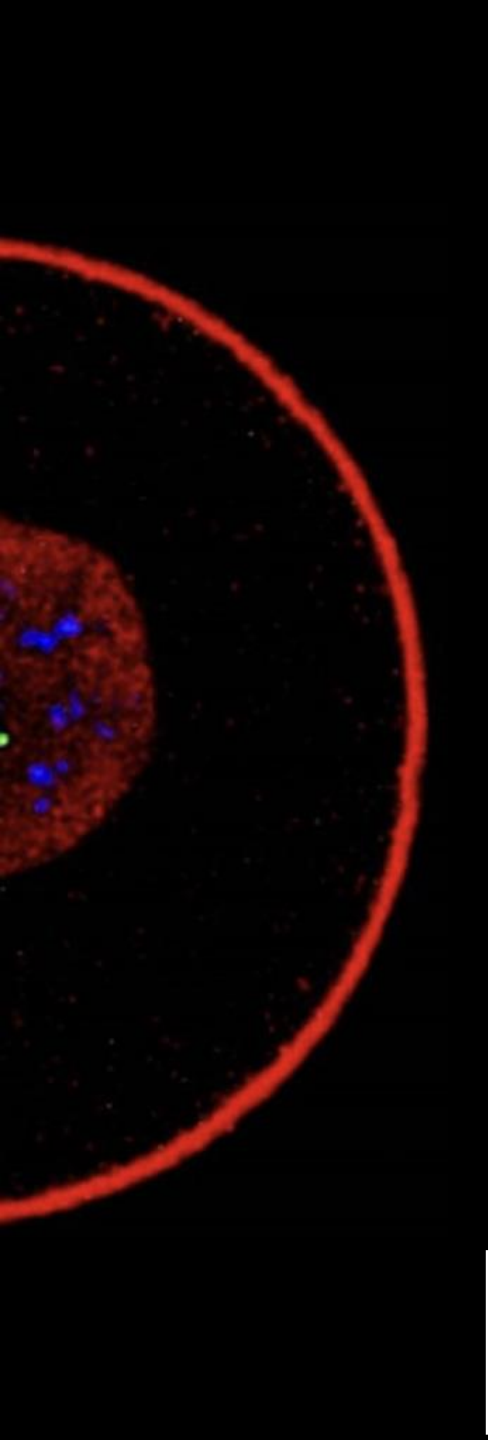


Studying the oocyte cortical domain: implications for the clinic

Giovanni Coticchio

IVIRMA ITALIA

IVIRMA Global Research Alliance

Outline

- Structure and function
- A major player: the subcortical maternal complex
- Practical implications for human IVF

