



***In vitro** derived oocytes (IVDOs)*

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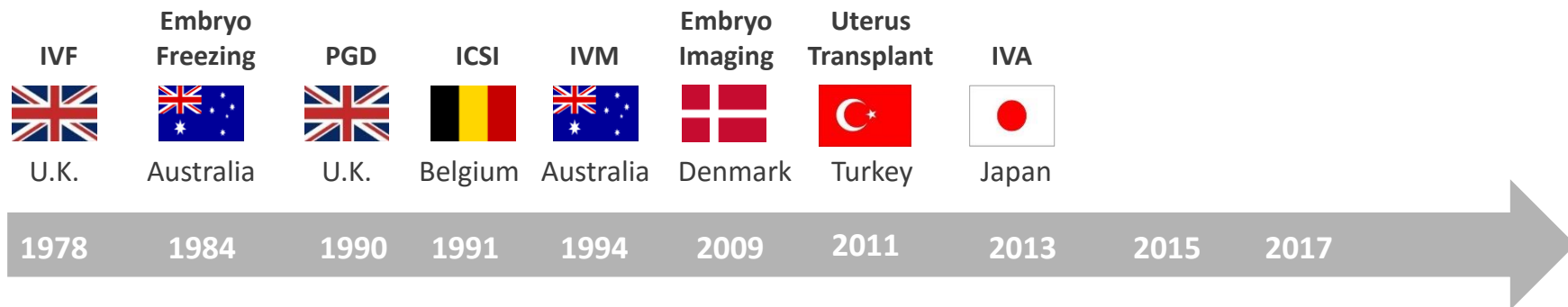
Determining maturation status of bovine and human *in vitro* derived oocytes from egg precursor cells by key morphological, genetic and metabolic indicators

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"This presentation provides an overview of current knowledge and research regarding *in vitro* derived oocytes (IVDOs). IVDOs, including egg precursor (EggPCSM) cells, are not currently available for clinical use in the United States. In addition, the OvaScience treatments described in this presentation (AUGMENTSM, OvaPrimeSM, and OvaTureSM), are not available in the United States."

Despite early innovation in IVF, significant unmet need remains



Term	Definition
IVF:	In Vitro Fertilization
PGD:	Pre-implantation Genetic Diagnosis
ICSI:	Intracytoplasmic Sperm Injection
IVM:	In Vitro Maturation
IVA:	In Vitro Activation

For IVF, Frozen Embryo, PGD, ICSI, and IVA: dates above indicate first baby born.

Recent additions to IVF treatment are largely complementary or incremental advances:

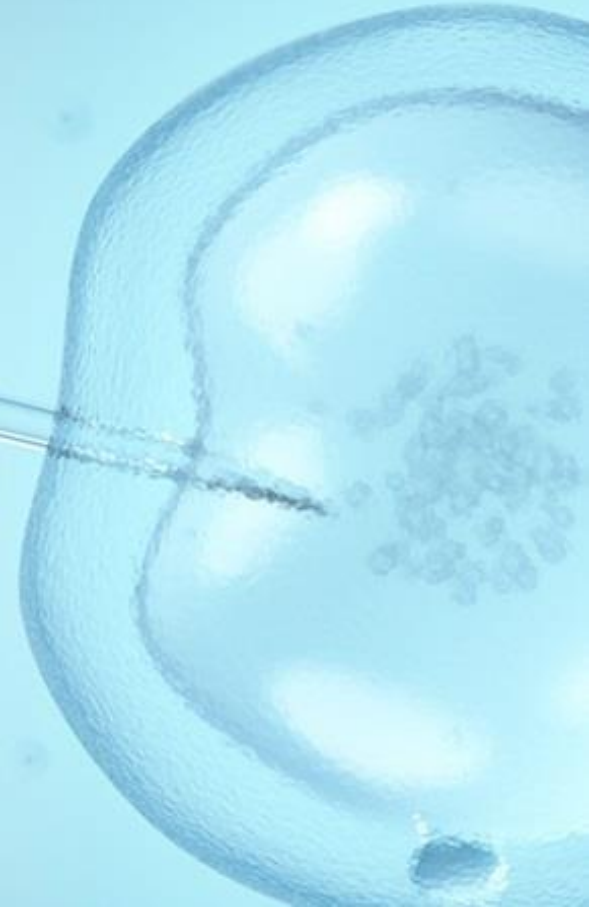
- Culture Media
- Egg quality/Embryo selection
- Fertility Apps
- Screening/monitoring/diagnostics
- Hormones
- Egg preservation/donor egg

