Artificial gametes: A game-changer?

In vitro growth and in vitro derivation of oocytes

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The University of Edinburgh, UK

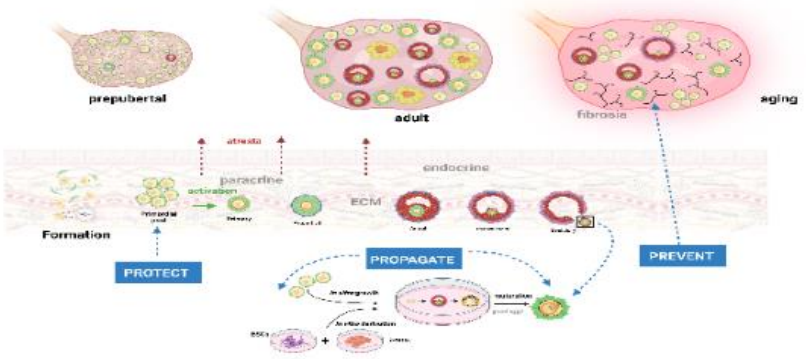
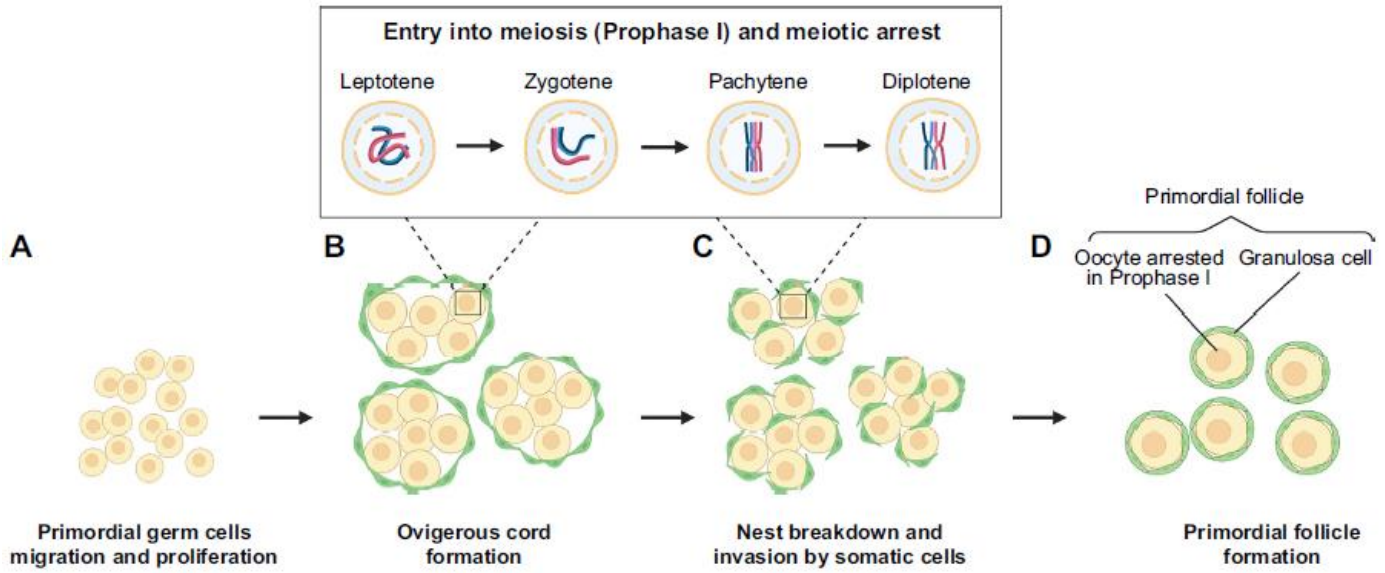
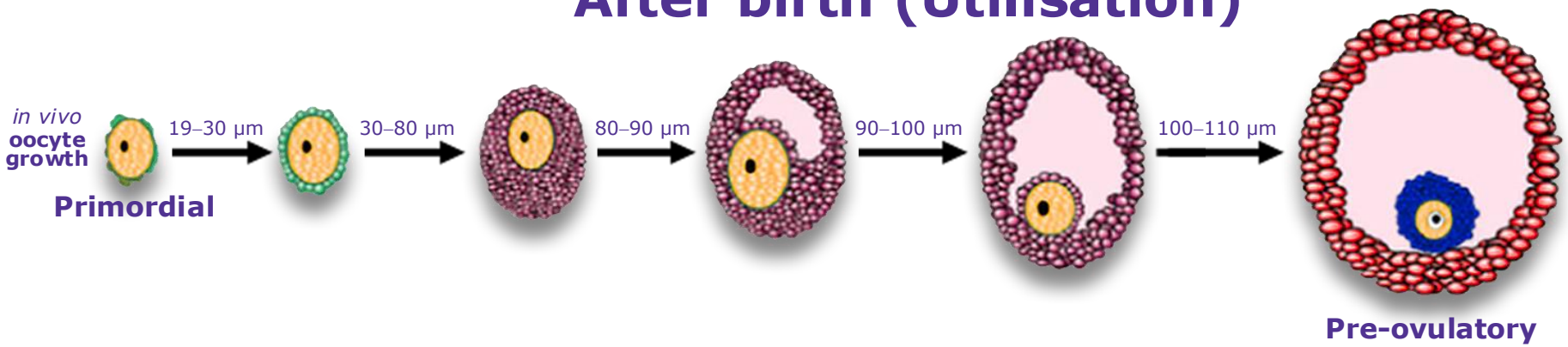


Figure taken from Telfer EE, et al. *Physiol Rev*. 2023;103:2623–7.

Formation of primordial follicles



After birth (Utilisation)



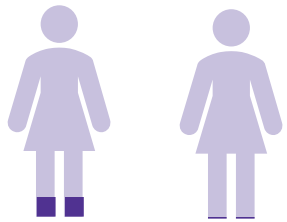
1. Telfer EE, et al. *Physiol Rev*. 2023;103:2623–77. 2. Telfer EE and Anderson RA. *Climacteric*. 2019;22:22–6.

Females are born with a fixed number of eggs

The key to your **fertility** is the age of your eggs

At birth, a woman is born with all of the eggs she will make in her **lifetime**[1,2]

Age 30 Age 40

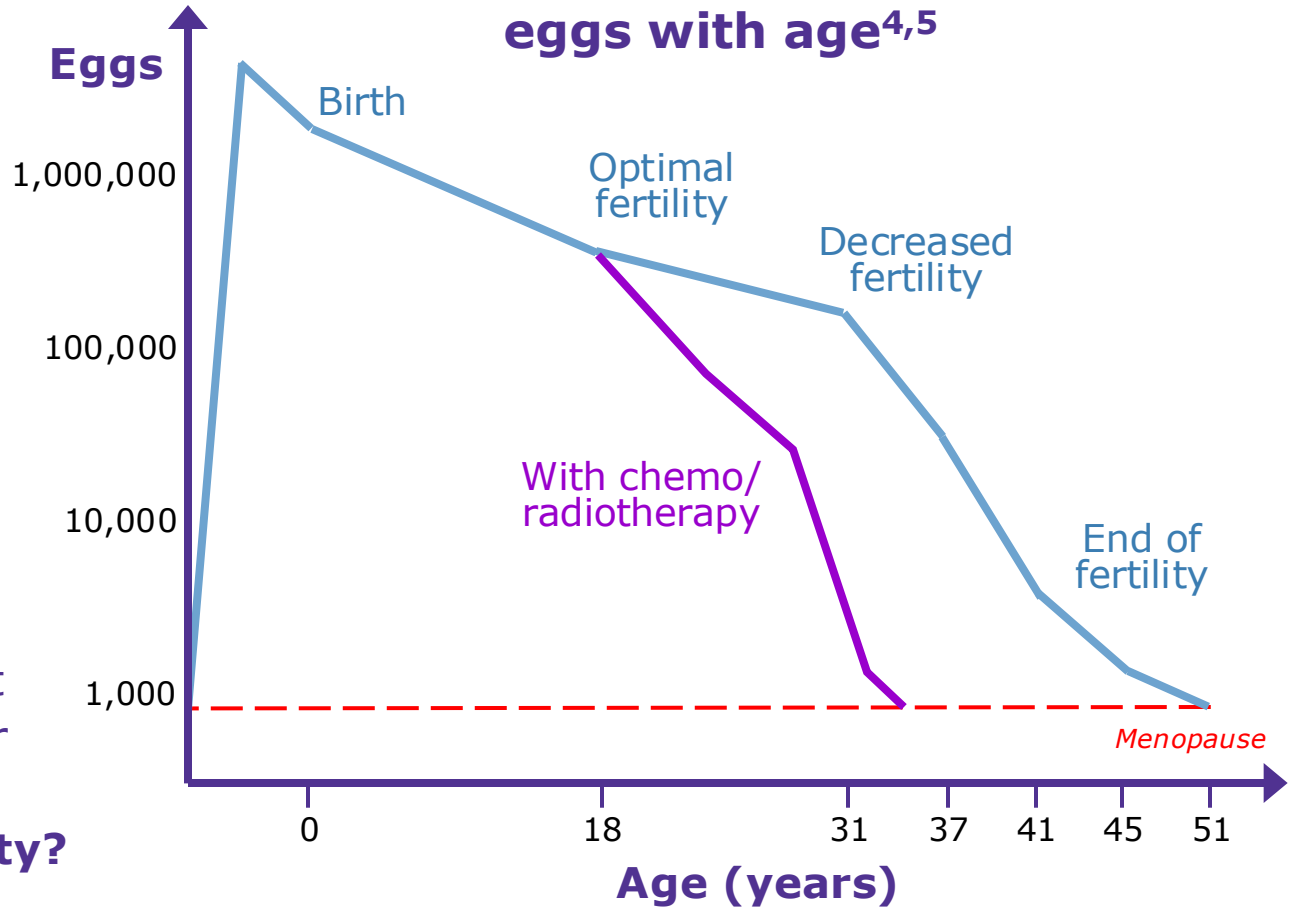


By the time a woman is 30, she has lost **90%** of her eggs, and by the time she is 40 she has lost **97%** of her eggs[3]

"Resting" immature eggs are used up throughout life with 99.9% degenerating and only 0.1% ever being ovulated[1]

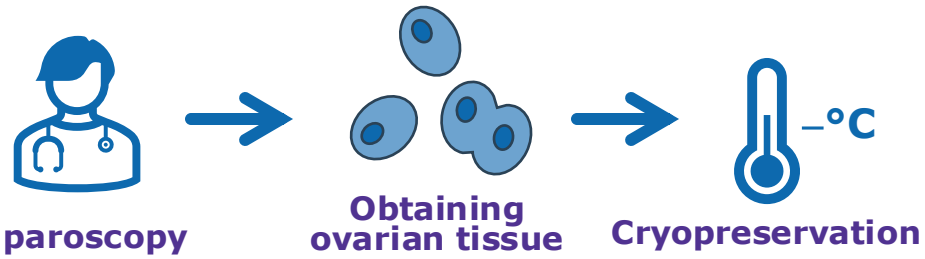
Can we save these eggs and preserve fertility?

Natural decline of immature eggs with age[4,5]



Fertility preservation

Ovarian biopsies can be taken and cryopreserved[1]



This preserves the most immature eggs and is the **ONLY** option for pre-pubertal girls[2,3]

Ovarian strips containing 100s/1,000s of immature eggs[2,4]

Transplantation of ovarian strips

>200 live births[6]

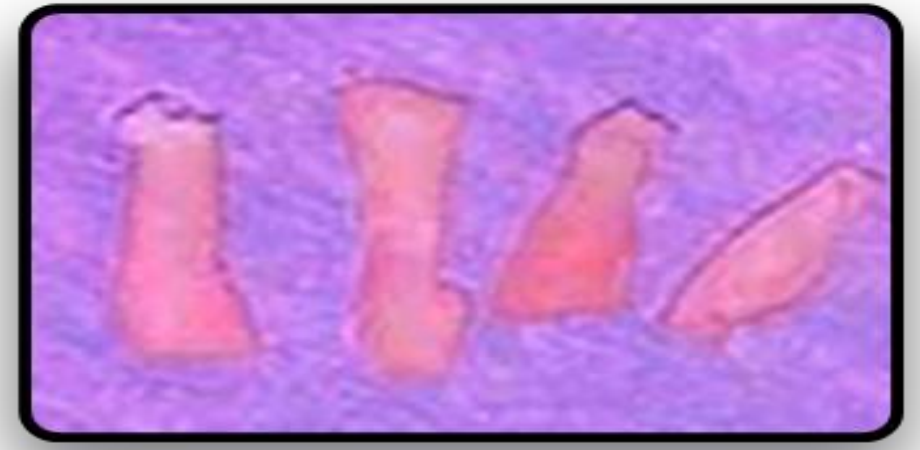
Transplantation is **NOT** suitable for everyone (e.g. women with blood borne cancers)[5]