No conflict of interest relevant to this talk's topic

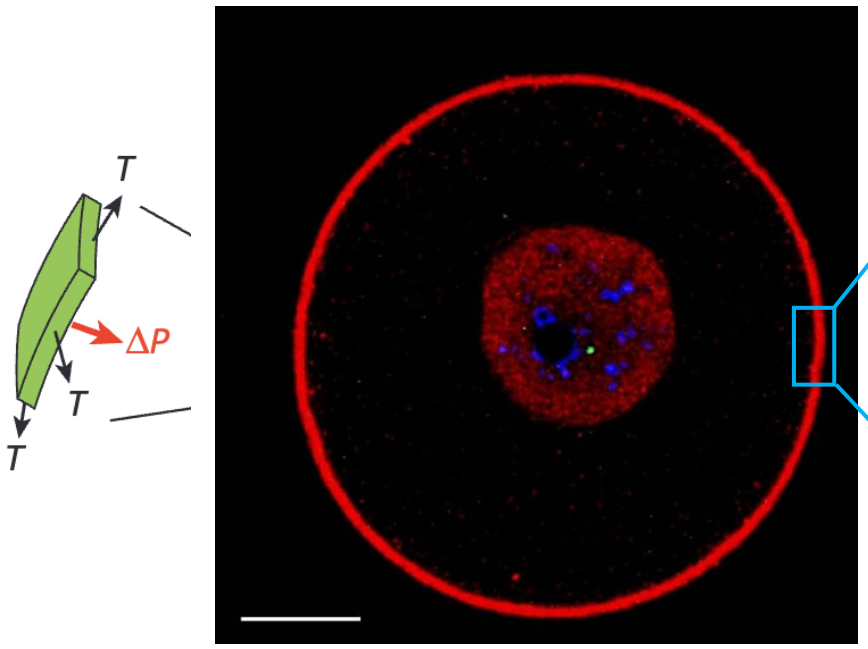
Structure and function

The Cell Cortex: Structure and General Functions

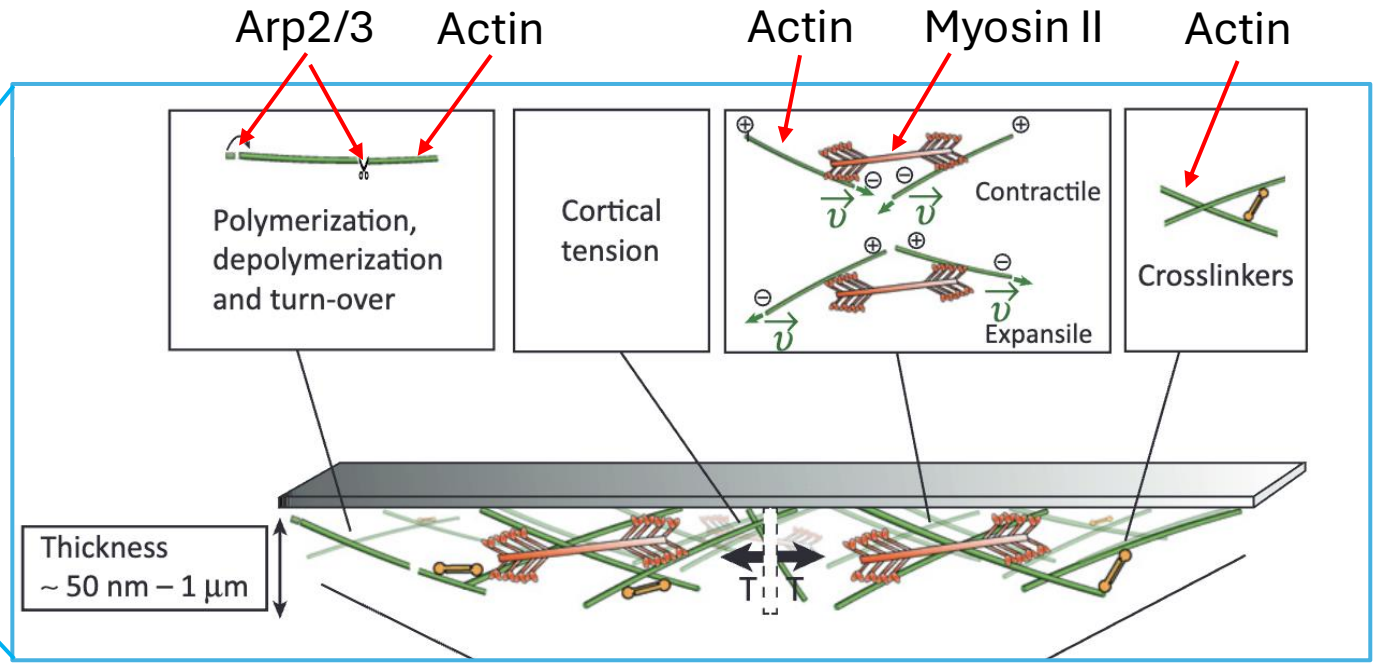
Present in most eukaryotic cells, it provides mechanical stability and defines cell shape

The Cortex in oocytes

The oocyte cortex is a thin, actin-rich layer located beneath the oolemma

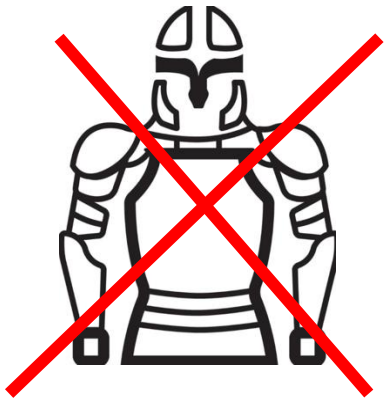


Coticchio et al., 2015a



Salbreux et al., 2012

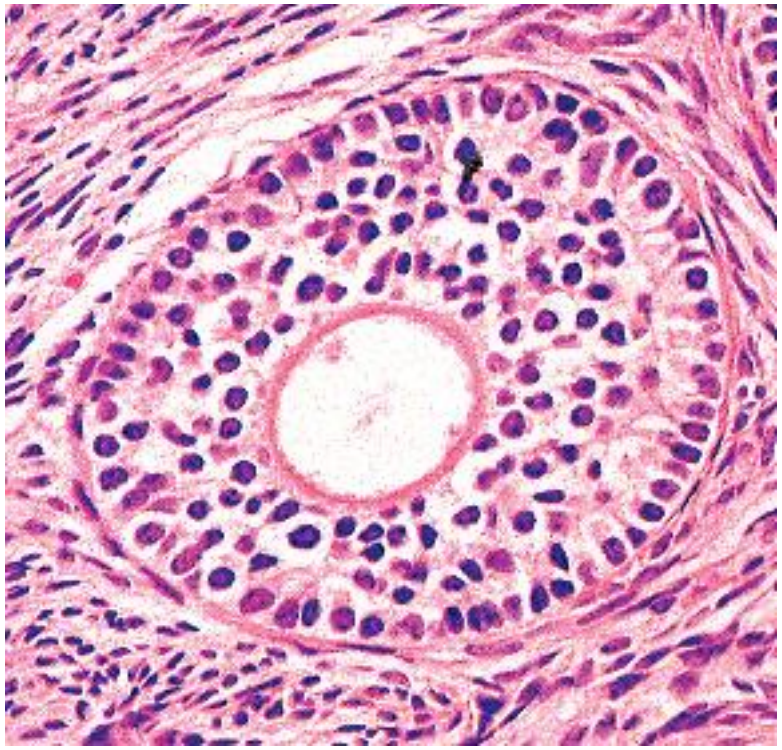
Not a passive structure, a central active player of oocyte quality and developmental competence

