WOMEN IN REPRODUCTIVE SCIENCE

Lessons from bioengineering the ovarian follicle: a personal perspective

Teresa K Woodruff

Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA

Correspondence should be addressed to T K Woodruff; Email: tkw@northwestern.edu

This paper forms part of a focus section on Women in Reproductive Science. The guest editor for this section was Professor Marilyn Renfree, Ian Potter Chair of Zoology, School of BioSciences, The University of Melbourne, Victoria, Australia

Abstract

The ovarian follicle and its maturation captivated my imagination and inspired my scientific journey – what we know now about this remarkable structure is captured in this invited review. In the past decade, our knowledge of the ovarian follicle expanded dramatically as cross-disciplinary collaborations brought new perspectives to bear, ultimately leading to the development of extragonadal follicles as model systems with significant clinical implications. Follicle maturation in vitro in an 'artificial' ovary became possible by learning what the follicle is fundamentally and autonomously capable of – which turns out to be quite a lot. Progress in understanding and harnessing follicle biology has been aided by engineers and materials scientists who created hardware that enables tissue function for extended periods of time. The EVATAR system supports extracorporeal ovarian function in an engineered environment that mimics the endocrine environment of the reproductive tract. Finally, applying the tools of inorganic chemistry, we discovered that oocytes require zinc to mature over time – a truly new aspect of follicle biology with no antecedent other than the presence of zinc in sperm. Drawing on the tools and ideas from the fields of bioengineering, materials science and chemistry unlocked follicle biology in ways that we could not have known or even predicted. Similarly, how today's basic science discoveries regarding ovarian follicle maturation are translated to improve the experience of tomorrow's patients is yet to be determined.


Introduction

Sitting proudly on my desk is a first edition of Regnier De Graaf's treatise on the ovarian follicle (de Graaf 1672), given to me by my great friend Najiba Lalou on the occasion of my investiture as the Thomas J Watkins Chair of Obstetrics and Gynecology at Northwestern University (Box 1). As strange as it may sound, I have loved the ovarian follicle and its cyclical rhythm since my first introduction to the structure in graduate school. My PhD thesis advisor, Dr Kelly Mayo, had cloned a gene expressed by the human ovarian follicle, the inhibin α-subunit (Mayo et al. 1986). My task was to clone the inhibin subunits from rats and sequence the associated genes, and then determine their molecular regulation during the reproductive cycle (Woodruff et al. 1987, 1988, 1989, 1991, D’Agostino et al. 1989, Woodruff & Mayo 1990, Makanji et al. 2014b). At the time, cloning of genes was reaching a fever pitch, with reagents like restriction enzymes available for purchase rather than needing to be purified by each lab. The chemically laborious Maxim–Gilbert sequencing method was also giving way to the enzymatically elegant Sanger dideoxy sequencing method. Within 4 years of publishing the rat inhibin sequence, graduate students like me, would no longer be cloning, sequencing, and publishing genetic data – those tasks would become the purview of The Human Genome Project.

A little over a decade passed in which I continued my work on the inhibins and activins, their cyclical regulation in and release from the ovarian follicle, and their function in the reproductive axis. I collaborated with Dr Tom Thompson to solve the crystal structure of activin with its receptor and binding proteins, elucidating key functional domains and shedding light on how the inhibin and activin ligands may have co-evolved (Thompson et al. 2003, 2005, Cook et al. 2005, Lin et al. 2006, 2011, Lerch et al. 2007a,b, Zhu et al. 2010). Having the right tools at the right time is a critical part of the advancement of science, and a
willingness to cross-disciplinary lines and be an early adopter or even an inventor is a winning combination. Here I provide a bit of background on some of the essential tools that my lab has utilized or invented to learn more about the ovarian follicle and the ways in which follicles develop over reproductive time. These tools have been prismatic – they have allowed us to peer through them as the light shines in, revealing multiple facets of follicle biology that feather out in a spectrum to advance our thinking.

Ovarian follicle maturation is autonomous to the follicle
The ovary can be seen as an artful box that holds individual organs called follicles. Each follicle contains a single germ cell – the oocyte – and is responsible for production of the hormones necessary for oocyte maturation and for endocrine feedback to the higher brain centers within the reproductive axis. Several labs, including my own, have developed various two- and
Table 1  Summary of eIVFG progress across species, follicle type and time.

<table>
<thead>
<tr>
<th>Article</th>
<th>Species</th>
<th>Follicle class</th>
<th>Material</th>
<th>Results</th>
<th>Reproductive outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rios et al. (2018)</td>
<td>Mouse</td>
<td>Aggregates of primordial, primary, and secondary co-cultured follicles</td>
<td>0.5% Alginate</td>
<td>Survival of multiple follicle populations was confirmed via histology, with the notable development of the antral follicles. Collected oocytes (63%) exhibited polar body extrusion and were fertilized by intracytoplasmic sperm injection and standard in vitro fertilization procedures. Successfully fertilized oocytes developed to the pronucleus (14%), two-cell (36%), and four-cell (7%) stages.</td>
<td>Development of embryos and mice</td>
</tr>
<tr>
<td>Jakus et al. (2017)</td>
<td>Human, primate</td>
<td>Cortical culture strips</td>
<td>Tissue paper</td>
<td>Ovarian tissue papers support mouse ovarian follicle adhesion, viability and health in vitro, as well as support, and maintain the viability and hormonal function of nonhuman primate and human follicle-containing, live ovarian cortical tissues ex vivo for 8 weeks postmortem.</td>
<td>Development of humans and primates</td>
</tr>
<tr>
<td>Laronda et al. (2017)</td>
<td>Mouse</td>
<td>In vitro: multilayer secondary (150–180 μm); in vivo: primordial, primary and small secondary co-cultured follicles</td>
<td>3DP 10% gelatin</td>
<td>30° and 60° scaffolds provide corners that surround follicles on multiple sides while 90° scaffolds have an open porosity that limits follicle–scaffold interaction. Follicle-seeded scaffolds become highly vascularized and ovarian function is fully restored when implanted in surgically sterilized mice. Moreover, pups are born through natural mating and thrive through maternal lactation.</td>
<td>Pups</td>
</tr>
<tr>
<td>Kniazeva et al. (2015)</td>
<td>Mouse</td>
<td>Primordial and primary co-cultured follicles</td>
<td>Fibrin vs fibrin/alginate vs fibrin/collagen ECM</td>
<td>Aggregated follicles encapsulated within fibrin had enhanced survival and integration with the host tissue following transplantation relative to the fibrin–alginate and fibrin–collagen composites. All mice transplanted with fibrin-encapsulated follicles resumed cycling and live births were achieved only for follicles transplanted within VEGF-loaded fibrin beads.</td>
<td>Primordial follicles develop into pups</td>
</tr>
<tr>
<td>Xiao et al. (2015b)</td>
<td>Human</td>
<td>Multilayer secondary follicles co-cultured follicles</td>
<td>Two-step: 0.5% alginate until diameter reached 400–500 μm with antrum, then released from alginate and cultured in the low attachment plates</td>
<td>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).</td>
<td>Progression to MII</td>
</tr>
<tr>
<td>Xiao et al. (2015a)</td>
<td>Mouse</td>
<td>Multilayer secondary co-cultured follicles (150–180 μm in diameter)</td>
<td>0.25% alginate</td>
<td>Our study demonstrates that size-specific follicle selection can be used as a noninvasive marker to identify high-quality oocytes and improve reproductive outcomes during eIVFG. When grown in this system, 96% of mouse follicles ovulated in response to hCG and released meiotically competent eggs.</td>
<td>Progression to MII</td>
</tr>
<tr>
<td>Skory et al. (2015)</td>
<td>Mouse</td>
<td>Multilayered secondary co-cultured follicles (180–210 μm in diameter)</td>
<td>0.5% (w/v) alginate</td>
<td>Ovulated follicles recapitulated transcriptional, morphologic and hormone synthesis patterns post-luteinizing hormone (LH/hCG). Recellularized grafts initiated puberty in mice that had been ovarioctomized.</td>
<td>Progression to puberty</td>
</tr>
<tr>
<td>Laronda et al. (2015)</td>
<td>Mouse</td>
<td>Whole ovary prep</td>
<td>Bovine de-cell strips ECM</td>
<td>Recellularized grafts initiated puberty in mice that had been ovarioctomized.</td>
<td>Progression to puberty</td>
</tr>
</tbody>
</table>