Lab-grown eggs

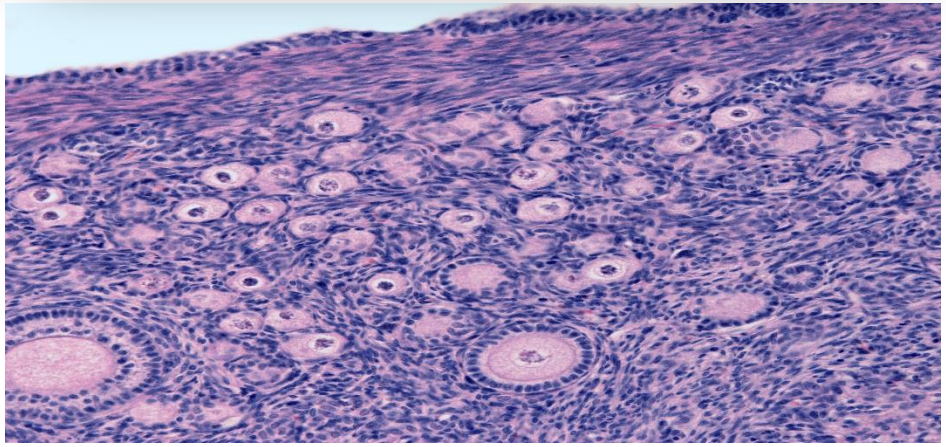
Avoids transmission of malignant cells[1]

Ovarian tissue freezing developed in Edinburgh (1994) for fertility preservation[1]

Human ovarian strips – Frozen for fertility preservation



Human ovarian biopsies taken for fertility preservation contain mainly primordial follicles **IMMATURE EGGS**[1]



The challenge is to develop these immature eggs in the lab to maturation and fertilisation[2]

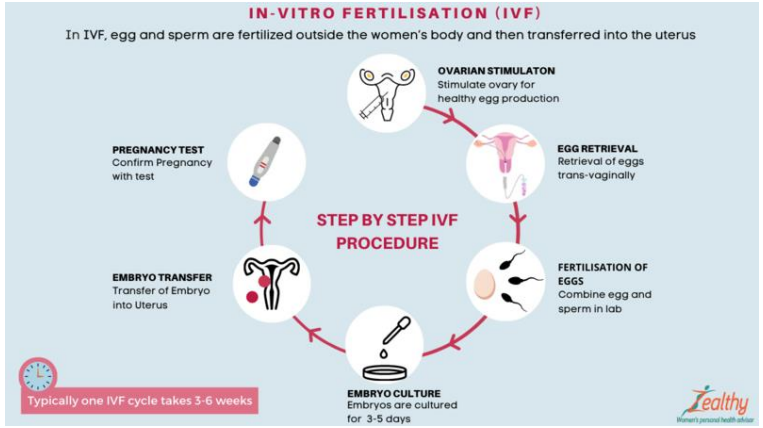
“Artificial” gametes: Oocytes

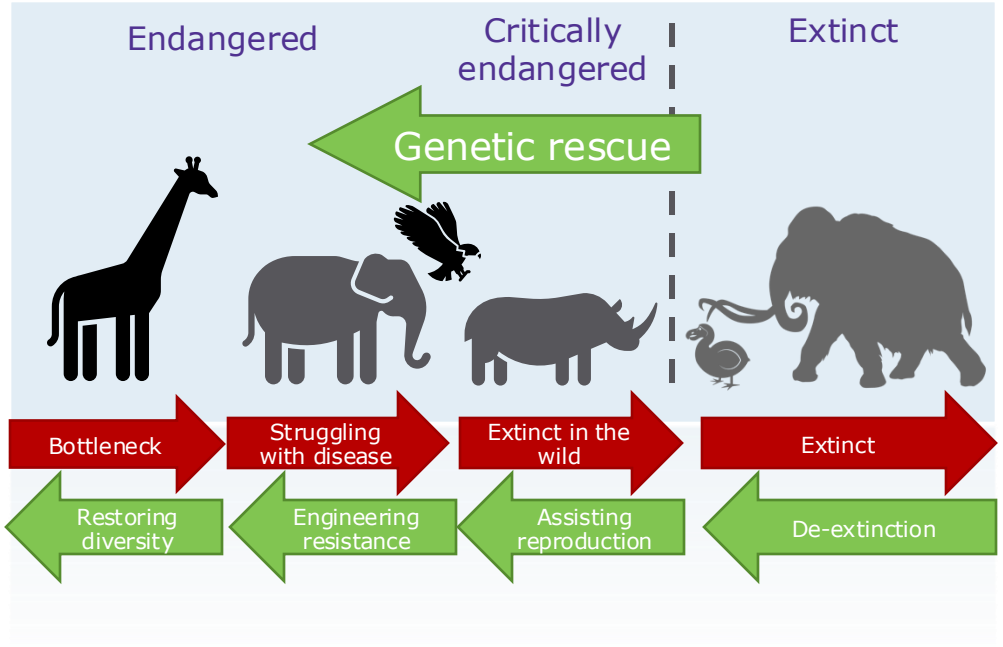
Producing mature in vitro-derived gametes either from immature gametes or from alternative sources (stem cells) would allow insights into the basic science of oogenesis, folliculogenesis and meiosis and may also offer the potential for new ART

Gametes derived in this way have been described as “**artificial gametes**” and if they are shown to be safe, they would alleviate the need for donor eggs and sperm and would enable people who cannot produce mature gametes the possibility of genetically-related children

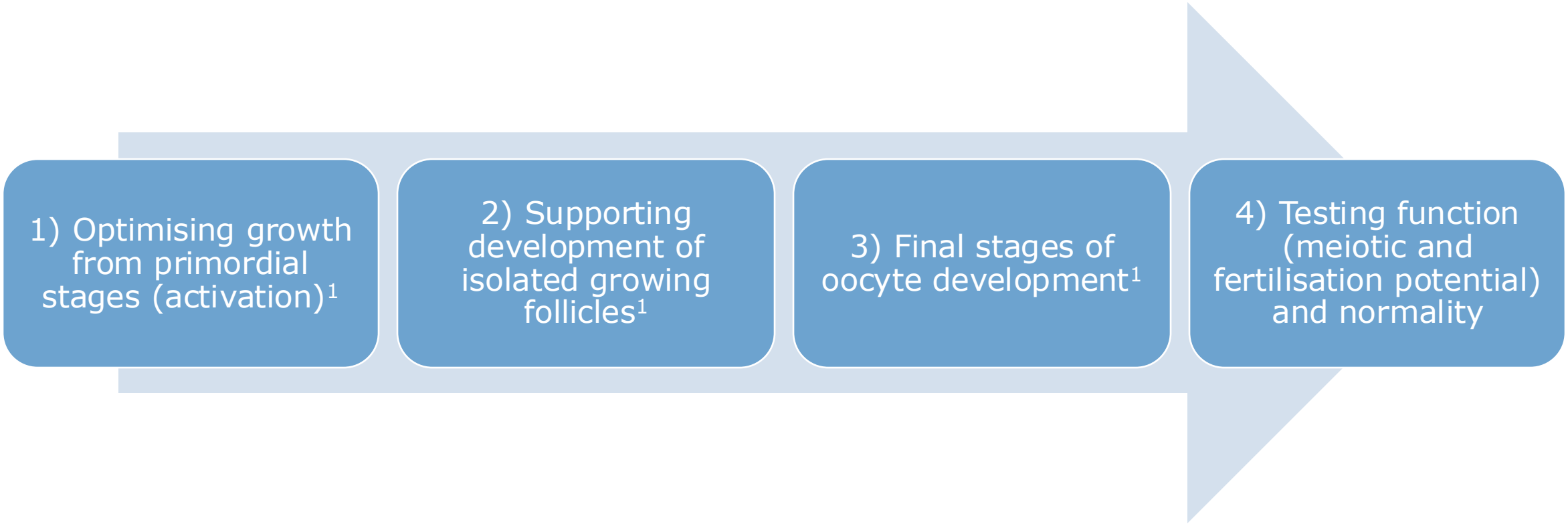
Lab-grown eggs have many applications

FERTILITY PRESERVATION IN CANCER PATIENTS





Developing IVG systems for human oocytes: Multi-step system required



IVG, in vitro growth.

1. Telfer EE. *Acta Obstet Gynecol Scand*. 2019;98:653–8.

Sources of human ovarian tissue for research

Small strip of ovarian cortex donated after informed consent:

Caesarean section (healthy women)

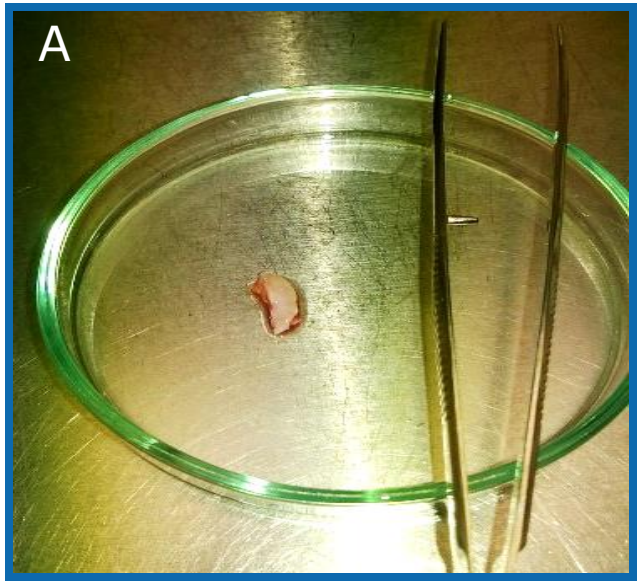
Fertility preservation (various cancers and Turner syndrome) – some tissue obtained after chemotherapy treatment

Tissue from 15 months to 45 years (fresh and cryopreserved)

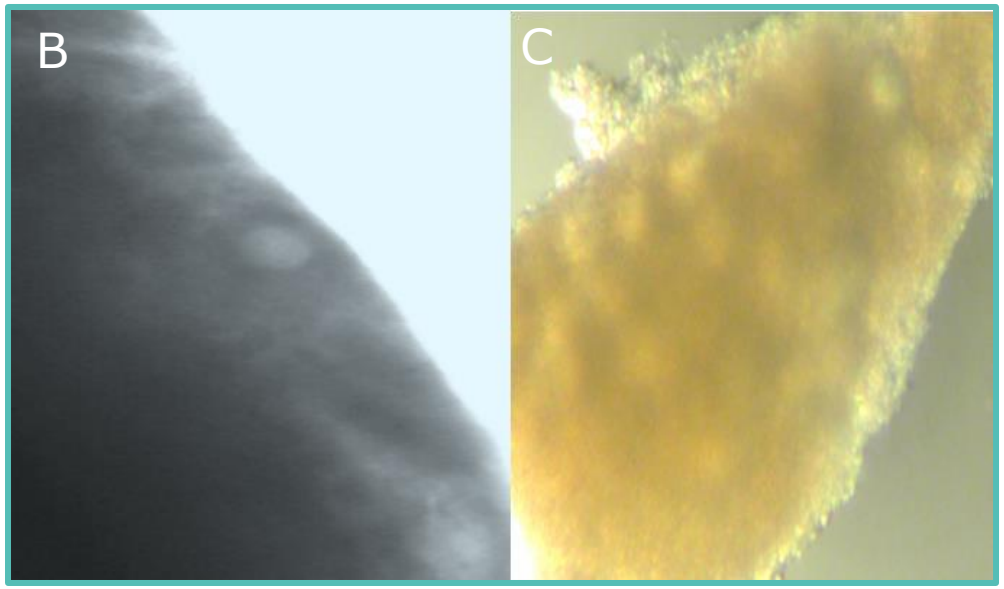
Transgender patients (whole ovaries at time of gender reassignment surgery)

Clinical collaborators: Richard Anderson, Hamish Wallace and Neale Watson

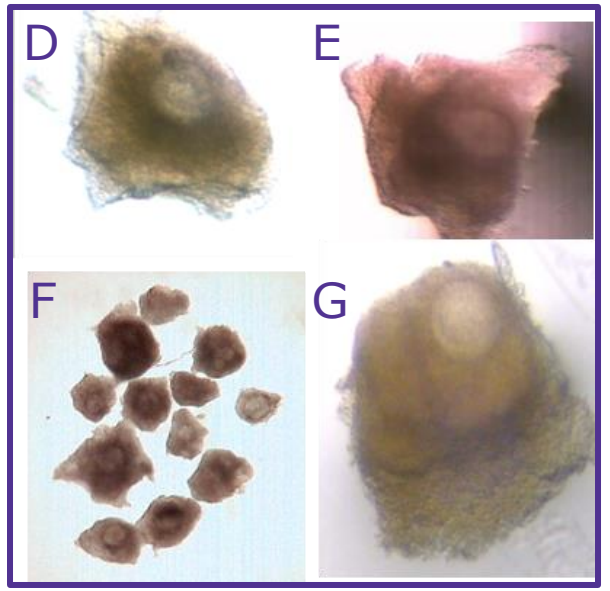
Lab grown human eggs – Step 1: Activation and growth of resting follicles



Piece of human ovarian tissue donated for research – This could contain hundreds of immature eggs

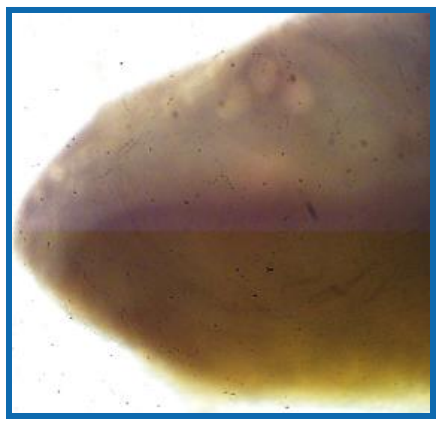


Tissue prepared to allow growth of the immature eggs outside the body in lab conditions