- IVF success rates range from 20-40%, however there are a high number of patients that are unable to benefit from current technology:
 - Few or no eggs (i.e., POR/POI/post-chemotherapy)
 - Poor egg quality
 - Patient intolerant to controlled ovarian hyperstimulation
 - Alternative to donor egg (genetically related offspring)

- *In vitro* derived oocytes (IVDOs) may bridge the gap to these underserved populations



***In vitro* derived oocytes (IVDOs) and their possibilities**



- What are *IVDOs*?
 - **Definition:** female gametes generated *in vitro* from already existing cells (Smajdor and Cutas, 2015)

 - Where can they come from?
 - **Embryonic stem cells (ESCs)** (Thomson et al., 1998; Reubinoff et al., 2000)
 - Original source: inner cell mass of blastocyst, followed by differentiation
 - Development potential: pluripotent
 - **Induced pluripotent stem cells (iPSCs)** (Yang et al., 2012; Hayashi et al., 2012)
 - Original source: adult somatic cells; reprogrammed to induce pluripotentiality, followed by differentiation
 - Development potential: pluripotent
- **Egg Precursor (EggPCSM) Cells** (Johnson et al., 2004; Zou et al., 2009; White et al., 2012)
 - Original source: ovarian cortex, followed by maturation
 - Development potential: lineage-specific

Egg precursor cells exist in many species

- What are *Egg Precursor cells* (*EggPCSM cell*; *female germline stem cells*; *oogonial stem cells*)?
 - **Definition:** Hypothesized to be residual, undifferentiated germ cells from fetal development that have the capacity to mature into eggs (White et al., 2012)
- EggPC cells have been identified in many species (from flies to humans)
 - **Widely accepted** that that EggPC cells function in less evolved species, such as flies and teleost fish, due to reproductive demands/strategy (high egg production) (Woods and Tilly, 2013)
 - **Widely debated** whether female mammals can produce new eggs postnatally resulted from lack of data (Powell, 2007; Tilly et al., 2009)

(“absence of evidence is not evidence of absence”)

- **Growing body of data** supporting the identification of EggPC cells in large mammals including humans (White et al., 2012; Dunlop et al., 2013; Bai et al., 2013; Wolff et al., 2014)

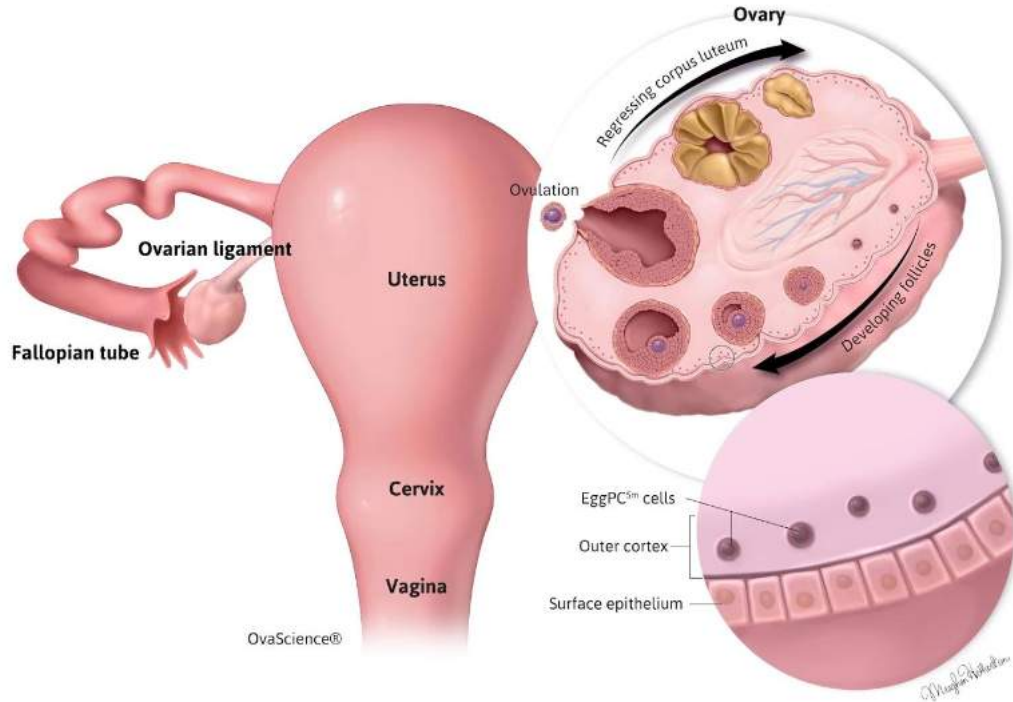
EggPCSM cells have been identified and characterized by top labs worldwide