(Continued)
<table>
<thead>
<tr>
<th>Article</th>
<th>Species</th>
<th>Follicle class</th>
<th>Material</th>
<th>Results</th>
<th>Reproductive outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laronda et al. (2014)</td>
<td>Human</td>
<td>Primordial</td>
<td>0.5% or 2% rigidity alginate</td>
<td>Human primordial follicles do not survive isolation and short-term culture; Encapsulated in situ culture of ovarian tissue supports the long-term survival of human primordial follicles.</td>
<td>Growing follicles</td>
</tr>
<tr>
<td>Smith et al. (2014)</td>
<td>Mouse</td>
<td>Mostly primordial (94%)</td>
<td>Fibrin</td>
<td>Primordial follicles were recruited into the growing pool.</td>
<td>Growing follicles</td>
</tr>
<tr>
<td>Makanji et al. (2014a)</td>
<td>Mouse</td>
<td>Early secondary (110 μm)</td>
<td>Oxygen alginate</td>
<td>Enhanced survival and growth for follicles cultured at 2.5% compared with 20% O₂ (hypoxia-mediated glycolysis is essential for growth and survival of early secondary follicles);</td>
<td>Progression to MII</td>
</tr>
<tr>
<td>Tagler et al. (2014)</td>
<td>Mouse</td>
<td>Secondary Media (diameter: 90–100 μm) and primary follicles (diameter: 60–80 μm)</td>
<td>0.25% alginate</td>
<td>The supplementation of ascorbic acid (50 μg/mL) significantly enhanced the survival of primary follicles (&lt;80 μm) cultured in alginate hydrogels, which coincided with improved structural integrity. Follicles developed antral cavities and increased to diameters exceeding 250 μm. Consistent with improved structural integrity, the gene/protein expression of ECM and cell adhesion molecules was significantly changed.</td>
<td>Progression to MII</td>
</tr>
<tr>
<td>Tagler et al. (2013)</td>
<td>Mouse</td>
<td>Late primary media (average initial diameter of 80 μm) and early secondary (average initial diameter of 90 μm) follicles</td>
<td>0.25% alginate</td>
<td>Supplemented αMEM/F12-based medium enables the survival and growth of primary ovarian follicles encapsulated in alginate hydrogels.</td>
<td>Progression to MII</td>
</tr>
<tr>
<td>Skory et al. (2013)</td>
<td>Mouse</td>
<td>Multilayered secondary follicles (150–180 μm in diameter)</td>
<td>0.5% alginate</td>
<td>COMP has potential utility as a marker of follicle maturation.</td>
<td></td>
</tr>
<tr>
<td>Hornick et al. (2013)</td>
<td>Mouse</td>
<td>Primary</td>
<td>0.5% alginate</td>
<td>Increased growth and survival with multiple follicle culture → maintained follicle integrity and resulted in the formation of antral stage follicles containing meiotically competent gametes. Our data suggest that the follicle unit is necessary to produce the secreted factors responsible for the supportive effects of multiple follicle culture, as neither denuded oocytes, nor oocyte-secreted factors, nor granulosa cells alone were sufficient to support early follicle growth in vitro. Therefore, there may be signaling from both the oocyte and the follicle that enhances growth but requires both components in a feedback mechanism.</td>
<td>Progression to MII</td>
</tr>
<tr>
<td>Hornick et al. (2012)</td>
<td>Primate</td>
<td>Primordial</td>
<td>2 vs. 0.5% alginate rigidity</td>
<td>Follicle survival and morphology were more optimal when follicles were cultured in 2% alginate compared with 0.5% alginate.</td>
<td>Progression to MII</td>
</tr>
<tr>
<td>Tagler et al. (2012)</td>
<td>Mouse</td>
<td>Early secondary (average diameter of 90–100 μm) and primary (average diameter of 70–80 μm) follicles</td>
<td>0.25% alginate</td>
<td>Co-culture with MEFs enabled the survival and growth of early secondary (average diameter of 90–100 μm) and primary (average diameter of 70–80 μm) follicles.</td>
<td>Progression to MII</td>
</tr>
</tbody>
</table>