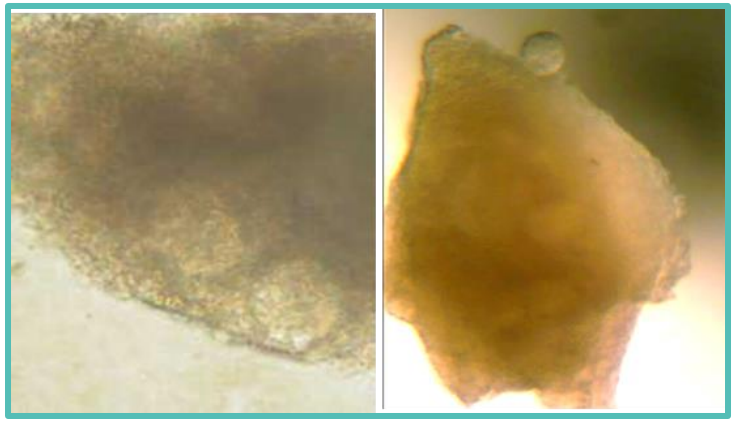


Isolated growing follicles (6–8 days) Grown in step 2 (7–10 days)

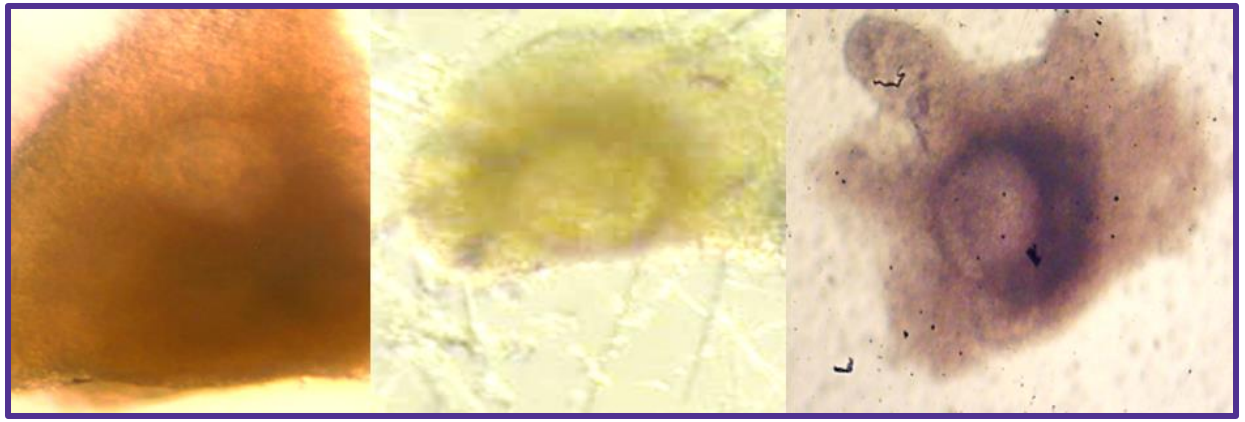
Step 2: Isolation of growing follicles and in vitro growth



Cultured micro-cortex



Follicles before isolation

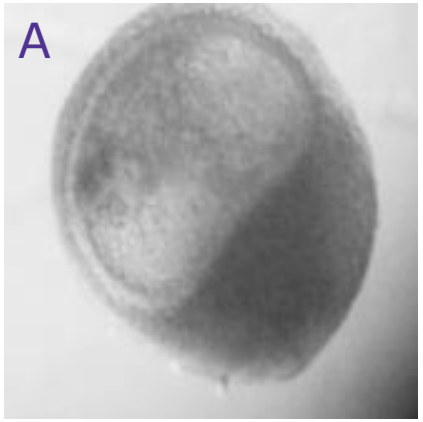


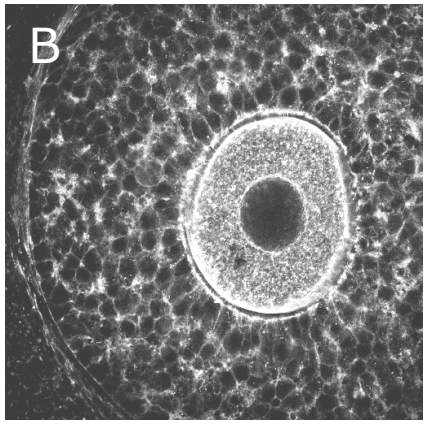
Isolated follicles

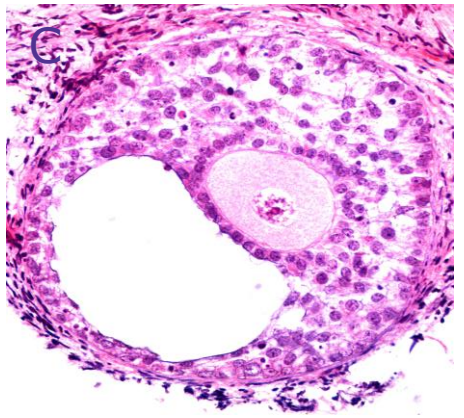
- Manual dissection using needles and fine scalpel (no enzymes)[1,2]
- Follicles cultured individually in V-shaped wells (no alginate)[1,2]
- Activin A supplementation of medium, additional 8–10 days in vitro[1,2]

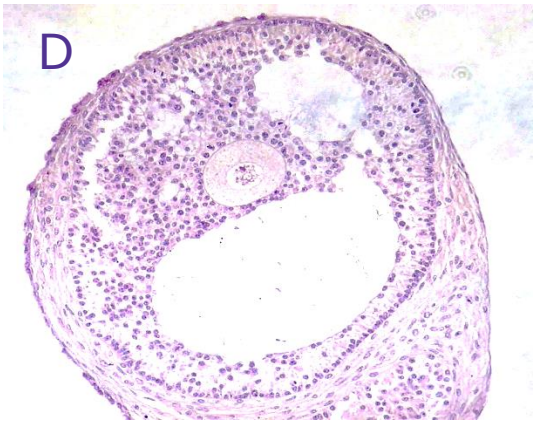
1. Telfer EE, et al. *Hum Reprod*. 2008;23:1151–8.

Antral follicle development from primordial follicles grown in vitro after step 1 (6–8 days) and step 2 (8–10 days)









Key to growth in step 2 is maintaining cell communication. The presence of Activin supports this and it is essential to ensure that high doses of FSH are not used at this stage as this disrupts contact[1,2]

Human Reproduction Vol.23, No.5 pp. 1151–1158, 2008
Advance Access publication on March 6, 2008
doi:10.1093/humrep/den070

A two-step serum-free culture system supports development of human oocytes from primordial follicles in the presence of activin

Evelyn E. Telfer[1,3], Marie McLaughlin[1], Christina Ding[2] and K. Joo Thong[2]

Molecular Human Reproduction, Vol.16, No.9 pp. 644–653, 2010
Advanced Access publication on March 4, 2010 doi:10.1093/molehr/gaq021

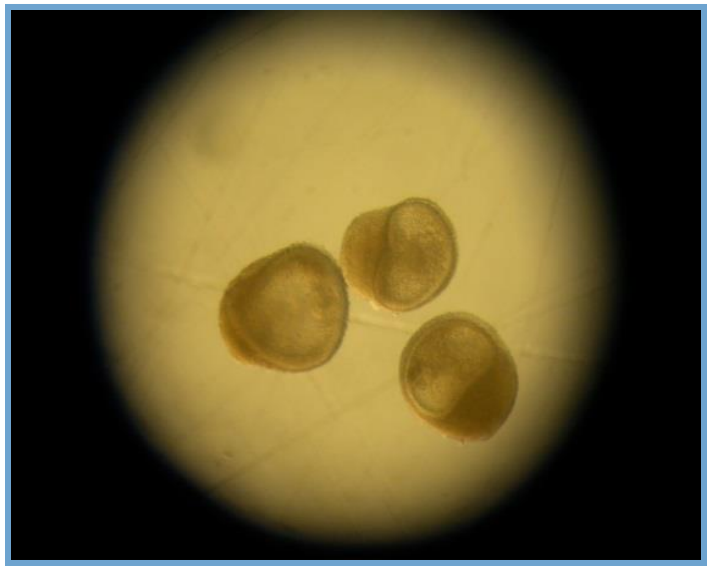
MHR ORIGINAL RESEARCH

Activin promotes follicular integrity and oogenesis in cultured pre-antral bovine follicles

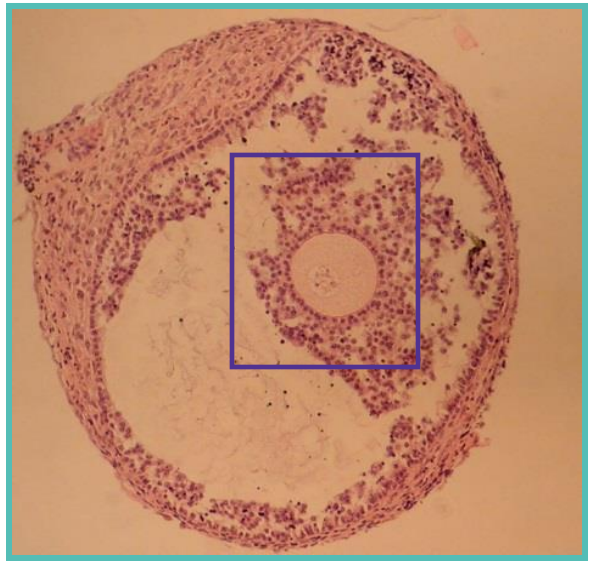
M. McLaughlin[1], J.J. Bromfield[2], D.F. Albertini[2], and E.E. Telfer[1,*]

1. Telfer EE, et al. *Hum Reprod*. 2008;23:1151–8. 2. McLaughlin M, et al. *Mol Hum Reprod*. 2010;16:644–53.

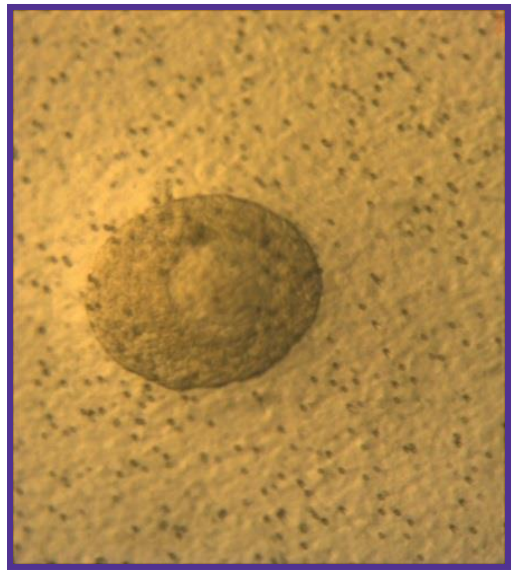
Step 3: Isolating oocyte-granulosa cell complexes



In vitro grown follicles (after 2 steps)[1]



Remove oocyte and surrounding cells[1]

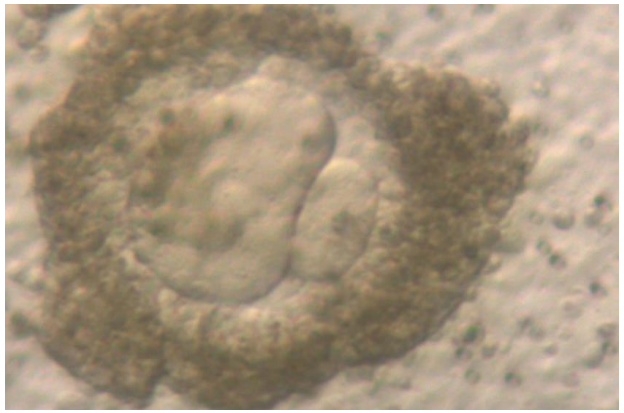


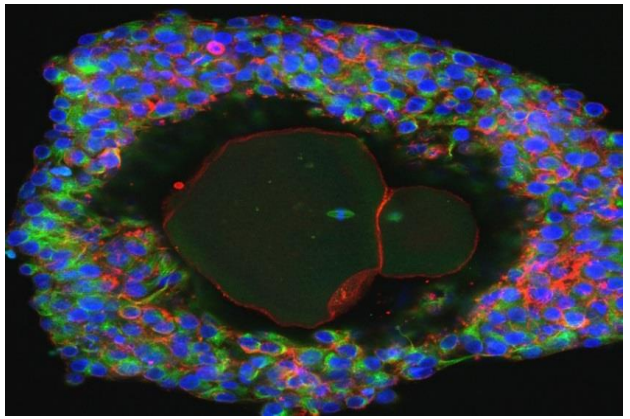
Step 3: Culture oocyte-granulosa cell complex on membranes[1]

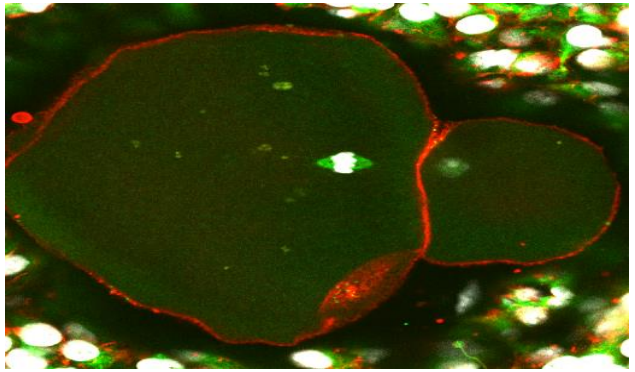
1. McLaughlin M, et al. *Mol Hum Reprod*. 2018;24:135–42.