- More than 50 peer reviewed publications from at least 17 top labs worldwide have identified and characterized EggPC cells
- Approximately ten primary peer reviewed publications have challenged the existence of EggPC cells in mammals potentially due to variations in evaluation methodologies (characterization techniques and cell isolation approaches)



Where are human EggPCSM cells located?



- Human EggPC cells were initially identified/characterized by Jonathan Tilly's laboratory at Harvard Medical School, MGH (White et al., 2012)
- Human EggPC cells are believed to be largely dormant *in situ*
- When isolated and cultured they become metabolically active and can form oocytes (White et al., 2012)

Human EggPC cells may potentially have application in the fertility field

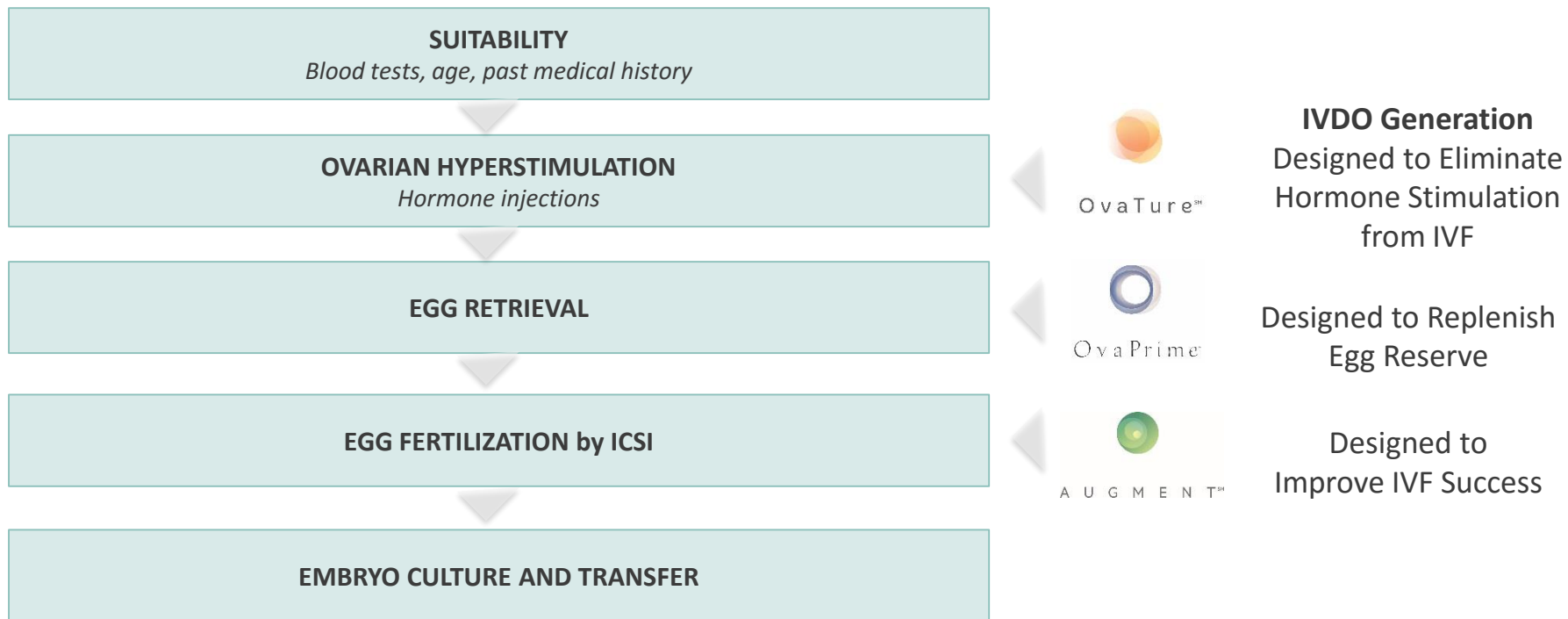
OvaScience founded based on discovery of EggPCSM cell platform