(Continued)
<table>
<thead>
<tr>
<th>Article</th>
<th>Species</th>
<th>Follicle class</th>
<th>Material</th>
<th>Results</th>
<th>Reproductive outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hirschfeld-Cytron et al. (2011)</td>
<td>Mouse</td>
<td>Secondary follicles, ranging in average diameter from 150 to 163 µm</td>
<td>Fibrinogen-alginate (FA)-IPNs ECM</td>
<td>These results suggest that the original physical environment of the follicle within the ovary can impact its function when isolated and cultured. Secondary follicles isolated from different cohorts and grown in vitro had indistinguishable growth trajectories. However, the follicles isolated from older and heavier mice and those in diestrus had altered hormone profiles. These follicles contained oocytes with reduced meiotic competence and produced oocytes with greater spindle defects.</td>
<td>Progression to MII</td>
</tr>
<tr>
<td>Parrish et al. (2011)</td>
<td>Mouse</td>
<td>Two-layered co-cultured secondary follicles (100–130 µm) and multilayered secondary follicles (150–180 µm)</td>
<td>0.25% alginate</td>
<td>PCR of key developmental genes during folliculogenesis. These studies establish the similarities and differences between in vivo and in vitro cultured follicles, guiding the creation of environments that maximize follicle development and oocyte quality. Endocrine: Cyp19a1, Lhcgr, Fshr, Inhra, Inhra; Growth: Igf1, Gdf9, Kit, Kitl, Tgflr2, Bmp15; Oocyte: Vasa, Jag1, Mater, Nobox, Zp1, Zp2, Zp3, Figlu</td>
<td></td>
</tr>
<tr>
<td>Shikanov et al. (2011b)</td>
<td>Mouse</td>
<td>Secondary (130–150 mm)</td>
<td>ECM fibrinogen-alginate (FA)-IPNs</td>
<td>This combination provides a dynamic mechanical environment because both components contribute to matrix rigidity initially; however, proteases secreted by the growing follicle degrade fibrin in the matrix leaving only alginate to provide support.</td>
<td></td>
</tr>
<tr>
<td>Shikanov et al. (2011a)</td>
<td>Mouse</td>
<td>Immature secondary follicles (140–150 µm in diameter)</td>
<td>PEG-VS</td>
<td>For tri-functional crosslinkers, the hydrogels supported a 17-fold volumetric expansion of the tissue during culture, with expansion dependent on the ability of the follicle to rearrange its microenvironment, which is controlled through the sensitivity of the cross-linking peptide to the proteolytic activity of plasmin.</td>
<td></td>
</tr>
<tr>
<td>Xu et al. (2011)</td>
<td>Primate</td>
<td>Preantral</td>
<td>Fibrin–alginate–matrigel ECM</td>
<td>Follicles grown in the absence of FSH produced MII oocytes with normal spindle structure.</td>
<td>Progression to MII</td>
</tr>
<tr>
<td>Jin et al. (2010a)</td>
<td>Primate</td>
<td>Tissue</td>
<td>Tissue culture × 4 days, then isolation of secondary in alg or alg-fib ECM × 12 days</td>
<td>This study provides evidence that markers of early follicle growth and development are preserved after slow cryopreservation and thaw, with little effect on follicle morphology and function.</td>
<td></td>
</tr>
<tr>
<td>Jin et al. (2010b)</td>
<td>Mouse</td>
<td>Tissue, secondary</td>
<td>FA IPN ECM</td>
<td>A strategy combining whole ovary culture of early-stage follicles and subsequent FA hydrogel in vitro follicle culture produced a high percentage of oocytes competent for fertilization.</td>
<td>Progression to MII</td>
</tr>
<tr>
<td>Shikanov et al. (2009)</td>
<td>Mouse</td>
<td>Two-layered secondary follicles</td>
<td>FA IPN ECM</td>
<td>The rate of meiotically competent oocytes produced by culture in FA IPN was 82%, which was significantly greater than in alginate alone. This increase in oocyte quality is an important step in identifying 3D culture systems.</td>
<td>Progression to MII</td>
</tr>
<tr>
<td>Xu et al. (2009b)</td>
<td>Human</td>
<td>Secondary</td>
<td>0.5% alginate bead or Matrigel ECM</td>
<td>No significant differences between alginate and matrigel. Our data support the notion that human follicle development can be achieved in vitro in a bio-engineered culture system.</td>
<td>Progression to MII</td>
</tr>
<tr>
<td>Article</td>
<td>Species</td>
<td>Follicle class</td>
<td>Material</td>
<td>Results</td>
<td>Reproductive outcome</td>
</tr>
<tr>
<td>-------------------------</td>
<td>----------------</td>
<td>----------------------</td>
<td>------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Xu et al. (2009c)</td>
<td>Primate</td>
<td>Secondary</td>
<td>0.5 and 0.25% rigidity alginate</td>
<td>Regardless of gonadotropin treatment, follicles produced estradiol, androstenedione and progesterone by 14–30 days in vitro. Thus, an alginate hydrogel maintains the 3D structure of individual secondary macaque follicles, permits follicle growth and supports steroidogenesis for &lt;30 days in vitro.</td>
<td>Progression to MII</td>
</tr>
<tr>
<td>Xu et al. (2009a)</td>
<td>Mouse</td>
<td>Two-layered secondary follicles (100–130 μm, type 4)</td>
<td>Alginate</td>
<td>Overall, the present study demonstrated that mouse preantral follicles, either within ovarian tissues or individually isolated, could be successfully cryopreserved by the slow-freezing method, as evidenced by post-thaw follicle development and steroidogenesis, oocyte maturation and molecular markers for oocyte and/or granulosa cells connection.</td>
<td></td>
</tr>
<tr>
<td>West-Farrell et al. (2009)</td>
<td>Mouse</td>
<td>Multilayered secondary follicles (150–180 μm in diameter)</td>
<td>0.5 or 1.5% rigidity</td>
<td>Matrices with a shear modulus of less than 250 Pa are regarded as permissive, as a large percentage of follicles cultured in these matrices survive and increase in diameter, develop an antrum, have a steroidogenic profile similar to that of follicles in vivo, and produce metaphase II stage oocytes.</td>
<td></td>
</tr>
<tr>
<td>West et al. (2007)</td>
<td>Mouse</td>
<td>Two-layered (100–130 μm) and multilayered (150–180 μm)</td>
<td>3% rigidity alginate; 1.5% rigidity alginate; 0.7% rigidity alginate; 1.5% rigidity oxidized alginate; 1.5% rigidity irradiated alginate</td>
<td>Concentration enhanced follicle growth, and coordinated differentiation of the follicle cell types, as evidenced by antral cavity formation, theca cell differentiation, oocyte maturation, and relative hormone production levels. While a stiff environment favored high progesterone and androgen secretion, decreasing alginate stiffness resulted in estrogen production, which exceeded progesterone and androgen accumulation.</td>
<td></td>
</tr>
<tr>
<td>Xu et al. (2006a)</td>
<td>Mouse</td>
<td>Multilayered secondary follicles (150–180 μm, type 5b)</td>
<td>1.5% (w/v) alginate</td>
<td>Embryos derived from cultured oocytes fertilized in vitro and transferred to pseudopregnant female mice were viable, and both male and female offspring were fertile.</td>
<td></td>
</tr>
<tr>
<td>Xu et al. (2006b)</td>
<td>Mouse</td>
<td>Two-layered follicle growth</td>
<td>0.25, 0.5, 1, and 2% rigidity alginate</td>
<td>The present study showed that the alginate scaffold consistency affects folliculogenesis and oocyte development in vitro and that the alginate culture system can and should be tailored to maximally support follicle growth depending on the size and stage of the follicles selected for culture.</td>
<td></td>
</tr>
<tr>
<td>Kreeger et al. (2006)</td>
<td>Mouse</td>
<td>Two-layered secondary follicles (100–130 μm, oocyte &lt;63 μm) and multilayered secondary follicles (150–180 μm)</td>
<td>RGD-alginate or alginate/ECM protein blends</td>
<td>Morphology of the follicle and provides an environment that supports follicle development. The ECM components signal the somatic cells of the follicle, affecting their growth and differentiation, and unexpectedly also affect the meiotic competence of the oocyte. These effects depend upon both the identity of the ECM components and the initial stage of the follicle, indicating that the ECM is a dynamic regulator of follicle development.</td>
<td></td>
</tr>
</tbody>
</table>

Table 1 Continued.
Table 1 Continued.

<table>
<thead>
<tr>
<th>Article</th>
<th>Species</th>
<th>Follicle class</th>
<th>Material</th>
<th>Results</th>
<th>Reproductive outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kreeger et al. (2005)</td>
<td>Mouse</td>
<td>Two-layered secondary (100-130 micron) and multilayered secondary (150-180 micron)</td>
<td>1.5% rigidity (w/v) alginate or alginate-collagen I matrices composed of 1.5% (w/v) alginate and 0.2 mg/mL of collagen I ECM</td>
<td>Responsive when cultured in alginate-collagen I matrices, exhibiting FSH dose-dependent increases in follicle growth, lactate production, and steroid secretion. Multilayered secondary follicles were FSH dependent, with follicle survival, growth, steroid secretion, metabolism, and oocyte maturation all regulated by FSH. However, doses greater than 25mIU/mL of FSH negatively impacted multilayered secondary follicle development (reduced follicle survival). The present results indicate that the hormonal and environmental needs of the follicular complex change during the maturation process.</td>
<td>Progression to MII</td>
</tr>
<tr>
<td>Pangas et al. (2003)</td>
<td>Mouse</td>
<td>COCs</td>
<td>1.9% (w/v)</td>
<td>The architecture of the follicular complex was maintained during the encapsulation and the subsequent culture. The granulosa cells proliferated, and the oocytes also grew in volume and obtained the structural characteristics of mature oocytes including cortical granule formation, a well-developed zona pellucida with microvilli, normal mitochondria, and lattice-like structures in the cytoplasm. Oocytes retrieved and matured were able to resume meiosis, a necessary step for proper development. Thus, this system represents a new in vitro methodology for growth of individual granulosa cell-oocyte complexes.</td>
<td>Progression to MII</td>
</tr>
<tr>
<td>Kreeger et al. (2003)</td>
<td>Mouse</td>
<td>GRM02 and primary granulosa cells</td>
<td>2% alginate ECM modified with RGD</td>
<td>Surfaces (0.02–0.2 ng/cm²) attached and spread, with morphologies specific to the peptide identity and density. Additionally, progesterone and estradiol secretion was a function of peptide density, with up to threefold increases compared to controls. These results indicate that the density and identity of adhesion peptides regulate granulosa cell function.</td>
<td></td>
</tr>
</tbody>
</table>