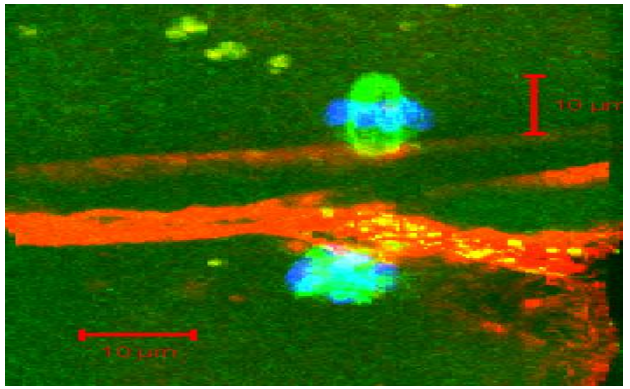
Step 4: In vitro maturation of in vitro grown oocytes

Metaphase II oocytes obtained from human IVG (19–21 days) follicles following 24 h IVM[1]









IVG, in vitro growth; IVM, in vitro maturation.

1. McLaughlin M, et al. *Mol Hum Reprod*. 2018;24:135–42.

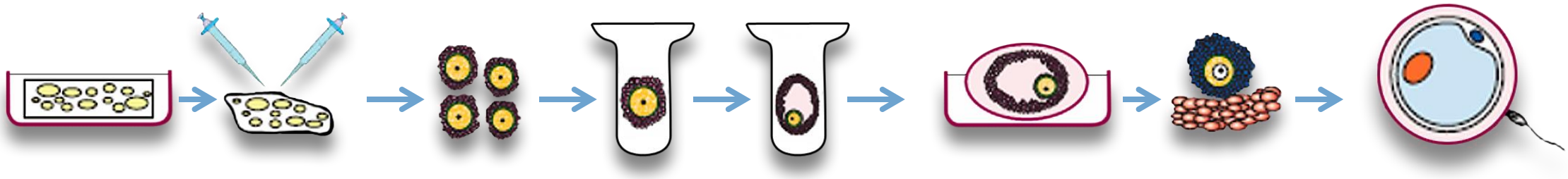
Multi-step culture system for human oocytes[1,2]

Step 1:

Step 2:

Step 3:

Step 4:



Activating growth of immature eggs from a small piece of ovary. Then isolating the growing eggs

Individual growing eggs placed with factors that support growth and development

Eggs matured in preparation for fertilisation

Fertilisation

Approximately 30% of oocytes that complete the culture process can reach maturity (metaphase II)[1]

Figure adapted from reference 2.

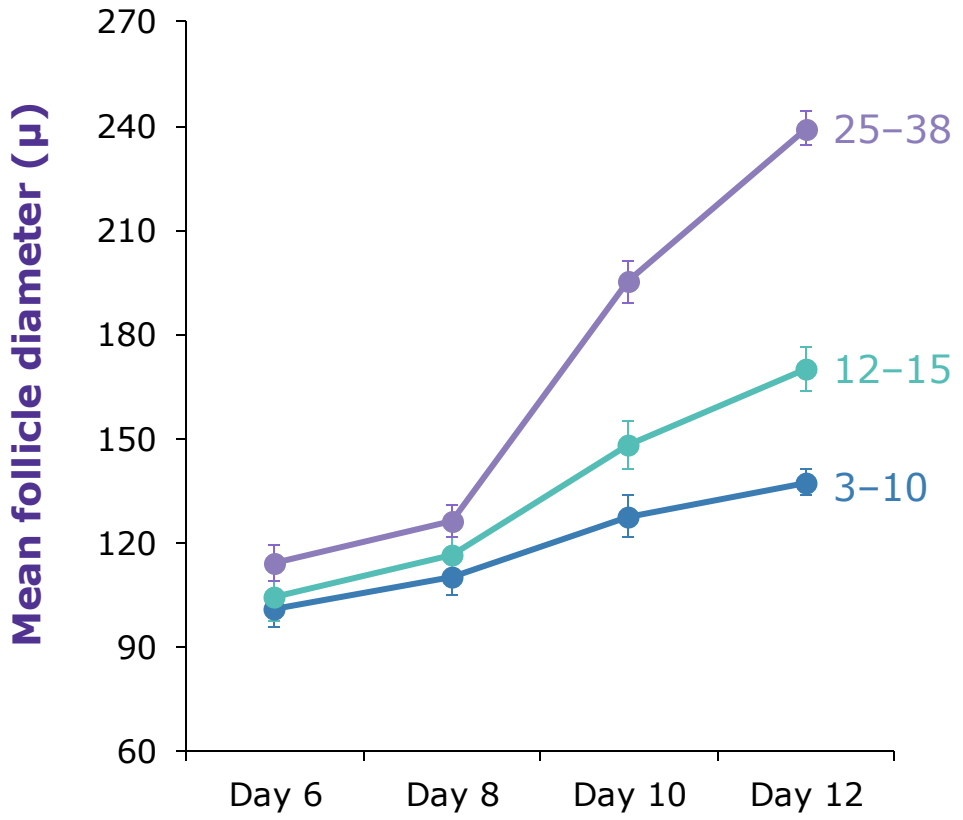
1. McLaughlin M, et al. *Mol Hum Reprod*. 2018;24:135–42. 2. Telfer EE. *Acta Obstet Gynecol Scand*. 2019;98:653–8.

Moving towards application: Testing a range of tissue sources

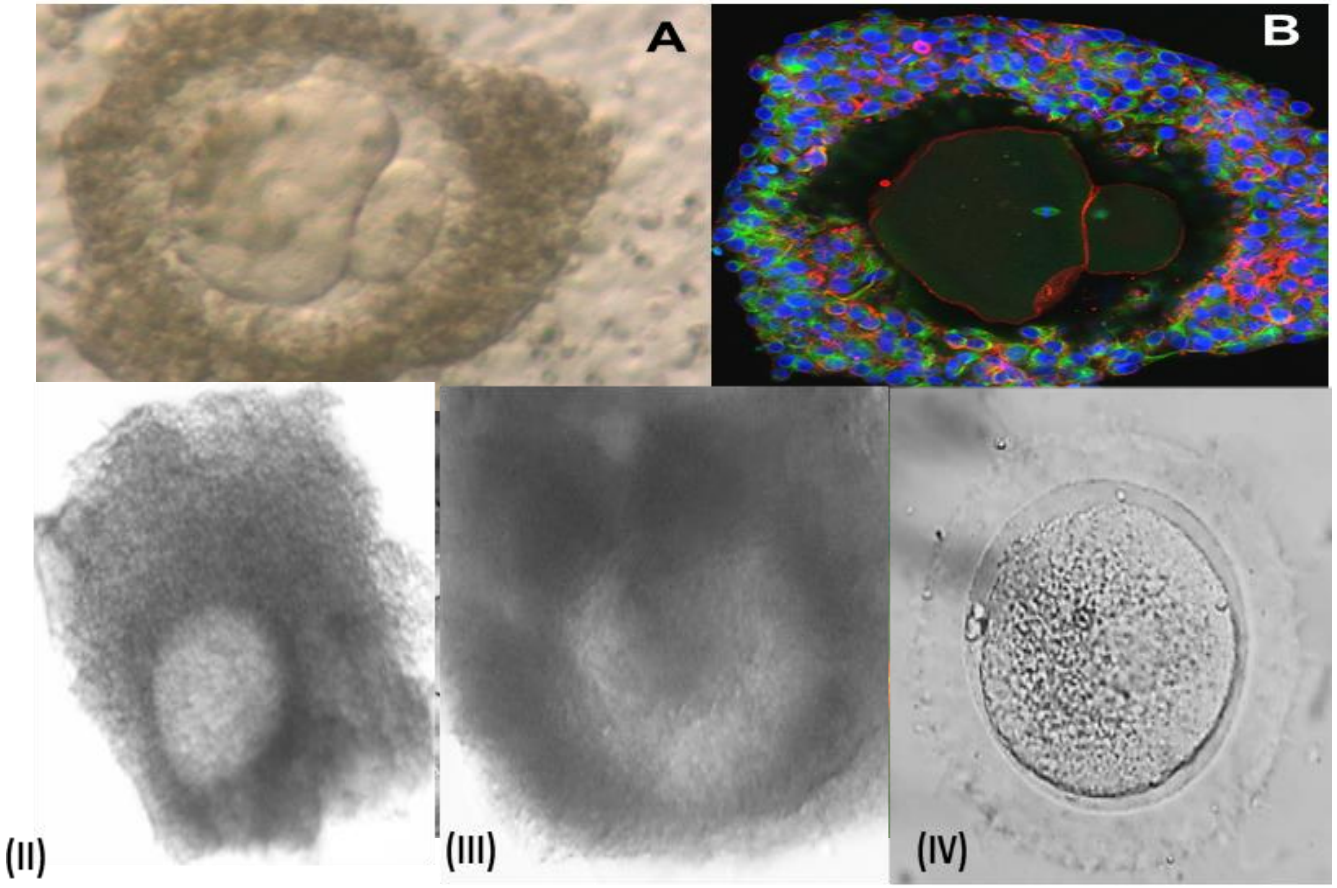
Tissue source[1]	Endpoint achieved: Lab-grown eggs[1]
Healthy women	Metaphase II oocytes (Mature eggs)
Prepubertal girls	Multilaminar stages (Midway mature)
Turner syndrome patients	Early antral (Almost mature)
Chemotherapy-treated	Variable: Depends on age and treatment
Gender reassignment	Metaphase II oocytes (Mature eggs)

One size does not fit all: System has to be adapted for tissue type (patient group)[1]

Growth rate during step 2 of culture[2]



Moving towards application: Improving culture conditions



All human IVG oocytes that formed metaphase II spindles had large polar bodies[1]

Adapting physical conditions of the culture system: Lower oxygen tension appears to improve polar body size and spindle

IVG, in vitro growth.

1. McLaughlin M, et al. *Mol Hum Reprod*. 2018;24:135–42.

Next steps towards clinical application

Determining health and developmental competence of IVG oocytes (sequencing, epigenome, metabolome)

Fertilisation of IVG human oocytes: HFEA approval

Embryo testing

Parallel studies on a large animal model (sheep and cow) embryo testing and transfer. Live young

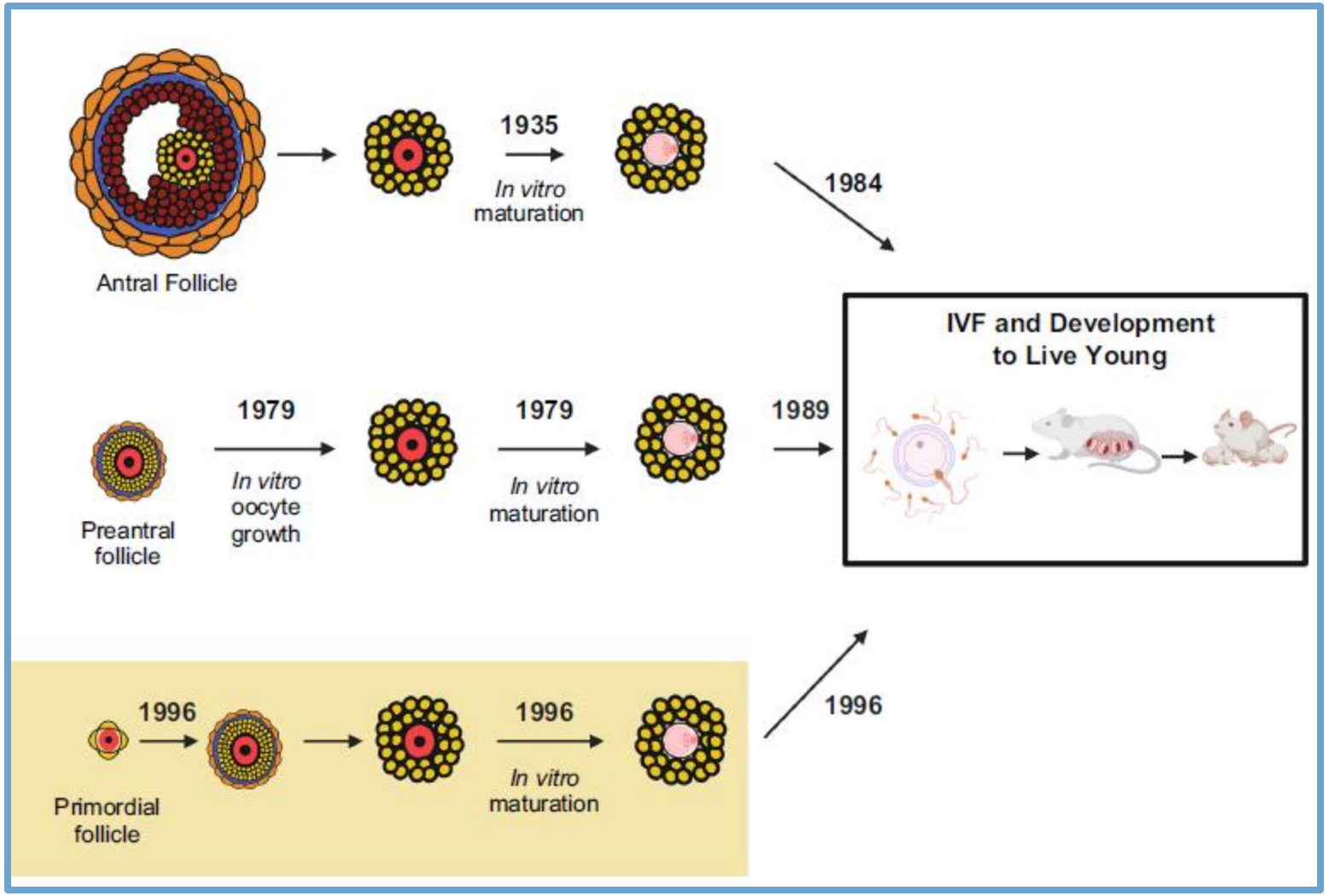
HFEA, Human Fertilisation and Embryology Authority; IVG, in vitro growth.

