


Note: Physician-reported patient experience

Clinical pregnancy determined by transvaginal ultrasound to detect gestational sac, fetal heartbeat approx. 3-4 weeks post-transfer
Ongoing clinical pregnancy rate/live birth rate defined as at least one documented fetal heartbeat and at least 12 weeks of gestation
No factors controlled for in this analysis
Patient as Own Control: Patient IVF History vs AUGMENT℠ Treatment

- AUGMENT℠ treatment showed an increase in ongoing clinical pregnancy rates vs. historic live birth rate

- Patient experience consistent across
  - Distinct centers
  - Distinct patient populations
The AUGMENT℠ Treatment
Using Egg Allocation Approach

Arthur O. Tzianabos, Ph.D.
President and Chief Scientific Officer, OvaScience

The AUGMENT℠ Treatment is Not Available in the United States
The AUGMENTSM Treatment: Physician Reported Outcomes of the Initial Global Patient Experience

Michael H Fakih1*, Mohamad El Shmoury1, Julia Szeptycki2, Dennis B dela Cruz2, Caroline Lux2, Suleman Verjee3, Colleen M Burgess4, Gabriel M Cohn4 and Robert F Casper2*

1Fakih IVF, Dubai, UAE
2TCART Fertility Partners, Toronto, ON M5S 2X9, Canada
3Versante International, LLC Oakland, CA 94606, USA
4OvaScience Cambridge, MA 02421, USA
Patient as Own Control: Egg Allocation Overview at Fakih IVF

- **Egg Retrieval**
  - Same patient, cycle & laboratory conditions

- **Egg Allocation to Two Treatment Groups**

  - **Standard IVF Treated Eggs**

  - **AUGMENT Treated Eggs**

  - **Identical Laboratory and Culture Procedures**

  - **Pre-Implantation Genetic Screening to Select Embryos**
    - (e.g., aneuploidy)

  - **Standard Assessment of Morphology to Select Embryos**
    - (e.g. size, shape, symmetry, fragmentation)

  - **Embryo Selection and Transfer from Either IVF Alone or AUGMENT Treatment Group**
    - (based on highest quality embryos)
Higher Rates of Selection from the AUGMENT\textsuperscript{SM} Treated Group Based on Genetics and Morphology

25 Women with Eggs Allocated Between Both Treatment Groups

<table>
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<tr>
<th>Standard IVF Treated Eggs</th>
<th>AUGMENT Treated Eggs</th>
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<tr>
<td>Aneuploidy Embryos</td>
<td>- 9 patients across both groups</td>
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<td>16 patients</td>
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</table>

Basis for Treatment Group Selection

- Genetic Selection
  - 2 patients
  - 9 patients

- Morphokinetic Selection
  - 0 patients
  - 5 patients

Embryo Transfers

- 2 patients
- 14 patients

Ongoing Clinical Pregnancies

- 0 patients
- 8* patients

*4 each from the morphokinetic and genetic selection groups
The AUGMENT℠ Treatment: A New Innovation in Assisted Reproductive Technologies

- Higher embryo selection rates and pregnancy rates observed in this retrospective ITT analysis
  - Primarily driven by preimplantation genetic testing
  - Marked improvement over patients’ historic IVF performance

- May help address the unmet need for women

- Egg allocation may offer a controlled, real-world clinical and laboratory alternative to randomized, controlled trials
Overview of the OvaPrime℠ Treatment

Robert F Casper MD, FRCS(C)

University of Toronto and TCART Fertility Partners
OvaPrime Overview

- OvaPrime treatment is designed to enhance ovarian reserve
- Utilizes a woman’s own egg precursor (EggPC) cells to promote the development of new follicles
- EggPC cells have the potential to develop into new follicles and mature eggs when repositioned within the follicular development zone (cortex)
OvaPrime Overview

- Offers potential new option for:
  - Women with diminished ovarian reserve (DOR) or premature ovarian insufficiency (POI or POF)
  - Older women with increasing risk of aneuploidy in oocytes
  - Women for whom a donor egg or adoption only option
## Pre-Clinical Studies in Support of OvaPrime