three-dimensional in vitro culture methods to grow follicles outside the ovary. Biomaterials engineer Dr Lonnie Shea and I worked together to develop a variety of physical and biomaterials to support in vitro follicle growth (Table 1 and Pangas et al. 2003, Kreeger et al. 2005, 2006, Xu et al. 2006a,b, 2009a,b,c, 2011, 2013, West et al. 2007, Shikanov et al. 2009, 2011a,b,c, West-Farrell et al. 2009, Jin et al. 2010a,b, Hirshfeld-Cytron et al. 2011, Laronda et al. 2014, Makanji et al. 2014a, Smith et al. 2014, Tagler et al. 2014, Skory et al. 2015, Xiao et al. 2015a,b). In particular, we used the algae-produced material alginate to encapsulate follicles into beads that provide a simple structural support for long-term culture. This alginate-based encapsulated in vitro follicle growth (eIVFG) culture system supports coordinated granulosa cell proliferation and oocyte growth, the development of mural and cumulus granulosa cell layers and development of a theca-like layer at a precise distance from the central egg: the development of an antral cavity at the time of cumulus cell differentiation and follicle-stimulating hormone (FSH)-induced expression of the luteinizing hormone (LH) receptors necessary for ‘ovulation’ of the mature egg from the follicle following human chorionic gonadotropin (hCG) induction. This has been accomplished across multiple species including the mouse, cat, dog, sheep, cow, nonhuman primate and human (Fig. 1). Perhaps more profoundly, after ovulation, the surface of the follicle re-epithelializes and the granulosa cells transform to display a luteal cell phenotype and function (Skory et al. 2015). No prior system has been able to support the entire arc of follicle maturation from a follicular to luteal structure in vitro.

Importantly, because of eIVFG, we know that hormones are necessary to drive follicle maturation through each
of these stages; to this dogma, we can now add the fact that ovarian follicles are themselves autonomous in their ability to respond to these signals, with no additional innervation, vasculature or circulating factors aside from gonadotropins needed to support follicle development and differentiation. This is an important observation that enables new thinking about the manipulation of follicles for the purpose of contraceptive development, follicle preservation technologies and new approaches to human diseases like polycystic ovary syndrome (PCOS) and premature ovarian insufficiency (POI). We were the first two to show that oocytes can mature entirely in vitro, giving rise to live birth in the mouse and to human metaphase II (MII) eggs competent for fertilization (Fig. 1). While critical to our understanding of the ability of oocytes to mature in vitro, more work is necessary to determine the optimal conditions for supporting the intertwined developmental processes of follicle growth and oocyte maturation.

**Hormones, architecture and environment – a revised central dogma for follicle activation**

Ovarian follicles require the gonadotropins to drive growth and maturation. Our eIVFG cultures demonstrated the necessity for maintaining the follicle’s 3D architecture – the precise relationship of the somatic and germ cells – for the accurate development of each cell compartment, including the oocyte. In addition to hormones and architecture, we have learned more about the contribution of a third factor – the physical environment – to follicle fate determination. The primordial follicle pool and growing follicles are partitioned into regions of the ovary that have different physical properties. The cortex is relatively avascular and collagen rich with the medulla having more fibronectin and vascular investments (Laronda et al. 2015). The physical rigidity of the ovary changes with age and in cases of PCOS-related infertility. The aging ovary becomes more fibrotic with every ovulation cycle, which likely contributes to the loss of fertility in the late 30s in ways yet to be fully described. Physicians have described PCOS ovaries as more rigid; surgical wedge resections or drilling were used therapeutically for many years to activate follicles and achieve short-term resumption of cyclicity.

Determining the actual physical properties of soft organs is a challenge for the entire field of biomedical engineering, as tissue sizes and complexities are not yet fully amenable to the tools we have today. To study ovary
Ovarian follicle biology

We initially invented an MRE driver that focuses waves to the ovary, but eventually were able to collect and publish data using conventional MRE drivers, comparing ovarian rigidity in women with normal or PCOS ovaries. These data consistently showed a stiffer tissue profile associated with the PCOS ovary compared with normally cycling women. The ultimate utility of these metrics may emerge in the pediatric and adolescent population, where the PCOS phenotype is appreciated only after durable menstrual issues that may go unrecognized and complicate the spectrum of androgen-affected tissue fates. If ovarian rigidity is indeed a clinically relevant measure, having an alternative to transvaginal ultrasound for noninvasive assessment will be important for these populations.

We also modeled the physiological effects of physical rigidity in our eIVFG system, by changing alginate concentration, altering alginate length and introducing various combinations of biomaterials and extracellular matrix components or whole molecules (Table 2) (Pangas et al. 2003, Kreeger et al. 2005, 2006, Xu et al. 2006a,b, 2009a,b,c, 2011, 2013, West et al. 2007, Shikanov et al. 2009, 2011a,b,c, West-Farrell et al. 2009, Jin et al. 2010a,b, Hirshfield-Cyron et al. 2011, Laronda et al. 2014, Makanji et al. 2014a, Smith et al. 2014, Tagler et al. 2014, Skory et al. 2015, Xiao et al. 2015a,b). Fundamentally, we learned that the physical environment informs follicle development and, along with follicle architecture, is as important to follicle biology as the hormonal factors that have been known for decades.

### Follicles communicate with each other and likely between the ovaries in ways yet to be discovered

One of the most interesting studies we conducted was the assessment of follicle growth when multiple follicles are encapsulated together in a single bead. We learned that interfollicular factors support follicle health and development (Tingen et al. 2011, Hornick et al. 2012, 2013, Tagler et al. 2012, Laronda et al. 2014). This was particularly important for the growth of small primary follicles that were only able to grow autonomously when supplemented with factors produced by the surrounding stroma or supplemented with factors from mouse embryonic fibroblast (MEF) cultures. Identifying these factors is an important next step for the field. They may include peptide factors that are able to traffic between follicles, bioactive lipids that can act through tissues rather than via the bloodstream or miRNAs that modify or regulate RNAs that function in follicle health.

One reason ovarian follicles struggle to re-establish a cyclical hierarchy after chemotherapy or radiation therapy may be related to the disruption of intrafollicular signaling pathways. Learning about the factors involved in this interfollicular communication may provide new inroads into supporting ovarian health in cancer survivors. This work also informed our development of an ovarian bioprosthesis (Laronda et al. 2015, 2017, Jakus et al. 2017). The integration of multiple follicle types into the 3D printed bioprosthesis enabled the resulting ovarian tissue to function through ovulation and lactation and have remaining subordinate follicles ready for a new round of selection. These bioprosthetics are creating new ways for soft tissue function to be restored and for fundamental new knowledge to be created using anatomically inclusive models of ovarian function. While reductionism works to understand first principles (the autonomous nature of follicles once activated), to understand the overall workings of the ovary, an intact tissue architecture is critical.

### Primordial follicle activation is the next frontier

While primordial follicles represent the dominant follicle type in the ovary, they are also the most elusive in terms of biology. We created a triple transgenic mouse to see if we could ‘fate map’ follicle activation.