Developing Mouse Follicle/Oocyte Culture Systems

Antral

Pre-antral

Primordial



Human Pre-antral Follicles cultured to Metaphase II

SCIENTIFIC REPORTS

Isolated Pre-antral follicles and grown in alginate can develop to Metaphase II

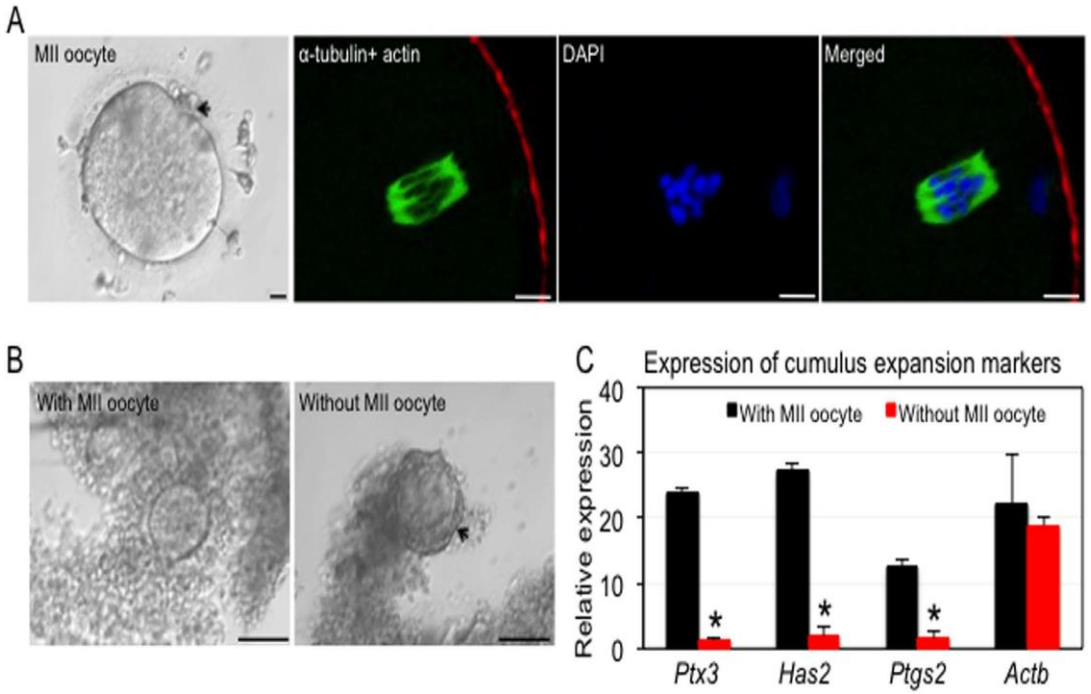
OPEN

In vitro follicle growth supports human oocyte meiotic maturation

Shuo Xiao[1,2], Jiyang Zhang[1,3], Megan M. Romero[1,2], Kristin N. Smith[4], Lonnie D. Shea[5] & Teresa K. Woodruff[1,2]

Received: 17 August 2015
Accepted: 23 October 2015
Published: 27 November 2015

In vitro follicle growth is a potential approach to preserve fertility for young women who are facing a risk of premature ovarian failure (POF) caused by radiation or chemotherapy. Our two-step follicle culture strategy recapitulated the dynamic human follicle growth environment *in vitro*. Follicles developed from the preantral to antral stage, and, for the first time, produced meiotically competent metaphase II (MII) oocytes after *in vitro* maturation (IVM).

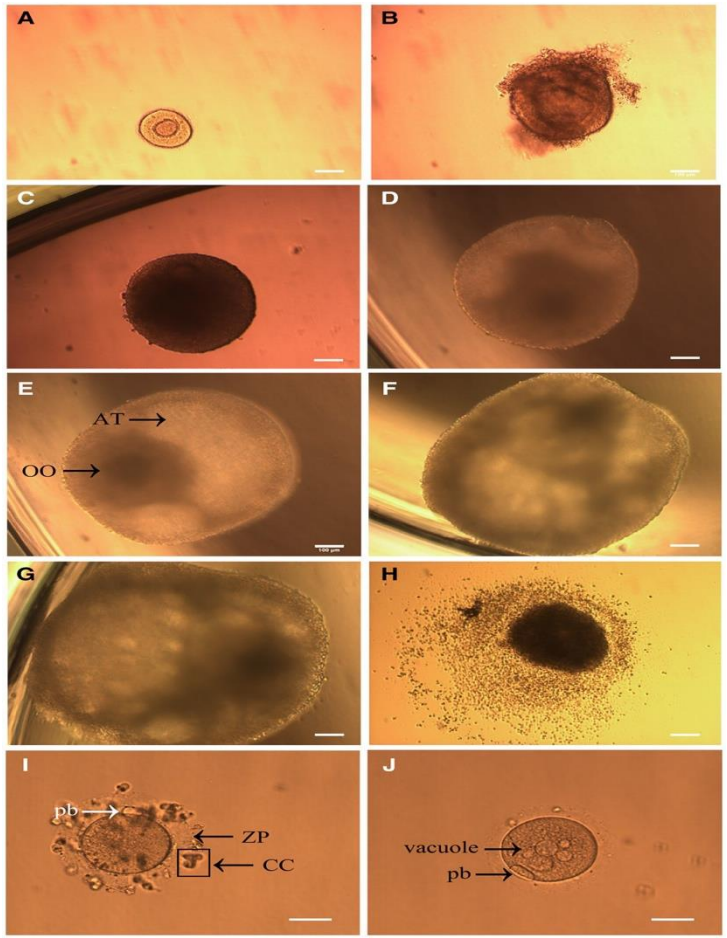


Neurotrophin-4 promotes *in vitro* development and maturation of human secondary follicles yielding metaphase II oocytes and successful blastocyst formation

Yingchun Guo[1,2], Lei Jia [1,2], Haitao Zeng[1,2], Peng Sun[1,2], Wenlong Su[1,2], Tingting Li [1,2,*,†], Xiaoyan Liang [1,2,*,†], and Cong Fang [1,2,*,†]

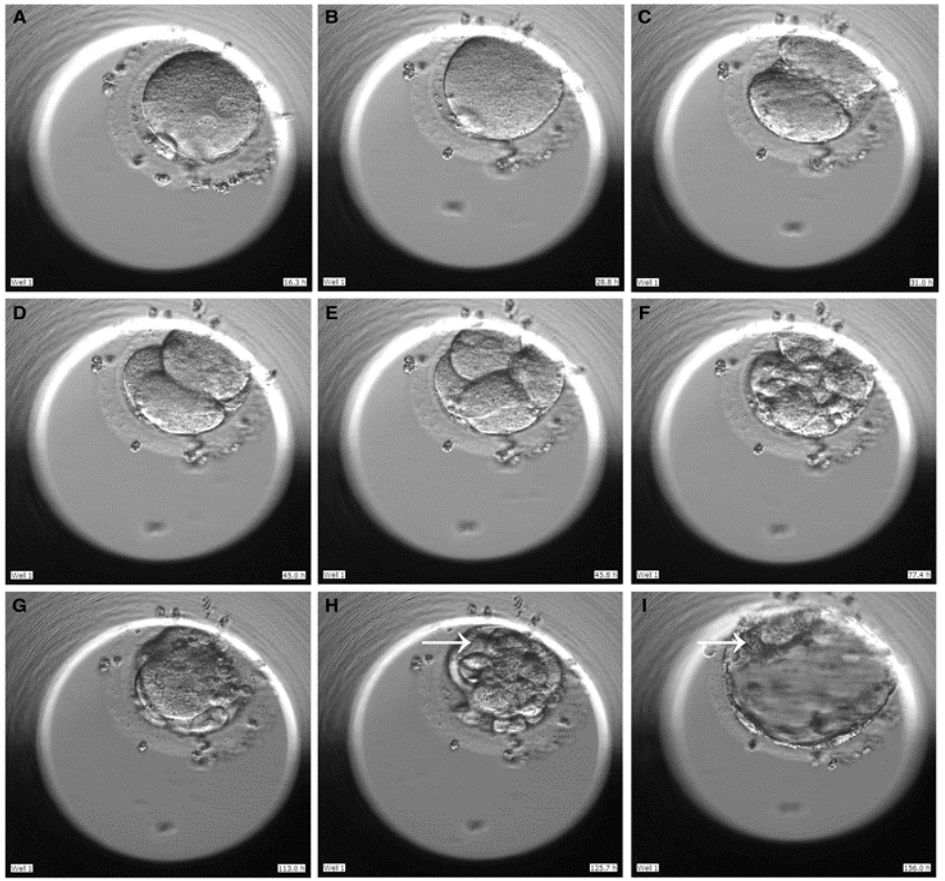
[1]Reproductive Medicine Research Center, The Sixth Affiliated Hospital, Sun Yat-Sen University, Guangdong, Guangzhou, China
[2]GuangDong Engineering Technology Research Center of Fertility Preservation, Guangdong, Guangzhou, China

Human Reproduction Open, 2024, 2024(1), hoae005
https://doi.org/10.1093/hropen/hoae005



Follicles Isolated from patients aged 6-21 years

Human secondary follicles cultured for up to 3 weeks



Embryo development after fertilization of an oocyte harvested from human follicle cultured in vitro with neurotrophic factor 4 (NT4).

Summary

Multi-step culture system supports human oocyte growth and development from primordial stages

Optimisation of each step required

Further testing required (epigenetic status)

Fertilisation potential has been shown in cultured preantral follicles next step PM

A model system for human oocyte development

Information from speaker's expertise and professional experience.

Multi-step culture system has been used to study effect of age, chemotherapy and role of signalling pathways[1–12]

Molecular Human Reproduction, Vol.20, No.8 pp. 736–744, 2014
Advanced Access publication on May 15, 2014 doi:10.1093/molehr/gau037

molecular human reproduction ORIGINAL RESEARCH

Inhibition of phosphatase and tensin homologue (PTEN) in human ovary *in vitro* results in increased activation of primordial follicles but compromises development of growing follicles

Marie McLaughlin[1,*], Hazel L. Kinnell[2], Richard A. Anderson[2], and Evelyn E. Telfer[1]

REPRODUCTION
RESEARCH

Oocyte development in bovine primordial follicles is promoted by activin and FSH within a two-step serum-free culture system

J Assist Reprod Genet (2010) 27:141–147
DOI 10.1007/s10815-010-9395-6

FERTILITY PRESERVATION

Activin A inhibits activation of human primordial follicles in vitro

Chi Christina Ding · K. Joo Thong · Archie Krishna · Evelyn E. Telfer

Molecular Human Reproduction, Vol.16, No.9 pp. 644–653, 2010
Advanced Access publication on March 4, 2010 doi:10.1093/molehr/gaq021

MHR ORIGINAL RESEARCH

Activin promotes follicular integrity and oogenesis in cultured pre-antral bovine follicles

M. McLaughlin[1], J.J. Bromfield[2], D.F. Albertini[2], and E.E. Telfer[1,*]

Human Reproduction Vol.23, No.5 pp. 1151–1158, 2008
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A two-step serum-free culture system supports development of human oocytes from primordial follicles in the presence of activin

Evelyn E. Telfer[1,3], Marie McLaughlin[1], Christina Ding[2] and K. Joo Thong[2]

FERTILITY PRESERVATION

mTOR kinase inhibition results in oocyte loss characterized by empty follicles in human ovarian cortical strips cultured in vitro

Marie McLaughlin, Ph.D.,[a] Pasquale Patrizio, M.D.,[b] Umit Kayisli, Ph.D.,[b] Janelle Luk, M.D.,[b] Travis C. Thomson, Ph.D.,[c] Richard A. Anderson, M.D., Ph.D.,[d] Evelyn E. Telfer, Ph.D.,[a] and Joshua Johnson, Ph.D.[b]