“When we started the company about five years ago, we had three main goals. The first was to come up with a fertility treatment that was safer for the mother (eliminate hormone) and baby (reduce rate of aneuploidy). The second was to improve IVF success rates, and the third was to make IVF more efficient. We looked for different technologies and came across a groundbreaking technology that was discovered at Harvard Medical School. It’s the discovery of egg precursor cells ...”

- In 2011, OvaScience was formed to translate the groundbreaking scientific discovery of EggPC cells into potential new treatments for female infertility
- The company identified and licensed its core technology from Harvard Medical School and Massachusetts General Hospital, and began to work on development of OvaTureSM treatment, a next generation treatment that could help a woman produce healthy, young fertilizable eggs outside of the body (and thus without the need for hormones)
- Two additional treatments, OvaPrimeSM treatment and AUGMENTSM treatment, have since been added to the company’s portfolio

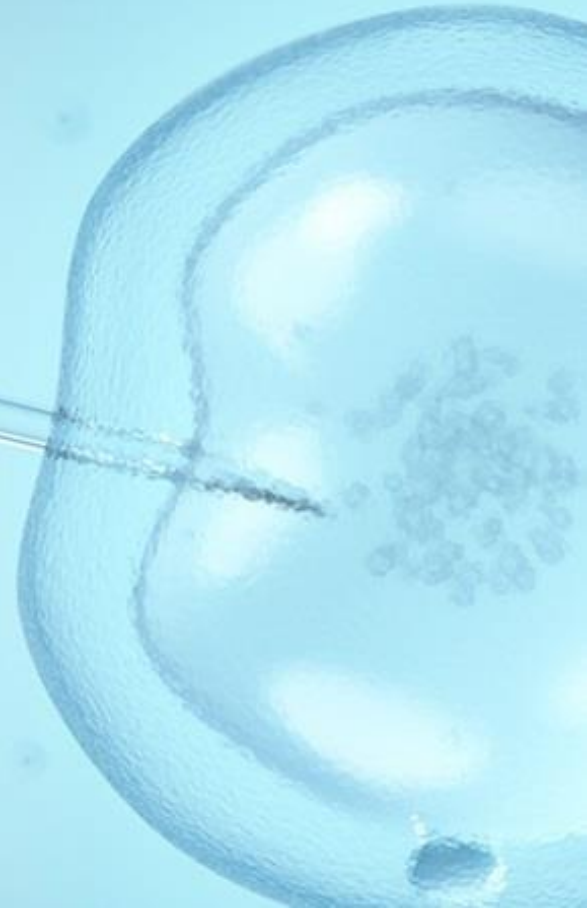
Treatments range from improving to transforming IVF



¹ AUGMENT available in select international regions/not available in the US.