---

Table 2  Milestones in follicle maturation ex vivo.

<table>
<thead>
<tr>
<th>Biomaterial environment</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Algin</td>
<td>Fibrin-alginate/interpenetrating networks (IPN)</td>
<td>PEG/PEG-X</td>
<td>Primordial follicles</td>
<td>Co-cultured cells</td>
<td>Co-cultured follicles</td>
</tr>
<tr>
<td>Animal environment</td>
<td>Age, metabolic status, weight</td>
<td>Species</td>
<td>Mouse</td>
<td>Nonhuman primate</td>
<td>Human</td>
<td></td>
</tr>
<tr>
<td>Physical environment</td>
<td>Rigidity</td>
<td>ECM</td>
<td>Media composition</td>
<td>Oxygen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellular environment</td>
<td>Stroma/theca</td>
<td>Co-cultured follicles</td>
<td>Co-cultured cells</td>
<td>Cortical cultures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human environment</td>
<td>Policy</td>
<td>Law</td>
<td>Religion</td>
<td>Ethics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicle stage</td>
<td>*</td>
<td>Primordial</td>
<td>**</td>
<td>Primary</td>
<td>***</td>
<td>Secondary</td>
</tr>
</tbody>
</table>

r agility, therefore, we adopted the emerging technique of magnetic resonance elastography (MRE), which uses sound waves to construct images of tissue-level stiffness in vivo (Wood et al. 2015). We initially invented an MRE driver that focuses waves to the ovary, but eventually were able to collect and publish data using conventional MRE drivers, comparing ovarian rigidity in women with normal or PCOS ovaries. These data consistently showed a stiffer tissue profile associated with the PCOS ovary compared with normally cycling women. The ultimate utility of these metrics may emerge in the pediatric and adolescent population, where the PCOS phenotype is appreciated only after durable menstrual issues that may go unrecognized and complicate the spectrum of androgen-affected tissue fates. If ovarian rigidity is indeed a clinically relevant measure, having an alternative to transvaginal ultrasound for noninvasive assessment will be important for these populations.

We also modeled the physiological effects of physical rigidity in our eIVFG system, by changing alginate concentration, altering alginate length and introducing various combinations of biomaterials and extracellular matrix components or whole molecules (Table 2) (Pangas et al. 2003, Kreeger et al. 2005, 2006, Xu et al. 2006a,b, 2009a,b,c, 2011, 2013, West et al. 2007, Shikanov et al. 2009, 2011a,b,c, West-Farrell et al. 2009, Jin et al. 2010a,b, Hirshfield-Cyron et al. 2011, Laronda et al. 2014, Makanji et al. 2014a, Smith et al. 2014, Tagler et al. 2014, Skory et al. 2015, Xiao et al. 2015a,b). Fundamentally, we learned that the physical environment informs follicle development and, along with follicle architecture, is as important to follicle biology as the hormonal factors that have been known for decades.

### Follicles communicate with each other and likely between the ovaries in ways yet to be discovered

One of the most interesting studies we conducted was the assessment of follicle growth when multiple follicles are encapsulated together in a single bead. We learned that interfollicular factors support follicle health and development (Tingen et al. 2011, Hornick et al. 2012, 2013, Tagler et al. 2012, Laronda et al. 2014). This was particularly important for the growth of small primary follicles that were only able to grow autonomously when supplemented with factors produced by the surrounding stroma or supplemented with factors from mouse embryonic fibroblast (MEF) cultures. Identifying these factors is an important next step for the field. They may include peptide factors that are able to traffic between follicles, bioactive lipids that can act through tissues rather than via the bloodstream or miRNAs that modify or regulate RNAs that function in follicle health.

One reason ovarian follicles struggle to re-establish a cyclical hierarchy after chemotherapy or radiation therapy may be related to the disruption of intrafollicular signaling pathways. Learning about the factors involved in this interfollicular communication may provide new inroads into supporting ovarian health in cancer survivors. This work also informed our development of an ovarian bioprosthesis (Laronda et al. 2015, 2017, Jakus et al. 2017). The integration of multiple follicle types into the 3D printed bioprosthesis enabled the resulting ovarian tissue to function through ovulation and lactation and have remaining subordinate follicles ready for a new round of selection. These bioprosthetics are creating new ways for soft tissue function to be restored and for fundamental new knowledge to be created using anatomically inclusive models of ovarian function. While reductionism works to understand first principles (the autonomous nature of follicles once activated), to understand the overall workings of the ovary, an intact tissue architecture is critical.

### Primordial follicle activation is the next frontier

While primordial follicles represent the dominant follicle type in the ovary, they are also the most elusive in terms of biology. We created a triple transgenic mouse to see if we could ‘fate map’ follicle activation.

---

Table 2  Milestones in follicle maturation ex vivo.

<table>
<thead>
<tr>
<th>Biomaterial environment</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Algin</td>
<td>Fibrin-alginate/interpenetrating networks (IPN)</td>
<td>PEG/PEG-X</td>
<td>Primordial follicles</td>
<td>Co-cultured cells</td>
<td>Co-cultured follicles</td>
</tr>
<tr>
<td>Animal environment</td>
<td>Age, metabolic status, weight</td>
<td>Species</td>
<td>Mouse</td>
<td>Nonhuman primate</td>
<td>Human</td>
<td></td>
</tr>
<tr>
<td>Physical environment</td>
<td>Rigidity</td>
<td>ECM</td>
<td>Media composition</td>
<td>Oxygen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellular environment</td>
<td>Stroma/theca</td>
<td>Co-cultured follicles</td>
<td>Co-cultured cells</td>
<td>Cortical cultures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human environment</td>
<td>Policy</td>
<td>Law</td>
<td>Religion</td>
<td>Ethics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicle stage</td>
<td>*</td>
<td>Primordial</td>
<td>**</td>
<td>Primary</td>
<td>***</td>
<td>Secondary</td>
</tr>
</tbody>
</table>

### Follicles communicate with each other and likely between the ovaries in ways yet to be discovered

One of the most interesting studies we conducted was the assessment of follicle growth when multiple follicles are encapsulated together in a single bead. We learned that interfollicular factors support follicle health and development (Tingen et al. 2011, Hornick et al. 2012, 2013, Tagler et al. 2012, Laronda et al. 2014). This was particularly important for the growth of small primary follicles that were only able to grow autonomously when supplemented with factors produced by the surrounding stroma or supplemented with factors from mouse embryonic fibroblast (MEF) cultures. Identifying these factors is an important next step for the field. They may include peptide factors that are able to traffic between follicles, bioactive lipids that can act through tissues rather than via the bloodstream or miRNAs that modify or regulate RNAs that function in follicle health.

One reason ovarian follicles struggle to re-establish a cyclical hierarchy after chemotherapy or radiation therapy may be related to the disruption of intrafollicular signaling pathways. Learning about the factors involved in this interfollicular communication may provide new inroads into supporting ovarian health in cancer survivors. This work also informed our development of an ovarian bioprosthesis (Laronda et al. 2015, 2017, Jakus et al. 2017). The integration of multiple follicle types into the 3D printed bioprosthesis enabled the resulting ovarian tissue to function through ovulation and lactation and have remaining subordinate follicles ready for a new round of selection. These bioprosthetics are creating new ways for soft tissue function to be restored and for fundamental new knowledge to be created using anatomically inclusive models of ovarian function. While reductionism works to understand first principles (the autonomous nature of follicles once activated), to understand the overall workings of the ovary, an intact tissue architecture is critical.