Molecular Human Reproduction, Vol.24, No.3 pp. 135–142, 2018
Advanced Access publication on January 30, 2018 doi:10.1093/molehr/gay002

molecular human reproduction ORIGINAL ARTICLE

Metaphase II oocytes from human unilaminar follicles grown in a multi-step culture system

M. McLaughlin[1], D.F. Albertini[2], W.H.B. Wallace[3], R.A. Anderson[4], and E.E. Telfer[1,*]

Reproduction & Fertility E Bailie *et al.* 4:2 e220102
RESEARCH

The ovaries of transgender men indicate effects of high dose testosterone on the primordial and early growing follicle pool

Emily Bailie[1], Mila Maidarti[1], Robert Hawthorn[2], Stuart Jack[3], Neale Watson[4], Evelyn E Telfer[1] and Richard A Anderson[5]

OXFORD human reproduction
Human Reproduction, 2024, 39(2), 382–392
https://doi.org/10.1093/humrep/dead255
Advance Access Publication Date: December 9, 2023
Original Article

Reproductive biology

Anti-Mullerian hormone attenuates both cyclophosphamide-induced damage and PI3K signalling activation, while rapamycin attenuates only PI3K signalling activation, in human ovarian cortex *in vitro*

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Advanced Access publication on October 17, 2013 doi:10.1093/humrep/det388

human reproduction ORIGINAL ARTICLE *Reproductive biology*

The immature human ovary shows loss of abnormal follicles and increasing follicle developmental competence through childhood and adolescence

R.A. Anderson[1,*], M. McLaughlin[2], W.H.B. Wallace[3], D.F. Albertini[4], and E.E. Telfer[2]

Human Reproduction, Vol.34, No.2 pp. 297–307, 2019
Advanced Access publication on December 6, 2018 doi:10.1093/humrep/dey354

human reproduction ORIGINAL ARTICLE *Reproductive biology*

Inhibition of PTEN activates bovine non-growing follicles *in vitro* but increases DNA damage and reduces DNA repair response

Mila Maidarti[1,2], Yvonne L. Clarkson[2], Marie McLaughlin[2], Richard A. Anderson[1], and Evelyn E. Telfer[2,*]

Human Reproduction, Vol.32, No.1 pp. 165–174, 2017
Advanced Access publication on December 5, 2016 doi:10.1093/humrep/dew260

human reproduction ORIGINAL ARTICLE *Reproductive biology*

Non-growing follicle density is increased following adriamycin, bleomycin, vinblastine and dacarbazine (ABVD) chemotherapy in the adult human ovary

M. McLaughlin[1,2], T.W. Kelsey[3], W.H.B. Wallace[4], R.A. Anderson[5], and E.E. Telfer[1,2,*]

Human Reproduction, Vol.38, No.3, pp. 444–458, 2023
Advance Access Publication on January 31, 2023 https://doi.org/10.1093/humrep/dead008

human reproduction ORIGINAL ARTICLE *Reproductive biology*

Spatio-temporal remodelling of the composition and architecture of the human ovarian cortical extracellular matrix during *in vitro* culture

Johanne Grosbois[1,*], Emily C. Bailie[1,2], Tom W. Kelsey[3], Richard A. Anderson[2], and Evelyn E. Telfer[1]

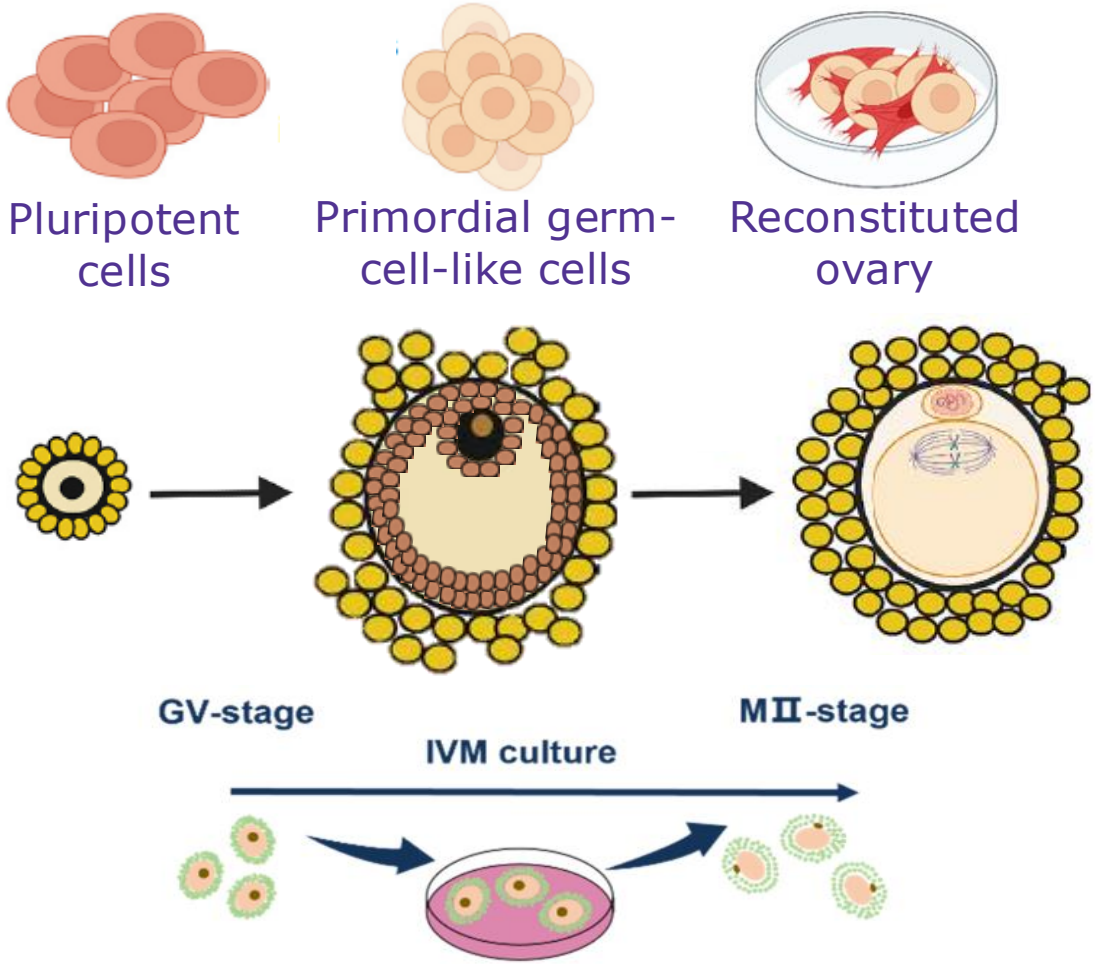
Now being used to study the potential of new oocytes generated from stem cells

Lab grown eggs:
In vitro gametogenesis/growth

Development of immature eggs/oocytes to maturity entirely in the lab (in vitro)
(*growing old eggs*)

Formation of new eggs/oocytes from stem cells (IVD)

Phases to producing oocytes from stem cells



Phase 1: Differentiation of stem cells to germline cells and combining with somatic cells to form primordial follicles. IVD

Phase 2: IVG of primordial follicles to produce fully grown oocytes

Phase 3: IVM of IVD/IVG oocytes to reach metaphase II stage

Oocytes have been produced entirely in vitro from somatic and germline cells derived from pluripotent stem cells

Approximately 5% of the oocytes produced by this process result in embryos and healthy pups have been produced

FOSLCs, foetal ovarian somatic cell-like cells; mESCs, mouse embryonic stem cells; miPSCs, mouse-induced pluripotent stem cells; PGCLCs, primordial germ cell-like cells; rOvary, reconstituted ovary.
Figure taken from Oqani RK, et al. *Cells*. 2022;11:1135 and used under a Creative Commons licence.
Yoshino T, et al. *Science*. 2021;373:eabe0237.

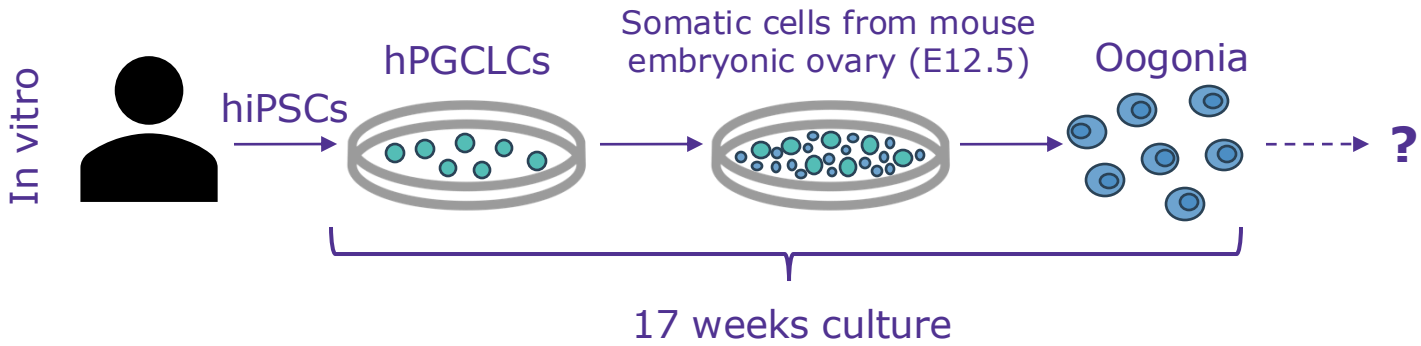
Where are we with IVD of human oocytes?

Pluripotent cells | Primordial germ cell-like cells | Reconstituted ovary

Phase 1: Differentiation of stem cells to germline cells and combining with somatic cells to form primordial follicles. IVD[1]

Human iPSCs have formed oogonia-like cells *in vitro*[2]

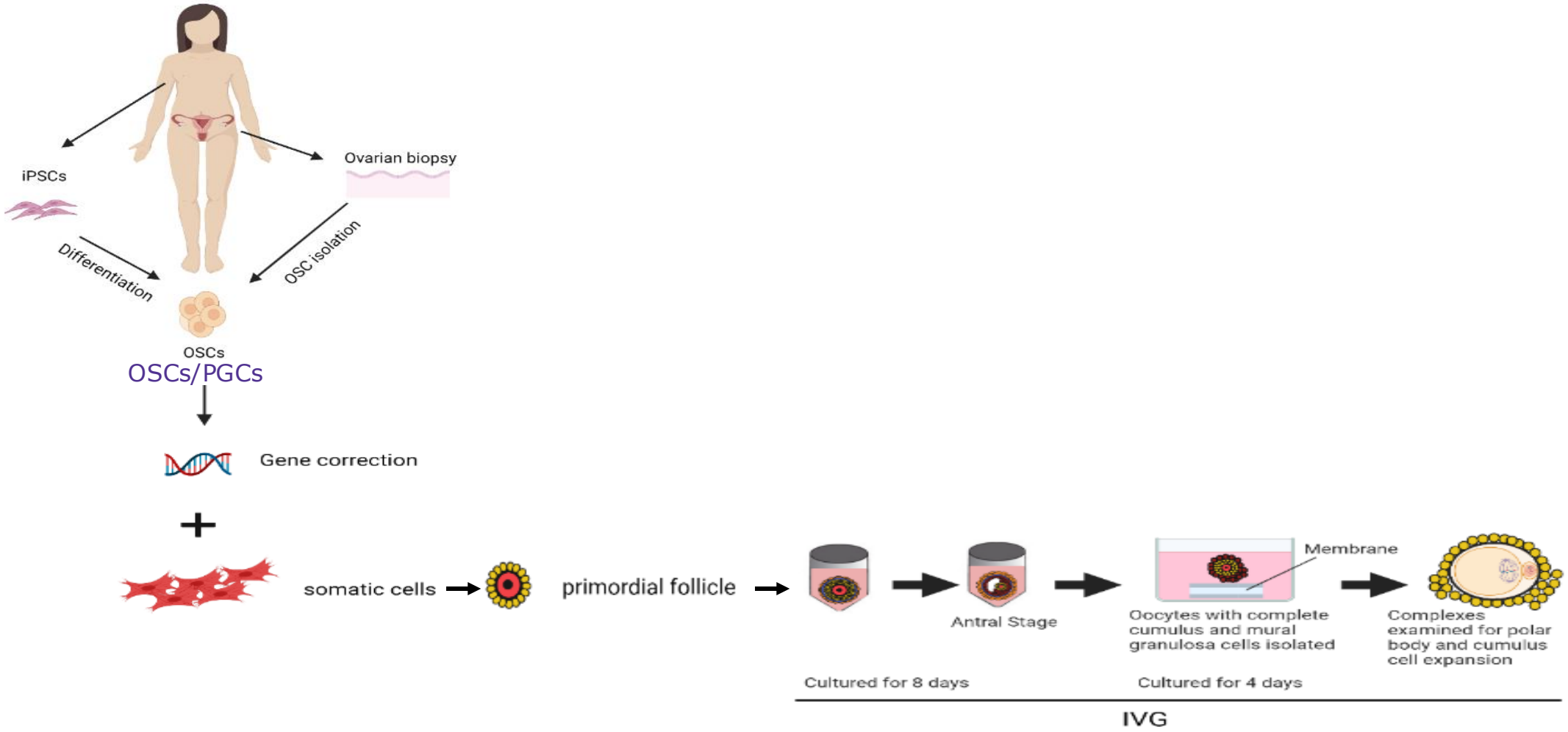
Human-induced pluripotent stem cells



Recent developments include: Directed differentiation of human iPSCs to functional ovarian granulosa-like cells[3]

(h)iPSCs, (human) induced pluripotent stem cells; hPGCLCs, human primordial germ cell-like cells; IVD, in vitro derivation.
Phase 1 figure adapted from reference 1. hIPSCs figure adapted from Stringer JM and Western PS. *Nat Biotechnol*. 2019;37:24-25.
1. Telfer EE, et al. *Physiol Rev*. 2023;103:2623–77. 2. Yamashiro C, et al. *Science*. 2018;362:356–60. 3. Pierson Smela MD, et al. *Elife*. 2023;12:e83291.