OvaTureSM Treatment Concept



OvaTureSM treatment: OvaScience's approach to IVDOs using EggPCSM cells

What it is:

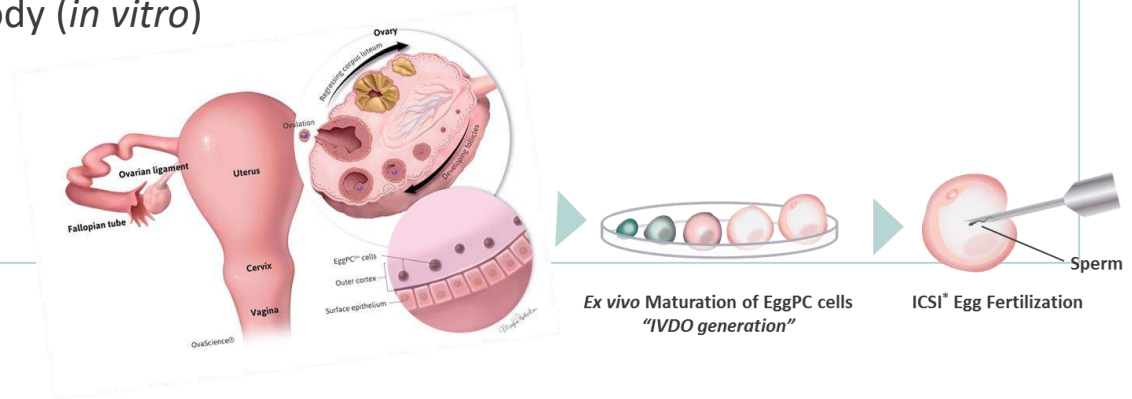
- Potential next generation treatment that could help a woman produce healthy, young fertilizable eggs without hormone stimulation injections

How it is designed to work:

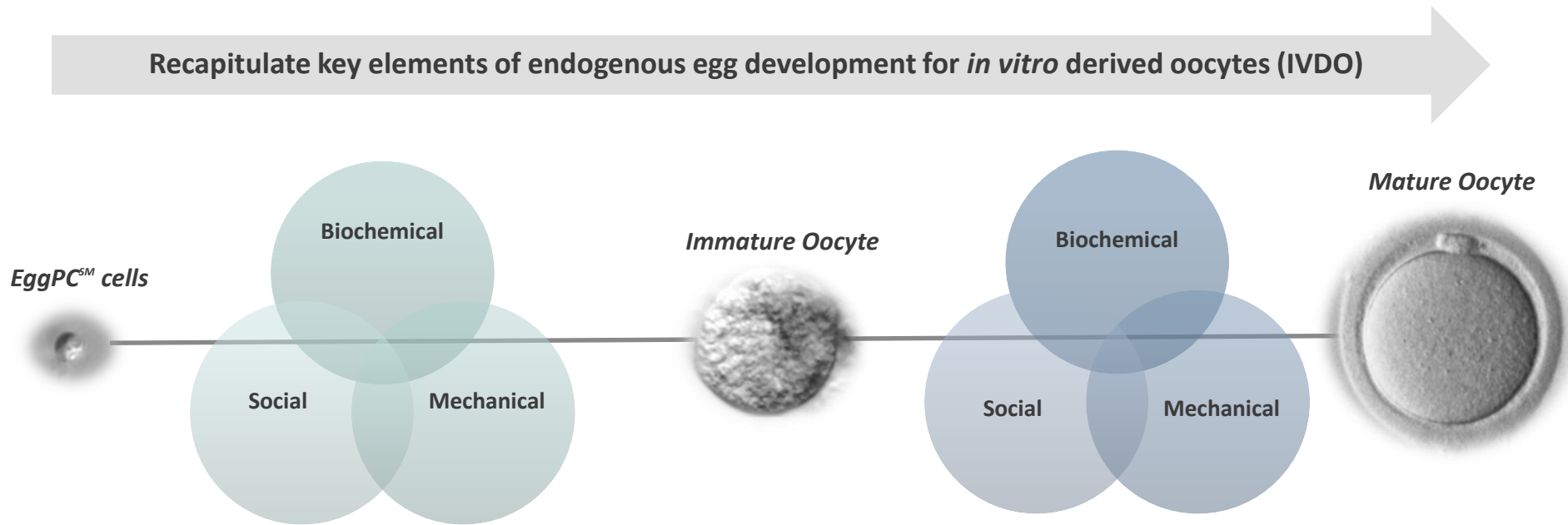
- We hypothesize that a woman's own EggPC cells can mature into healthy, young fertilizable eggs outside the body (*in vitro*)