### Primordial follicle activation is the next frontier

While primordial follicles represent the dominant follicle type in the ovary, they are also the most elusive in terms of biology. We created a triple transgenic mouse to see if we could ‘fate map’ follicle activation.
We showed that the first wave of follicles activated in the developing ovary is located in a specific region of the ovary (Cordeiro et al. 2015). We also created an animal model with a constitutively active allele of the PI3 kinase subunit (PI3K-Ca) (Kim et al. 2015), based on Dr So-Youn Kim’s efforts to create an oocyte-specific transgenic mouse. I was a bit reluctant to travel down this particular path, because I predicted the PI3K-Ca mouse follicle activation profile would phenocopy that of the PTEN knockout. Dr Kim wanted to test this hypothesis directly, predicting instead that the repressive pathway (PTEN) would be dominant over the activating (PI3K) pathway. In the end, she was correct. While knocking out the ‘break’ to follicle activation (PTEN) results in rapid activation of the entire follicle cohort with early POI, knocking in an active version of the ‘accelerator’ (PI3K) results in a larger number of follicles activated over unit time, but not a simultaneous activation of all follicles as seen in the PTEN knockout. The PI3K-Ca mouse ovarian reserve remained intact through 60 days of life, at which point the local effect of constitutively active PI3K leads to the onset of a fully penetrant granulosa cell cancer (Kim et al. 2016). This study points out how cagey ovarian follicle biology can be and also reinforces the notion that regulation of the primordial follicle pool is dominated by repressive factors. More is to be learned in this area of active research, and elucidating the specific way in which the break and the accelerator drive the ovarian reserve will be an important undertaking.

Important advances in primordial follicles came from altering the biomaterial environment and physical rigidity and led to the development of systems that support folliculogenesis from these earliest stage follicles in multiple species (Table 2).

### Integrating hormones into organ culture changes everything

Interestingly, our ovarian follicle models provided an opportunity to link the endocrine hormones to follicle biology, but we required the development of a new platform for linking reproductive tissue constructs in vitro. The key for this project was the fact that our ovarian follicles not only grow in culture, producing estradiol and inhibin along the way, they also ovulate in vitro and the granulosa cells transform into the luteal tissue that makes progesterone. We collaborated with the Draper Laboratories to invent and deploy four microfluidic platforms (MFPs): Solo-MFP, Duet-MFP, MyCure-MFP

### Table 3 Contributions to follicle biology.

<table>
<thead>
<tr>
<th>Follicle biology</th>
<th>Follicle maturation is autonomous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theca cells differentiation is autonomous to the follicle</td>
<td></td>
</tr>
<tr>
<td>Ovulation is autonomous to the follicle</td>
<td></td>
</tr>
<tr>
<td>Physical rigidity alone can alter the hormone synthesis ratio (increasing rigidity favors androgen over estrogen)</td>
<td></td>
</tr>
<tr>
<td>The ovulation rupture zone is a unique site</td>
<td></td>
</tr>
<tr>
<td>Follicles reconstruct a holostructure after rupture in an autonomous manner</td>
<td></td>
</tr>
<tr>
<td>The granulosa to luteal transition does not require exogenous cells</td>
<td></td>
</tr>
<tr>
<td>Mouse follicles can function on a 28-day cycle</td>
<td></td>
</tr>
<tr>
<td>First live birth in mouse from in vitro-grown 3D follicles</td>
<td></td>
</tr>
<tr>
<td>First human MII eggs from in vitro-grown human follicles</td>
<td></td>
</tr>
<tr>
<td>First ovarian bioprosthetic</td>
<td></td>
</tr>
<tr>
<td>First microfluidic system that integrates ovarian function with reproductive tract tissues</td>
<td></td>
</tr>
<tr>
<td>Zinc biology</td>
<td>20 billion zinc atoms are concentrated during meiotic maturation in the egg</td>
</tr>
<tr>
<td>Telophase includes a zinc-depending zinc switch</td>
<td></td>
</tr>
<tr>
<td>Zinc is exocytosed at the time of fertilization (zinc spark)</td>
<td></td>
</tr>
<tr>
<td>Zinc is the cytoplasmic maturation factor</td>
<td></td>
</tr>
<tr>
<td>Zinc is the factor responsible for the fast block to polyspermy</td>
<td></td>
</tr>
<tr>
<td>Egg biology</td>
<td>Meiotic chromosomes are ten times stiffer than mitotic chromosomes</td>
</tr>
<tr>
<td>Meiotic chromosomes become more stiff with age</td>
<td></td>
</tr>
<tr>
<td>Genes in polar body 1 and polar body 2 are indicative of egg maturity</td>
<td></td>
</tr>
<tr>
<td>Terms coined</td>
<td>Oncofertility – field of medicine at the intersection of cancer and reproductive care</td>
</tr>
<tr>
<td>Bioprosthetics – tissue construct that restores soft organ function; originally ovarian bioprosthetic but term appropriate to any biologically constructed tissue</td>
<td></td>
</tr>
<tr>
<td>Repropedia – short definitions, images or videos of reproductive terms; can be linked to any website</td>
<td></td>
</tr>
<tr>
<td>Repropedia – aggregate site for educational tools in the reproductive sciences</td>
<td></td>
</tr>
<tr>
<td>EVATAR – name of microfluidic system connecting ovary, fallopian tube, uterus, cervix and liver tissue constructs; functions for 28 days</td>
<td></td>
</tr>
<tr>
<td>Policies addressed</td>
<td>Zinc spark – term for exocytotic release of zinc atoms at the time of fertilization</td>
</tr>
<tr>
<td>Inclusion of sex as a biological variable in biomedical research</td>
<td></td>
</tr>
<tr>
<td>Economic gaps for women in science</td>
<td></td>
</tr>
<tr>
<td>Need for rational policies on the use of human gametes in research</td>
<td></td>
</tr>
<tr>
<td>Insurance and reimbursement for oncofertility care</td>
<td></td>
</tr>
<tr>
<td>Coordinated oncofertility science and medicine</td>
<td></td>
</tr>
</tbody>
</table>
(a duet that recirculates) and a Quintet-MFP (Xiao et al. 2017). In these systems, we cultured mouse ovarian follicles and ovaries along with human fallopian tubes, uterus, cervix and liver organoids (Laronda et al. 2013, Eddie et al. 2014, Arslan et al. 2015, Eddie et al. 2015, Zhu et al. 2016, Olalekan et al. 2017). This was a fascinating project and it took 5 years to invent a device that could support tissue-level function using fluidic channels that could manage the long-term (over 30 days), accurate movement of media between tissues. This undertaking reminded me how remarkable our own circulatory system is, with arteries and veins, capillaries and tissues integrated into a microfluidic system. We were able to have all tissues in the system function for at least 30 days and produce the expected rise and fall of follicular and luteal hormones, punctuated by ovulation at midcycle. Downstream tissues functioned in response to the changing hormones, making this one of the most important and exciting studies my lab has done. The ability to study tissue-level function in the face of changing reproductive hormones is a true game-changer for biology. Up to this point, the fluctuating endocrine hormones in males and females have never been recapitulated in cell cultures, which are all usually composed of individual cell types grown on flat plastic. I predict a new level of biological discovery will come from this work, rewriting the textbooks regarding signal transduction events and leading to a better framework for understanding and interrogating the biological basis of health and disease.