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Outcome</th>
<th>Support for OvaPrime</th>
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<tbody>
<tr>
<td>Zou et al., 2009</td>
<td><strong>Mouse:</strong> Cultured GFP*-labeled EggPC cell line</td>
<td>Transplanted EggPCs matured into oocytes</td>
<td>Healthy live birth from EggPCs</td>
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<td>Transplanted into ovaries of chemotherapy-treated mice (infertile)</td>
<td>Fertilized after natural mating</td>
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<td>Natural mating post-transplant (No IVF)</td>
<td>Offspring expressing GFP</td>
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<td>75-110 days post-transplantation to produce EggPC derived offspring</td>
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<tr>
<td>Zhang et al., 2011</td>
<td><strong>Mouse:</strong> Cultured GFP*-labeled EggPC cell lines</td>
<td>Transplanted EggPCs matured into oocytes</td>
<td>Healthy live birth from EggPCs</td>
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<td>Transplanted into ovaries of chemotherapy-treated mice (infertile)</td>
<td>Fertilized producing heterozygous offspring after mating with wild-type male</td>
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<td>Natural mating post-transplant (No IVF)</td>
<td>2 months post-transplantation to produce EggPC derived offspring</td>
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<tr>
<td>White et al., 2012</td>
<td><strong>Mouse:</strong> Cultured GFP*-labeled EggPC cell line</td>
<td>Transplanted EggPCs matured into oocytes</td>
<td>Mouse: Mature and fertilizable EggPC-derived oocyte. F1 and F2 generation to be published</td>
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<tr>
<td></td>
<td>Transplanted into ovaries of untreated wild type mice (fertile)</td>
<td>Ovulated after endogenous gonadotrophins treatment between 7-8 months post transplantation.</td>
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<td>IVF used</td>
<td>Ovulated eggs fertilized in vitro, resulting in 34% (8/23) EggPCs derived embryos from 5 mice</td>
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<td>Each mouse releasing at least one EggPC derived oocyte at ovulation</td>
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<tr>
<td>Zhou et al., 2014</td>
<td><strong>Rat:</strong> Cultured GFP*-labeled EggPC cell line</td>
<td>Transplanted EggPCs matured into oocytes and fertilized producing heterozygous offspring after mating with wild-type male</td>
<td>Healthy live birth from EggPCs</td>
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<tr>
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<td>Natural mating post-transplant (No IVF)</td>
<td>GFP gene expression found in F2 generation</td>
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## Pre-Clinical Studies in Support of OvaPrime

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Outcome</th>
<th>Support for OvaPrime</th>
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</thead>
</table>
| White et al., 2012 | • **Human:**  
  - Cultured GFP*-labeled EggPC cell line transplanted into human ovarian cortical strips, xeno-grafted subcutaneously into immune compromised mice  
  - No hormonal stimulation | • Xeno-transplanted EggPCs matured into oocytes at 7-14 days post grafting  
  • Result: 15-21 GFP-positive oocytes per graft | • **Human:** Follicles were formed containing GFP-positive EggPC derived oocytes |
| Wolff et al., 2014 | • **Non-human primate (Rhesus macaque):**  
  - Cultured GFP*-labeled EggPC cell line transplanted into ovaries of two untreated recipients (autologous)  
  - IVF performed (No natural mating) | • Transplanted EggPCs matured into oocytes which were ovulated after endogenous gonadotrophins  
  • Resulting in ~12% (7/60) EggPCs derived oocytes-GFP oocytes were isolated from both animals  
  • 1/17 EggPC derived oocytes aspirated from follicles in vivo; 6/43 EggPC derived oocytes from frozen dissected cortex | • Mature EggPC-derived oocyte in a non-human primate |
| OVAS (GLP level feasibility study with CRO) | • **Non-human primate (Cynomolgus):**  
  - Isolated, non-labeled, primary (non-cultured) EggPCs from one ovary transplanted to contralateral ovary  
  - No mating studies performed | • Normal histological and clinical observations after 6 months  
  • Evaluation of ovaries, reproductive organs, and full anatomical assessment | • Repositioning of EggPCs (as much as tens of thousands) is safe in a non-human primate |
GFP Transfected EggPC Cells Injected into Human Ovarian Cortex

1 week post injection

White et al.
Nature Medicine, 2012
GFP Transfected EggPC Cells Injected into Human Ovarian Cortex

2 weeks post injection

GFP +ve

GFP -ve

White et al.
Nature Medicine, 2012
Summary of Preclinical Studies in Support of the OvaPrime Treatment

- Egg precursor cells can develop in the ovary into mature eggs capable of spontaneous ovulation and fertilization.
- Pregnancy and delivery of live offspring can result.
- This process is different from *in vitro* activation of existing primordial follicles (Stanford protocol).
In Vitro Activation

- Ovarian Hippo signaling inhibits follicle growth
- Fragmentation of ovarian cortex by mincing disrupts Hippo signaling
- PTEN inhibitors and PI-3-K augment AKT signaling and promote the growth of pre-antral follicles
- New infertility treatment for patients with premature ovarian insufficiency

Hsueh et al, Endocrine Rev, Feb 2015
**In Vitro Activation**

- **Cryopreservation**
- Preparation of ovarian strips for vitrification and histology
- Remove ovaries
- **POI patients**

- (Hippo Signaling Disruption)
- Fragmentation of ovarian strips to cubes
- Culture of ovarian cubes
- Return activated ovarian cubes beneath serosa of Fallopian tubes