Making new eggs from stem cells would reduce the need for donor eggs and open up possibility of germline editing



iPSCs, induced pluripotent stem cells; IVG, in vitro growth; OSCs, oogonial stem cells; PGCs, primordial germ cells.

Putative human oogonial stem cells isolated from ovarian tissue of adult women

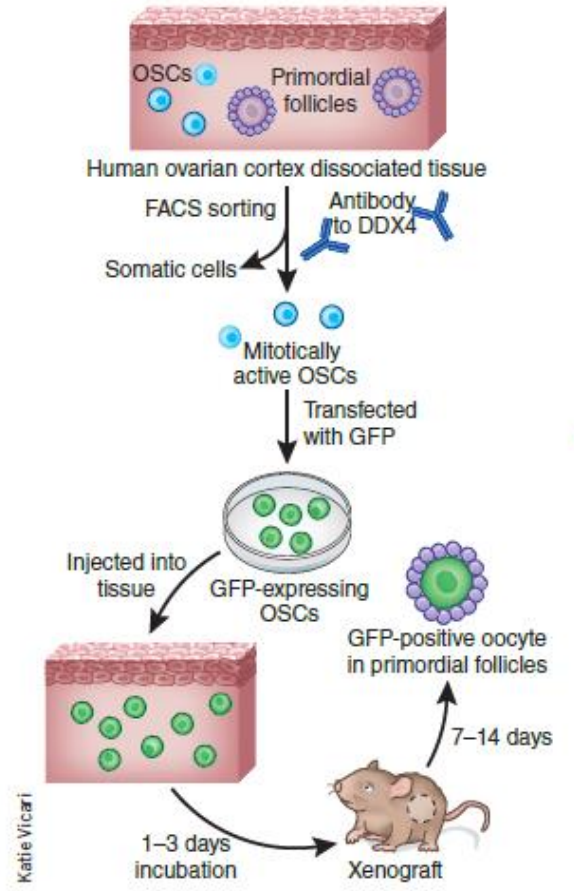
The quest for human ovarian stem cells

Evelyn E Telfer & David F Albertini

Researchers have isolated a rare population of germline stem cells from adult mouse and human ovaries that are capable of forming oocytes. The ability to harvest such cells from human ovaries could change the options available for fertility preservation and the treatment of infertility.

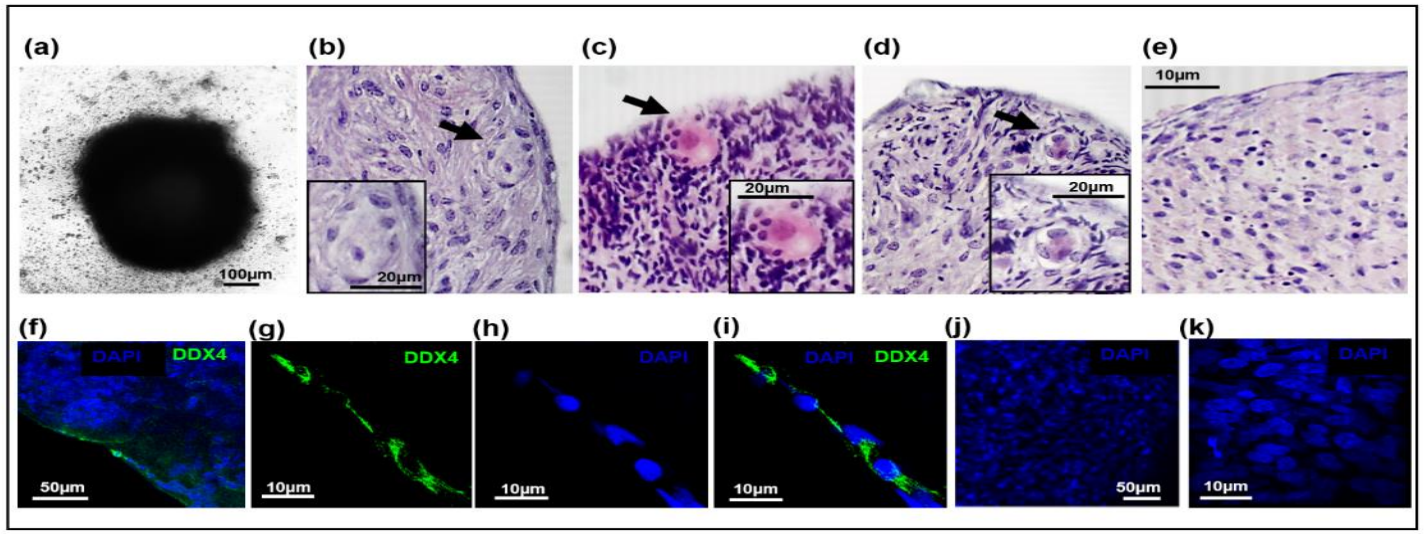
White et al, 2012 Oocyte formation by mitotically-active germ cells purified from ovaries of reproductive-age women[2]
[*Nature Medicine*;18:412–21]
Fluorescent Activated Cell Sorting (FACS) approach

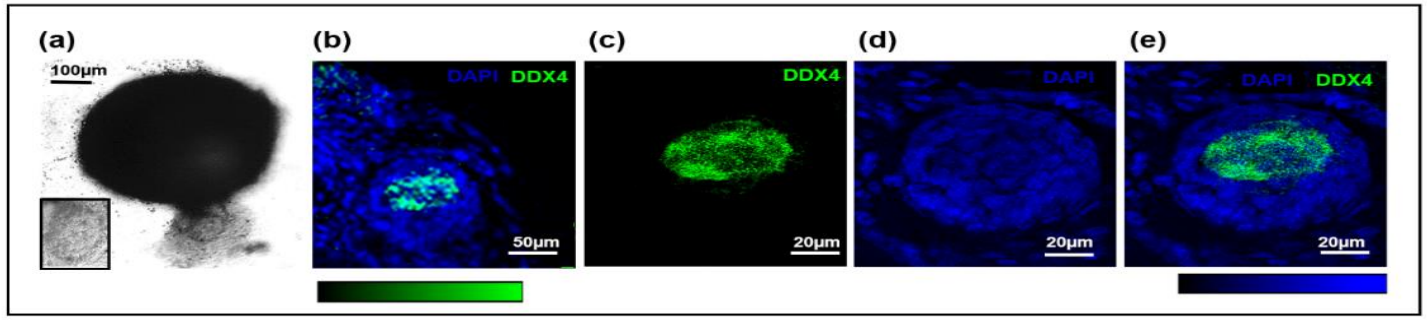
Isolation of OSCs based on the germline marker DDX4



DDX4, DEAD box polypeptide 4; FACS, fluorescent activated cell sorting; GFP, green fluorescent protein; OSCs, oogonial stem cells.
Screenshot and figure taken from reference 1.
1. Telfer EE and Albertini DF. *Nat Med*. 2012;18:353–4. 2. White YA, et al. *Nat Med*. 2012;18:413–21.

Making new human oocyte/follicle-like structures?



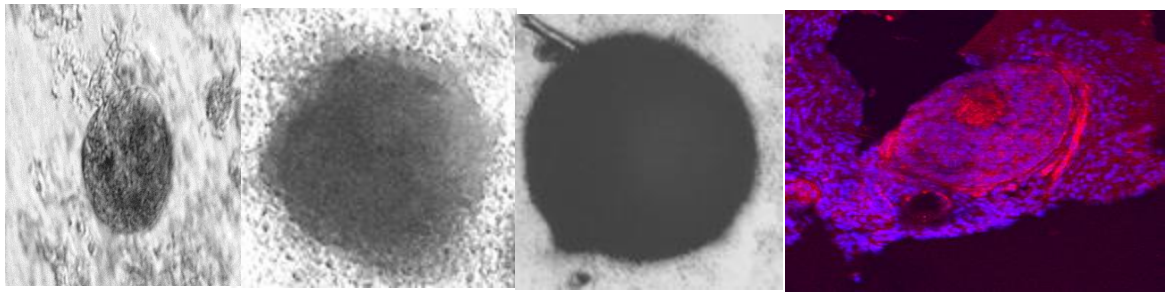


Combining DDX+ sorted cells with human foetal somatic cells results in the formation of oocyte/follicle-like structures

No structures are formed when DDX4 negative cells are combined with foetal somatic cells

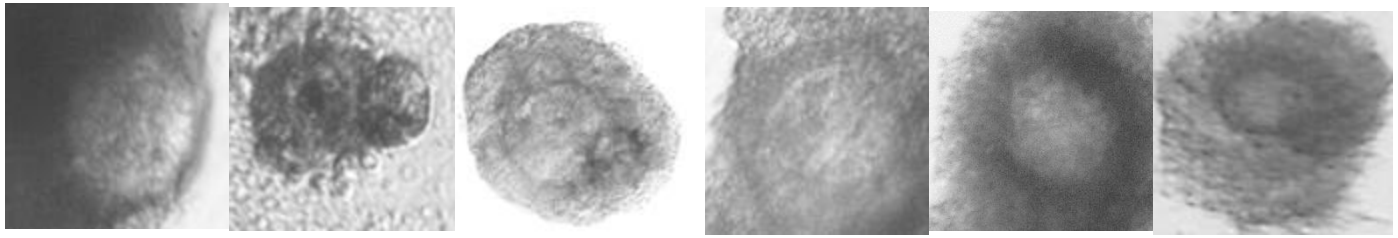
DAPI, 4’,6-diamidino-2-phenylindole; DDX4, DEAD box polypeptide 4.
Images taken from reference and used under a Creative Commons licence.
Clarkson YL, et al. *Sci Rep*. 2018;8:6953.

Making new eggs from stem cells entirely in vitro

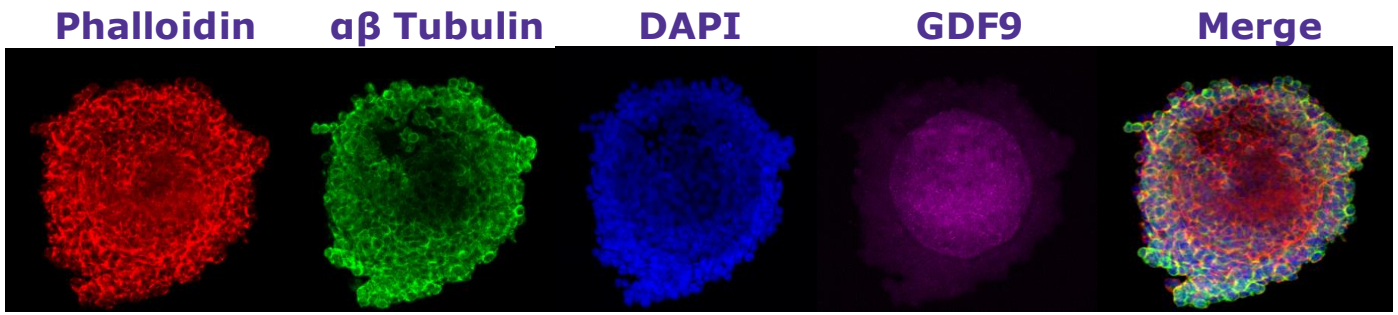


Mixing ovarian stem cells with isolated somatic cells to form mini ovaries and new eggs/follicles

Aggregates cultured for 28 days form mini ovaries



Some "new eggs" show markers that eggs within the ovary show (GDF9). Need to test their full potential and safety



DAPI, 4',6-diamidino-2-phenylindole; GDF9, growth differentiation factor 9.

Telfer Lab Unpublished

Summary (1/2)

"Artificial" oocytes (or oocyte-like structures) can be formed from cells with germline and stem cell markers isolated from adult human ovaries (OSCs) when combined with somatic cells[1]

Options to produce new oocytes from ovarian cells and iPSCs: Need to define the populations of cells within the ovary and understand their in vitro and in vivo potential[2]

Somatic cell stem cells and in vitro growth systems can bridge the gap in developing oocytes from iPSCs[2]

Robust testing of human "artificial" oocytes required[2]

iPSCs, induced pluripotent stem cells; OSCs, oogonial stem cells.

1. Telfer EE, et al. *Physiol Rev*. 2023;103:2623–77.

Summary (2/2)

- Multi-step in vitro growth systems can bridge the gap in developing oocytes from iPSCs and OSCs
- Extends options to start with primordial follicles or precursor cells
- Detailed testing of IVG oocytes required
- Results from large animal models (sheep and cows) will inform next steps towards human application

iPSCs, induced pluripotent stem cells; IVG, in vitro growth; OSCs, oogonial stem cells.