Discovery of zinc fluxes during meiotic maturation

There are few discoveries that have no antecedent experimental basis. The release of zinc from a mature human egg at the time of fertilization is that rare discovery that came about because an inorganic chemist and a reproductive biologist had a conversation about sperm. Zinc is known to concentrate in the semen, and when pressed with the teleological question about why this should be, a remarkable collaboration began that provided not only evidence of a new meiotic checkpoint in the oocyte, but a series of tools that are now poised to unlock the biology of zinc as a cell signal within the cell and a paracrine factor outside the cell (Kim et al. 2010, 2011, Bernhardt et al. 2011, 2012, Kong et al. 2012, 2014, 2015, Hong et al. 2014, Que et al. 2015, Duncan et al. 2016, Zhang et al. 2015, Mendoza et al. 2017, Que et al. 2017, 2019). Perhaps most importantly, this new area of chemical biology revealed an extracellular marker (the ‘zinc spark’) that predicts the potential of an egg to become an embryo. The development and application of this observation may provide IVF clinics with earlier and more precise insights into the quality of eggs and a way to select a single embryo for transfer.

Fluctuations in the intracellular concentration of inorganic elements are essential signaling mechanisms in a variety of cell types. While these phenomena are well documented for alkali and alkaline earth metals such as potassium and calcium, a similar signaling role for transition metals like zinc has not been documented until now. We described the zinc physiology of the mammalian oocyte as it completes maturation and initiates early embryonic development. We made three fundamental discoveries regarding the physiology of zinc in the egg. First, single-cell elemental analysis revealed that zinc is the most abundant transition metal in the fully grown mouse oocyte, egg and early embryo, and its total intracellular levels fluctuate dynamically during the developmental transitions between stages. We discovered that the pathways controlled through MTF-1 and MTF-2 are downregulated after the oocyte becomes meiotically competent, which mechanistically explains the unrestrained zinc influx. Second, we found that an increase in the zinc quota during meiotic maturation is necessary to drive exit from meiosis I; induction of zinc insufficiency in maturing oocytes via small-molecule chelators results in premature meiotic arrest at telophase I. We showed that zinc is the developmental switch that controls the activity of the zinc-binding meiotic regulator Emi2. Fundamentally, Emi2 acts as a zinc sensor, with fluxes in cellular zinc driving meiotic transitions. This is the first case in which zinc was shown to bind directly to a protein that transmits its signal associated with a cellular function that is not related to transcriptional or translational regulation. Finally, an abrupt decrease in the zinc quota upon egg activation is achieved through an intense exocytosis event called a ‘zinc spark’. The lower intracellular level of zinc after the spark is necessary to permit cell cycle resumption; an artificial increase in intracellular zinc levels leads to the maintenance of a metaphase arrest in activated eggs. This represents a new biological paradigm for zinc and identifies a key signaling role for this ligand during meiotic progression.

We also found that zinc is the most abundant transition metal in the mammalian preimplantation embryo; perturbation of zinc homeostasis in cleavage stage embryos disrupts mitotic progression; and zinc chelation in the early embryo causes altered chromatin structure and global transcription defects. Taken together, we have identified a new biology, a new signal and new signaling mechanisms that are necessary for oocyte meiosis, at a time when transcription is quiescent.

Summary

My work in the field of ovarian follicle biology and oocyte health has relied on cross-disciplinary collaborations that span material science, chemistry, bioengineering and pure engineering. By integrating new perspectives and applying new tools to biological questions, we have learned much more than we might have if we had focused only on one kind of scientific inquiry (Table 3). Ultimately, it is my hope that this work will be translated to
inform the field of onc fertility and fertility preservation, and the basic science discoveries associated with zinc fluxes in meiotic maturation, the live birth from the first-ever soft tissue ovarian bioprosthetic and the creation of the microfluidic platform and the EVATAR reproductive tract will be lasting imprints on the most important field for our literal future — reproduction.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding

This work did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector.

Acknowledgements

I am delighted to be included in this special edition of Reproduction and acknowledge that all of the works described above were done collaboratively with my mentors, collaborators and my tremendous lab members over 24 years of running an independent lab. Every co-author is a friend and colleague and has created a meaningful narrative of friendship and learning over my career. I want to note, in particular, my mentors Kelly Mayo and Neena Schwartz, longstanding collaborator Lonnie Shea and my zinc co-discoverer, Tom O’Halloran. This narrative review is told from the perspective of my laboratory and its contributions to the discipline of reproduction and there are also many groups worldwide who have made leaps in discoveries that aligned and propel this field forward.

References


Duncan FE, Que EL, Zhang N, Feinberg EC, O’Halloran TV & Woodruff TK 2016 The zinc spark is an inorganic signature of human egg activation. Scientific Reports 6 24737. (https://doi.org/10.1038/srep24737)


Hornick JE, Duncan FE, Shea LD & Woodruff TK 2013 Multiple follicle culture supports primordial follicle growth through paracrine-acting signals. Reproduction 145 19–32. (https://doi.org/10.1530/REP-12-0233)


Kim AM, Bernhardt ML, Kong BY, Ahn RW, Vogt S, Woodruff TK & O’Halloran TV 2011 Zinc sparks are triggered by fertilization and facilitate cell cycle resumption in mammalian eggs. ACS Chemical Biology 6 716–723. (https://doi.org/10.1021/cb200084v)


Kniazeva E, Hardy AN, Boukaidi SA, Woodruff TK, Jeruss JS & Shea LD 2015 Primordial follicle transplantation within designer biomaterial grafts produce live births in a mouse infertility model. Scientific Reports 5 17709. (https://doi.org/10.1038/srep17709)