Status:

- In pre-clinical development

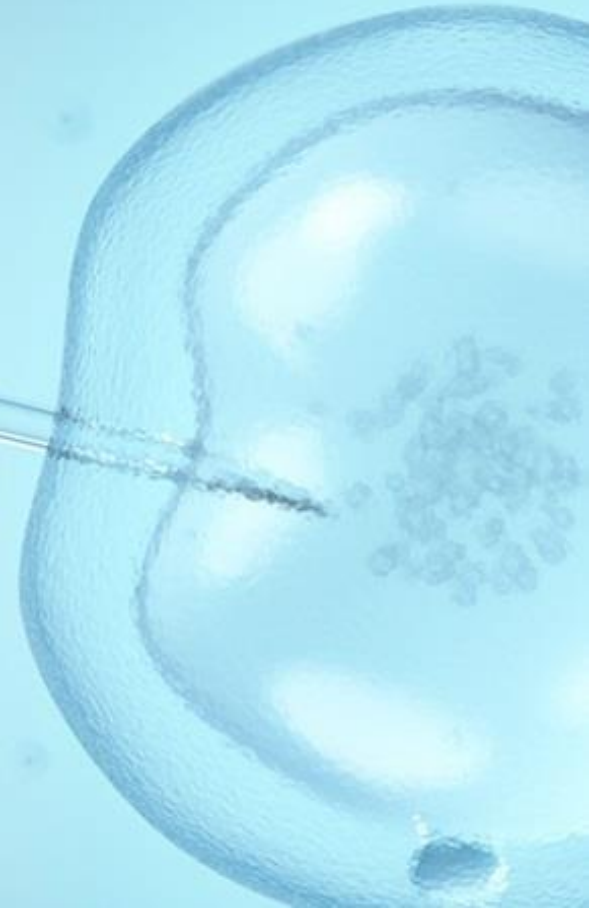


OvaTureSM treatment culture process development (current approach)





**OvaTureSM treatment IVDO
characterization/evidence of developmental
competence**



OvaScience's innovative technology: Progress with OvaTureSM treatment



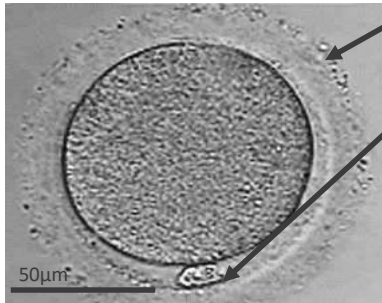
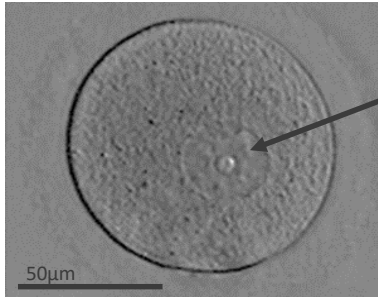
- The OvaTure Treatment
 - **Status:** pre-clinical development
 - **Progress:** routinely generates maturing IVDOs

- Success criteria of the IVDOs are focused on oocyte characterization and acquisition of developmental competence
 - Characterization based on morphological and genetic hallmarks
 - Developmental competence criteria include cytoplasmic and nuclear maturation

OvaTureSM treatment derived IVDOs present with key oocyte characteristics

Nuclear maturation: OvaTure IVDOs presenting with distinct GV and PB structures

Endogenous oocytes (controls)