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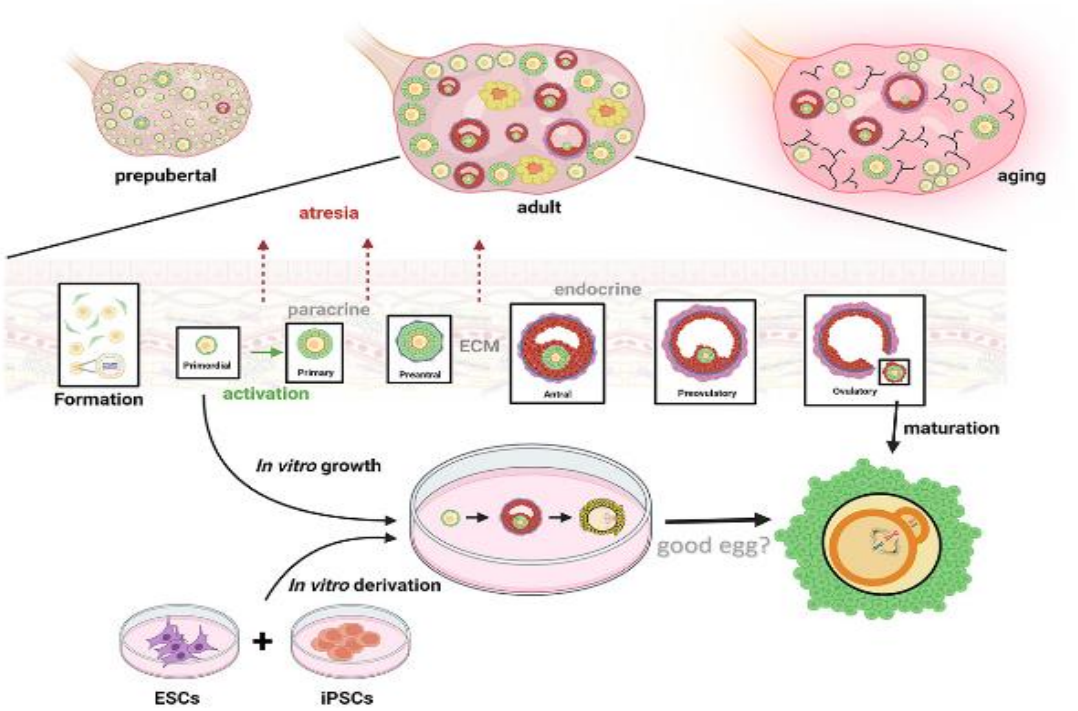
american physiological society | PHYSIOLOGICAL REVIEWS

REVIEW ARTICLE

MAKING A GOOD EGG: HUMAN OOCYTE HEALTH, AGING, AND IN VITRO DEVELOPMENT



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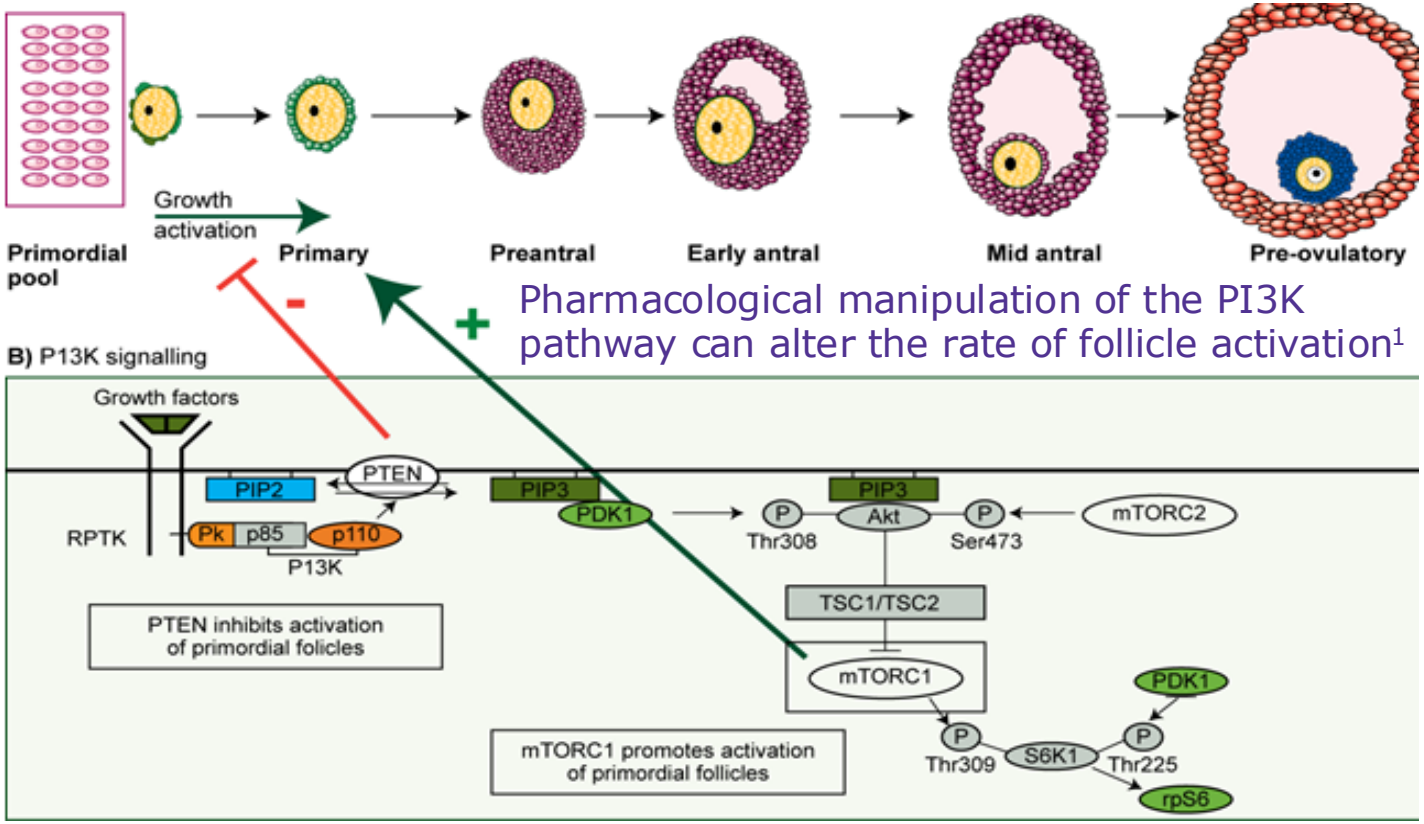
KEY WORDS

follicle culture; meiosis; oocyte maturation; ovary; reproductive aging; stem cells

ECM, extracellular matrix; ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells.
Image taken from Telfer EE, et al. *Physiol Rev*. 2023;103:2623–77 and used under a Creative Commons licence.

Initiation of primordial follicle growth in vitro

Regulation of primordial follicle activation[1]



Pharmacological manipulation of the PI3K pathway can alter the rate of follicle activation[1]

Molecular Human Reproduction, Vol.20, No.8 pp. 736–744, 2014
Advanced Access publication on May 15, 2014 doi:10.1093/molehr/gau037

molecular human reproduction ORIGINAL RESEARCH

Inhibition of phosphatase and tensin homologue (PTEN) in human ovary *in vitro* results in increased activation of primordial follicles but compromises development of growing follicles

Marie McLaughlin[1,*], Hazel L. Kinnell[2], Richard A. Anderson[2], and Evelyn E. Telfer[1]

Human Reproduction, Vol.34, No.2 pp. 297–307, 2019
Advanced Access publication on December 6, 2018 doi:10.1093/humrep/dey354

human reproduction ORIGINAL ARTICLE *Reproductive biology*

Inhibition of PTEN activates bovine non-growing follicles *in vitro* but increases DNA damage and reduces DNA repair response

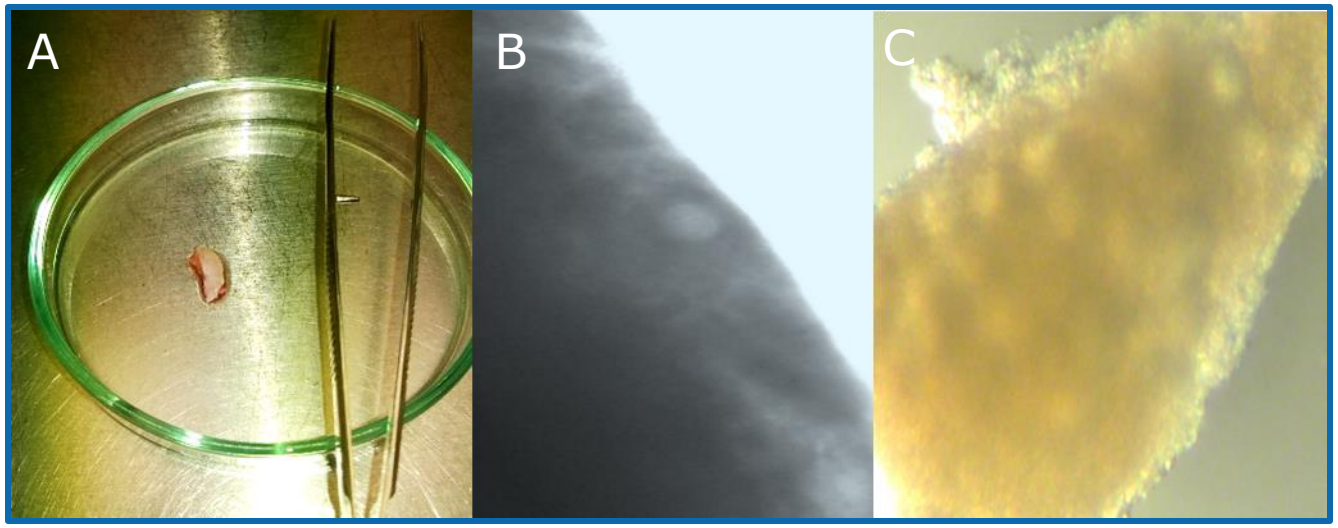
Mila Maidarti[1,2], Yvonne L. Clarkson[2], Marie McLaughlin[2], Richard A. Anderson[1], and Evelyn E. Telfer[2,*]

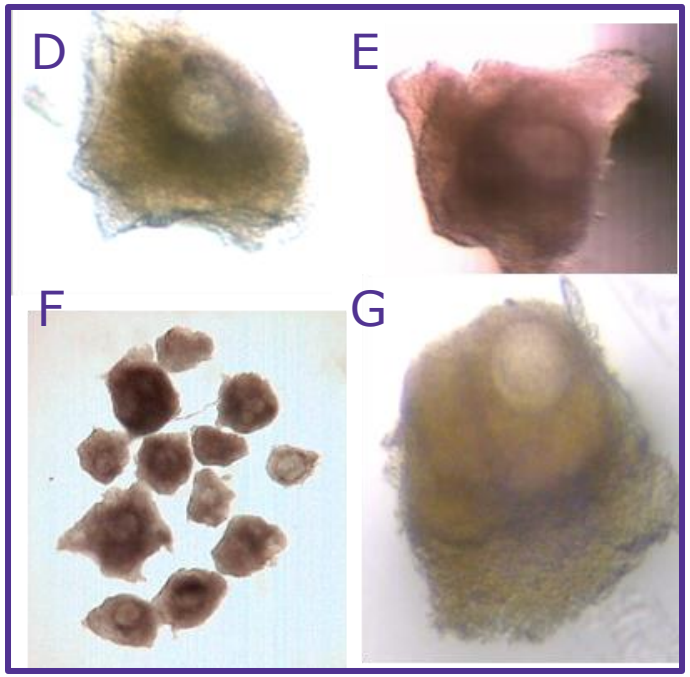
Safety of in vitro activation[3]

Akt, protein kinase B; mTORC1, mechanistic target of rapamycin complex 1; PDK, pyruvate dehydrogenase kinase; PI3K, phosphoinositide 3-kinases; PIP2, phosphatidylinositol (4,5)-bisphosphate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PTEN, phosphatase and tensin homolog; rpS6, ribosomal protein S6; RPTK, receptor protein tyrosine kinases; Ser, serine; Thr, threonine; TSC, tuberous sclerosis complex subunit.
Figure taken from reference 1. Images of papers taken from references 2 and 3.

1. Telfer EE and Zelinski MB. *Fertil Steril*. 2013;99:1523–33. 2. McLaughlin M, et al. *Mol Hum Reprod*. 2014;20:736–44.
3. Maidarti M, et al. *Hum Reprod*. 2019;34:297–307.

Step 1: In vitro activation and growth of quiescent follicles





- Primordial follicles activate within a loose micro-cortex[1]
- Hippo signalling disruption (tissue architecture crucial)[2]
- Isolated primordial follicles do not activate in vitro[2]
- Optimal time and size to remove growing follicles from micro-cortex environment[1,2]
- 6–8 days; ≥100 µM mean diameter[1,2]
- Prolonging step 1 results in increased death and poor-quality follicles/oocytes[2]

Images A, C and G taken from Telfer EE, et al. *Physiol Rev*. 2023;103:2623–77. All other images courtesy of speaker.
1. Telfer EE, et al. *Hum Reprod*. 2008;23:1151–8. 2. Information from speaker's expertise and professional experience.