https://rep.bioscientifica.com
Ovarian follicle biology

2018 Retrievable hydrogels for ovulation transplantation.

2017 A bioprosthetic ovary created using 3D printed microporous scaffolds.

2017 Zinc sparks induce cell death.

2014 Bovine eggs release zinc in response to parthenogenetic activation.

2009 Interpenetrating fibrin matrix gels.

2011 Structure of collagen type I.

2011 Gene expression in mouse ovarian follicle development.

2013 Microarray analysis identifies COMP as the most differentially regulated transcript.

2012 Embryonic fibroblasts enable the culture of primary ovarian follicles.

2010 The cell–oocyte complex.

2019 Gene expression in mouse ovarian follicle development in vivo versus an ex vivo algin system.

2014 Fibrin-mediated delivery of an ovarian follicle pool.

2012 Embryonic fibroblasts enable the culture of primary ovarian follicles.

2010 Gene expression in mouse ovarian follicle development.

2009 The three-dimensional culture of granulosa cell-oocyte complexes.

2019 Bovine eggs release zinc in response to parthenogenetic and sperm-induced egg activation.

2014 Gene expression in mouse ovarian follicle development.

2013 Microarray analysis identifies COMP as the most differentially regulated transcript.

2012 Embryonic fibroblasts enable the culture of primary ovarian follicles.

2010 The cell–oocyte complex.

2019 Gene expression in mouse ovarian follicle development in vivo versus an ex vivo algin system.

2014 Fibrin-mediated delivery of an ovarian follicle pool.

2012 Embryonic fibroblasts enable the culture of primary ovarian follicles.

2010 Gene expression in mouse ovarian follicle development.

2009 The three-dimensional culture of granulosa cell-oocyte complexes.

2019 Bovine eggs release zinc in response to parthenogenetic and sperm-induced egg activation.

2014 Gene expression in mouse ovarian follicle development.

2013 Microarray analysis identifies COMP as the most differentially regulated transcript.

2012 Embryonic fibroblasts enable the culture of primary ovarian follicles.

2010 Gene expression in mouse ovarian follicle development.

2009 The three-dimensional culture of granulosa cell-oocyte complexes.

2019 Bovine eggs release zinc in response to parthenogenetic and sperm-induced egg activation.

2014 Gene expression in mouse ovarian follicle development.

2013 Microarray analysis identifies COMP as the most differentially regulated transcript.

2012 Embryonic fibroblasts enable the culture of primary ovarian follicles.

2010 Gene expression in mouse ovarian follicle development.

2009 The three-dimensional culture of granulosa cell-oocyte complexes.

2019 Bovine eggs release zinc in response to parthenogenetic and sperm-induced egg activation.

2014 Gene expression in mouse ovarian follicle development.

2013 Microarray analysis identifies COMP as the most differentially regulated transcript.

2012 Embryonic fibroblasts enable the culture of primary ovarian follicles.

2010 Gene expression in mouse ovarian follicle development.

2009 The three-dimensional culture of granulosa cell-oocyte complexes.

2019 Bovine eggs release zinc in response to parthenogenetic and sperm-induced egg activation.

2014 Gene expression in mouse ovarian follicle development.

2013 Microarray analysis identifies COMP as the most differentially regulated transcript.

2012 Embryonic fibroblasts enable the culture of primary ovarian follicles.

2010 Gene expression in mouse ovarian follicle development.

2009 The three-dimensional culture of granulosa cell-oocyte complexes.

2019 Bovine eggs release zinc in response to parthenogenetic and sperm-induced egg activation.

2014 Gene expression in mouse ovarian follicle development.

2013 Microarray analysis identifies COMP as the most differentially regulated transcript.

2012 Embryonic fibroblasts enable the culture of primary ovarian follicles.

2010 Gene expression in mouse ovarian follicle development.

2009 The three-dimensional culture of granulosa cell-oocyte complexes.

2019 Bovine eggs release zinc in response to parthenogenetic and sperm-induced egg activation.

2014 Gene expression in mouse ovarian follicle development.

2013 Microarray analysis identifies COMP as the most differentially regulated transcript.

2012 Embryonic fibroblasts enable the culture of primary ovarian follicles.

2010 Gene expression in mouse ovarian follicle development.

2009 The three-dimensional culture of granulosa cell-oocyte complexes.

2019 Bovine eggs release zinc in response to parthenogenetic and sperm-induced egg activation.

2014 Gene expression in mouse ovarian follicle development.

2013 Microarray analysis identifies COMP as the most differentially regulated transcript.

2012 Embryonic fibroblasts enable the culture of primary ovarian follicles.

2010 Gene expression in mouse ovarian follicle development.

2009 The three-dimensional culture of granulosa cell-oocyte complexes.

2019 Bovine eggs release zinc in response to parthenogenetic and sperm-induced egg activation.

2014 Gene expression in mouse ovarian follicle development.

2013 Microarray analysis identifies COMP as the most differentially regulated transcript.

2012 Embryonic fibroblasts enable the culture of primary ovarian follicles.

2010 Gene expression in mouse ovarian follicle development.

2009 The three-dimensional culture of granulosa cell-oocyte complexes.

2019 Bovine eggs release zinc in response to parthenogenetic and sperm-induced egg activation.

2014 Gene expression in mouse ovarian follicle development.

2013 Microarray analysis identifies COMP as the most differentially regulated transcript.

2012 Embryonic fibroblasts enable the culture of primary ovarian follicles.

2010 Gene expression in mouse ovarian follicle development.

2009 The three-dimensional culture of granulosa cell-oocyte complexes.

2019 Bovine eggs release zinc in response to parthenogenetic and sperm-induced egg activation.

2014 Gene expression in mouse ovarian follicle development.

2013 Microarray analysis identifies COMP as the most differentially regulated transcript.

2012 Embryonic fibroblasts enable the culture of primary ovarian follicles.

2010 Gene expression in mouse ovarian follicle development.

2009 The three-dimensional culture of granulosa cell-oocyte complexes.

2019 Bovine eggs release zinc in response to parthenogenetic and sperm-induced egg activation.

2014 Gene expression in mouse ovarian follicle development.

2013 Microarray analysis identifies COMP as the most differentially regulated transcript.

2012 Embryonic fibroblasts enable the culture of primary ovarian follicles.

2010 Gene expression in mouse ovarian follicle development.

2009 The three-dimensional culture of granulosa cell-oocyte complexes.

2019 Bovine eggs release zinc in response to parthenogenetic and sperm-induced egg activation.

2014 Gene expression in mouse ovarian follicle development.

2013 Microarray analysis identifies COMP as the most differentially regulated transcript.

2012 Embryonic fibroblasts enable the culture of primary ovarian follicles.

2010 Gene expression in mouse ovarian follicle development.

2009 The three-dimensional culture of granulosa cell-oocyte complexes.

2019 Bovine eggs release zinc in response to parthenogenetic and sperm-induced egg activation.

2014 Gene expression in mouse ovarian follicle development.

2013 Microarray analysis identifies COMP as the most differentially regulated transcript.

2012 Embryonic fibroblasts enable the culture of primary ovarian follicles.

2010 Gene expression in mouse ovarian follicle development.

2009 The three-dimensional culture of granulosa cell-oocyte complexes.

2019 Bovine eggs release zinc in response to parthenogenetic and sperm-induced egg activation.

2014 Gene expression in mouse ovarian follicle development.

2013 Microarray analysis identifies COMP as the most differentially regulated transcript.

2012 Embryonic fibroblasts enable the culture of primary ovarian follicles.

2010 Gene expression in mouse ovarian follicle development.

2009 The three-dimensional culture of granulosa cell-oocyte complexes.

2019 Bovine eggs release zinc in response to parthenogenetic and sperm-induced egg activation.

2014 Gene expression in mouse ovarian follicle development.

2013 Microarray analysis identifies COMP as the most differentially regulated transcript.

2012 Embryonic fibroblasts enable the culture of primary ovarian follicles.

2010 Gene expression in mouse ovarian follicle development.

2009 The three-dimensional culture of granulosa cell-oocyte complexes.

2019 Bovine eggs release zinc in response to parthenogenetic and sperm-induced egg activation.

2014 Gene expression in mouse ovarian follicle development.

2013 Microarray analysis identifies COMP as the most differentially regulated transcript.

2012 Embryonic fibroblasts enable the culture of primary ovarian follicles.

2010 Gene expression in mouse ovarian follicle development.

2009 The three-dimensional culture of granulosa cell-oocyte complexes.

2019 Bovine eggs release zinc in response to parthenogenetic and sperm-induced egg activation.

2014 Gene expression in mouse ovarian follicle development.

2013 Microarray analysis identifies COMP as the most differentially regulated transcript.

2012 Embryonic fibroblasts enable the culture of primary ovarian follicles.

2010 Gene expression in mouse ovarian follicle development.

2009 The three-dimensional culture of granulosa cell-oocyte complexes.

2019 Bovine eggs release zinc in response to parthenogenetic and sperm-induced egg activation.

2014 Gene expression in mouse ovarian follicle development.

2013 Microarray analysis identifies COMP as the most differentially regulated transcript.

2012 Embryonic fibroblasts enable the culture of primary ovarian follicles.

2010 Gene expression in mouse ovarian follicle development.

2009 The three-dimensional culture of granulosa cell-oocyte complexes.


Zhang N, Duncan FE, Que EL, O’Halloran TV & Woodruff TK 2016 The fertilization-induced zinc spark is a novel biomarker of mouse embryo quality and early development. Scientific Reports 6 22772. (https://doi.org/10.1038/srep22772)


Received 25 April 2019
First decision 17 May 2019
Revised manuscript received 25 June 2019
Accepted 15 July 2019