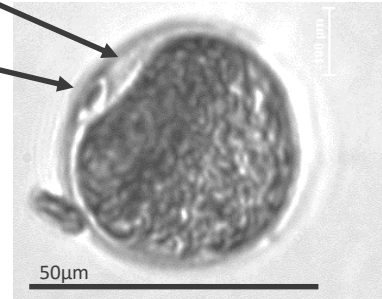
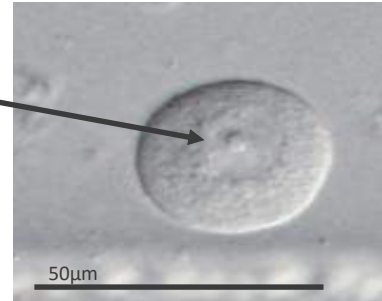


Germinal Vesicle (GV)
enlarged nucleus of an oocyte before meiotic division is completed.

Zona Pellucida (ZP)
Thickened outer membrane

Polar Body (PB)
one of the small cells produced during the two meiotic divisions of developing oocytes

OvaTure IVDOs



Approximate incidence of identification

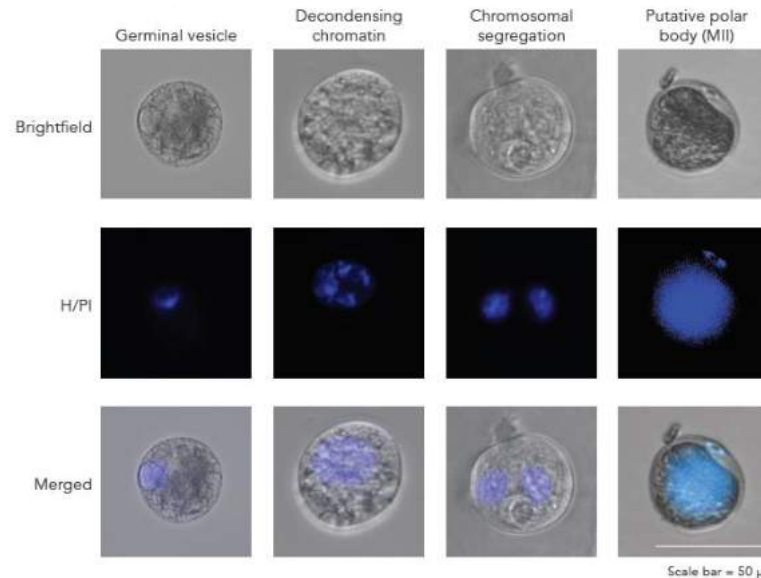
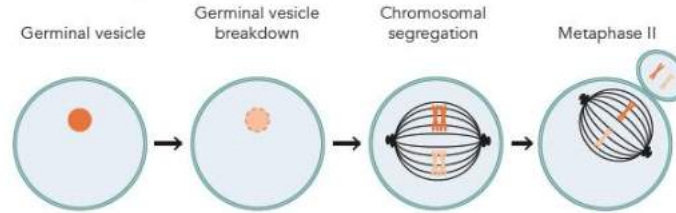
GV: 50%

ZP: 50%

PB: infrequent

Internal OVAS data 2016/2017
(representative examples)

OvaTureSM treatment derived IVDOs show evidence of nuclear maturation

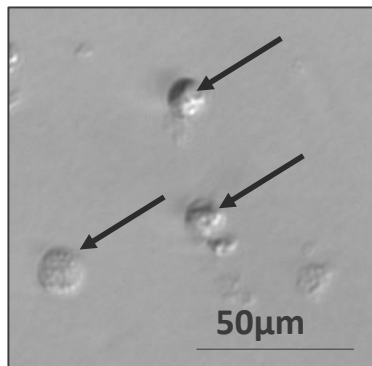


Internal OVAS data 2016/2017
(representative examples)

OvaTureSM treatment derived IVDOs show significant growth from EggPCSM cell to IVDO

Cytoplasmic maturation: IVDOs have grown from less than 10 μ m to greater than 100 μ m (fully matured endogenous oocytes \sim 120 μ m)