- (Akt stimulators)
- Retrieve mature eggs
- In vitro Fertilization with husbands’ sperm
- Embryos
- Embryo transfer
In Vitro Activation

Kawamura et al. PNAS 2013
In Vitro Activation
**Folliculogenesis**

**Pre-natal**

- **Follicle formation**

**Post-pubertal**

- **Cyclic follicle recruitment, activation, growth or atresia**

- **12+ months**

**PGCs Migrate to Ovary and Organize Into Quiescent Primordial Follicles**

- Recruitment → Activation
- AMH Produced
- Ovulation

- Maturing oocyte
- Growing recruited follicle

**Advantages of OvaPrime: Potential for Reduced Development Time**

- PGCs = primordial germ cells
- AMH = anti-müllerian hormone
- Atresia = Hormonally controlled apoptosis of (non-dominant) follicles
OvaPrime Clinical Evaluation

- Patients with non-detectable AMH and/or elevated FSH
- Laparoscopy and ovarian surface biopsy
- Freeze tissue and allow patient to recover from surgery
- Thaw tissue and isolate egg precursor cells
OvaPrime Clinical Evaluation

- Laparoscopy to inject cells into the ovarian cortex
- Injection of egg precursor cells into one ovary (treatment) and injection of carrier into other ovary (control)
- Follow with serial AMH, FSH and Estradiol levels, and ultrasounds for basal antral follicles (BAF)
- IVF when 5 to 10 BAF observed, followed by PGS
Conclusions

- Potential Benefits of OvaPrime Treatment
  - Utilizes a woman’s own egg precursor cells
  - May provide a way to re-initiate follicular development and restoration of ovarian reserve in DOR/POI patients
  - Offers potential advantages over IVA
    - No pharmacologic or molecular inhibitors (e.g., PTEN)
    - Egg precursor cells are re-introduced into the site of normal follicular development
    - May allow natural spontaneous conception without the need for IVF
Concluding Remarks

Arthur O. Tzianabos, Ph.D.
President and Chief Scientific Officer
Egg Precursor (EggPC<sup>SM</sup>) Cells

- The debate has been going on for decades
- Makes sense from an evolutionary perspective that women have precursor cells just as men do (spermatogonial)
- Paradigm shifts in science and medicine take time and innovation is always met with skepticism – IVF itself saw this kind of resistance
- Methods and reagents DO matter
Role of Mitochondria

- Extensive evidence in humans and animals that mitochondrial insufficiency leads to poor reproductive outcomes

- Clinical experience to date suggests that supplementation with mitochondria may improve these outcomes

- Multiple studies in multiple species demonstrate that the supplementation of eggs with mitochondria can improve success rates and result in healthy live births

- Egg precursor (EggPC\textsuperscript{SM}) cells represent an autologous (egg derived from the same women) source of mitochondria
AUGMENT℠ Treatment Patient Experience

**Woman’s Own IVF History**
- IVF clinics demonstrated improvements in pregnancy rates above baseline in very poor prognosis women
- Consistent results across clinics despite differences in laboratory practice and patient backgrounds

**Egg Allocation: AUGMENT treatment vs. Standard IVF**
- Statistically significant higher embryo selection and transfer rates with AUGMENT treatment; higher embryo selection rate was primarily based on genetic screening (objective measure)
- Statistically significant higher ongoing clinical pregnancy rate with AUGMENT treatment
- Study had limitations

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Egg Allocation Represents a Rigorous Approach to Evaluate AUGMENT Benefit vs. IVF

- Limitations in future assessments of AUGMENT treatment can be addressed
- Potential to control for randomization and blinded embryo assessment

The AUGMENT treatment is not available in the United States
OvaPrime℠ Treatment Background

- Potential for a woman to use her own egg precursor (EggPC℠) cells in order to replenish ovarian reserve
- Designed for women who make few eggs or no eggs at all (25-30% of women seeking fertility treatment)
- Substantial preclinical studies support the concept in multiple species
- Clinical feasibility has been demonstrated in non-human primates
- Will be introduced in patients by the end of this year in at least one of our international clinics
OvaScience Remains Dedicated to Delivering New Fertility Treatments to Patients

- **AUGMENT<sup>SM</sup> Treatment**
  - Continues to demonstrate clinical impact
  - 14 babies born to date with ongoing clinical pregnancies expected to result in additional babies

- **OvaPrime<sup>SM</sup> Treatment**
  - Strong preclinical evidence
  - To be introduced via preceptorship by end of 2015 in at least one international region

- **OvaTure<sup>SM</sup> Treatment**
  - *In vitro* maturation of egg precursor (EggPC<sup>SM</sup>) cells into eggs
  - Goal is to define development plan by year end for introduction to humans
  - Progress being made by multiple research groups

OvaScience treatments are not available in the United States