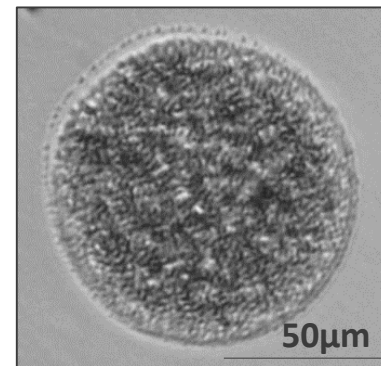
Single EggPC cells



Immature IVDO
(frequently identified)



Maturing IVDO
(maturation under investigation)



Internal OVAS data 2016/2017
(representative examples)

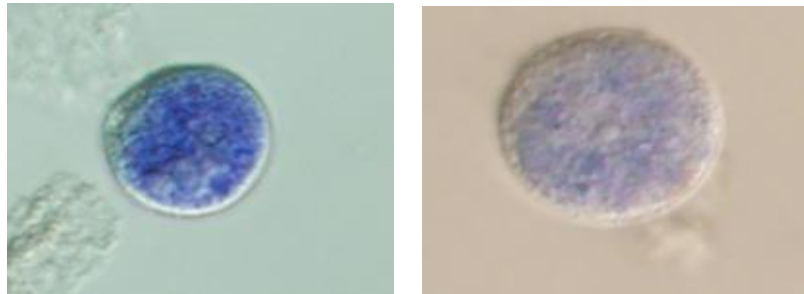
OvaTureSM treatment derived IVDOs demonstrate developmental competence based on Glucose-6-Phosphate Dehydrogenase activity



Brilliant Cresyl Blue (BCB) dye is converted by Glucose-6-Phosphate Dehydrogenase (G6PD) in developing oocytes; (used as an indicator of developmental competence for oocyte selection resulting in increased embryo development)

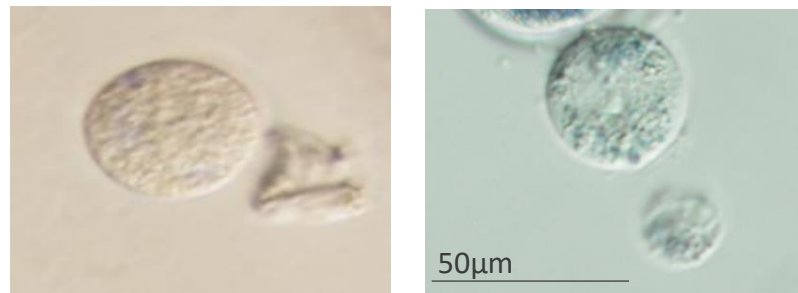
Developmentally Competent

BCB dye **NOT** converted by G6PD → resulting in blue color



Developmentally Incompetent

BCB dye converted by G6PD → resulting in **NO** blue color



Internal OVAS data 2016/2017
(representative examples, frequency
analysis ongoing)

Where is OvaScienceSM on the journey from human EggPCSM cell to IVDO?



OvaTure IVDO Characterization

- ✓ Acquisition of oocyte related marker expression
- ✓ Appearance of zona pellucida

Developmental Competence

- ✓ Nuclear maturation
 - Development of germinal vesicle
 - Putative polar body structures identified
 - Evidence for chromosomal segregation
- ✓ Cytoplasmic maturation
 - Increase in cytoplasmic volume
 - Brilliant cresyl blue (BCB) positive

Considerations for IVDO clinical development

- Guidelines for the creation, manipulation and destruction of embryos for research
- In parallel, emerging bioethical discussion regarding IVDOs and clinical introduction
 - Controversial area
- Regulatory challenges unknown
 - Innovations in the IVF field have not previously been exposed to rigorous regulatory approval
 - Where do IVDOs fit?
- Rights of embryo/physical and physiological health of children conceived with IVDOs

New innovation is happening, potentially influencing the future of fertility treatment

- Fertility innovations/technology advancements
- *In vitro* derived oocytes (IVDOs) and their possibilities
- The journey of an Egg Precursor (EggPCSM) cell to an IVDO

New innovation is happening, potentially influencing the future of fertility treatment