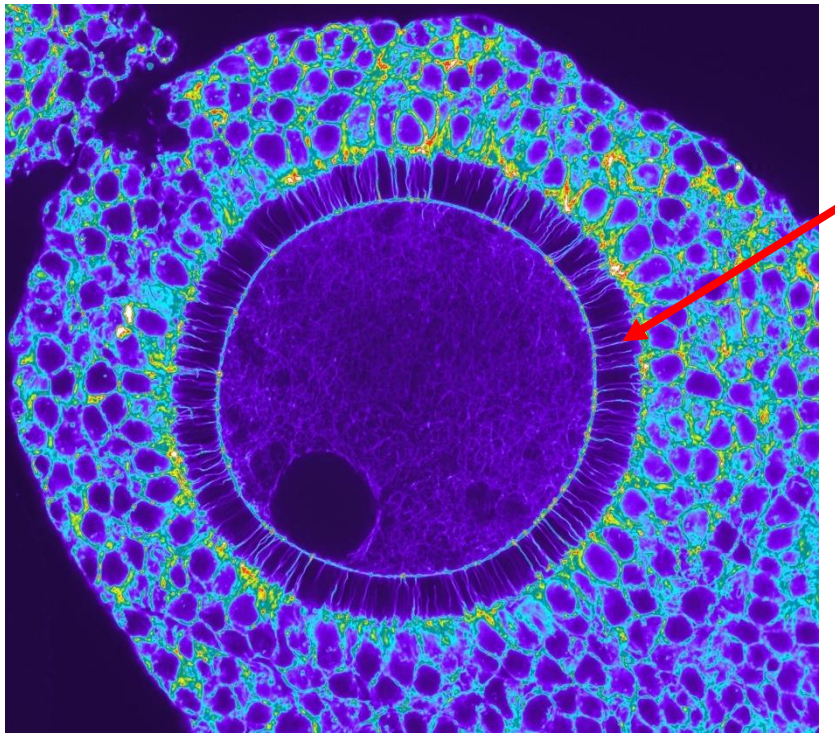


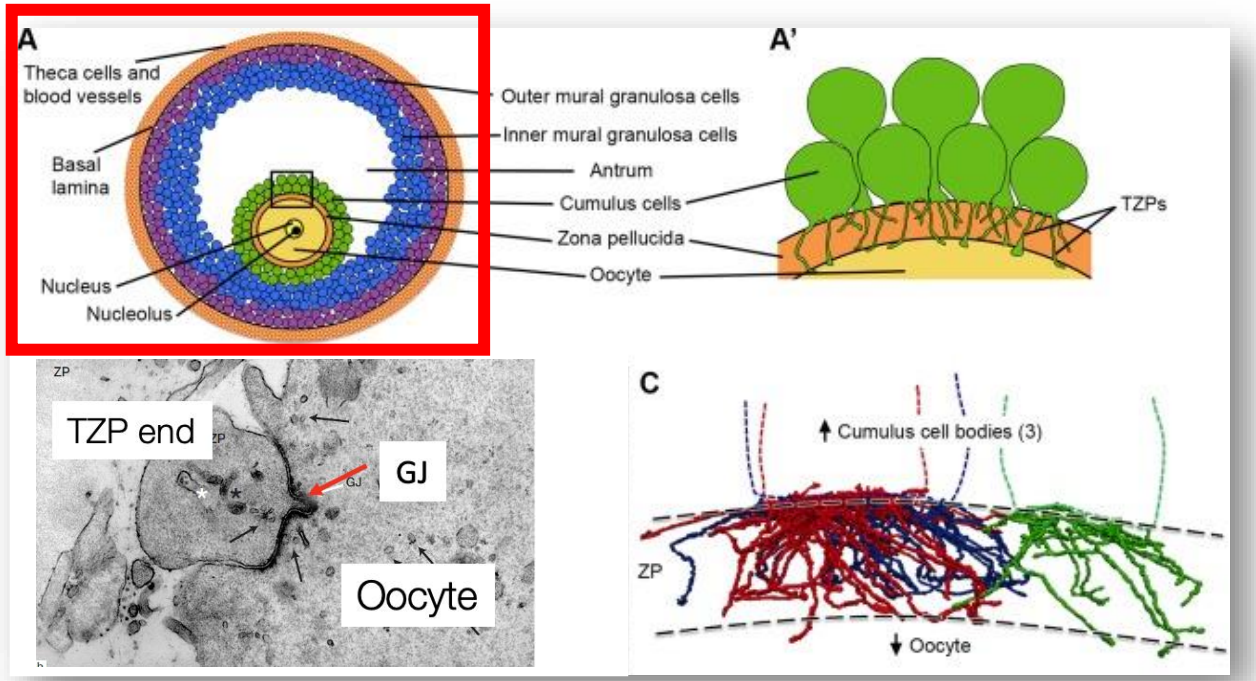
Key involvements in:

- Oogenesis
- Cell shape
- Cell Design
- Female meiosis
- Fertilization
- Early development

Trans-zonal projections (TZP) become crucial from the preantral stage

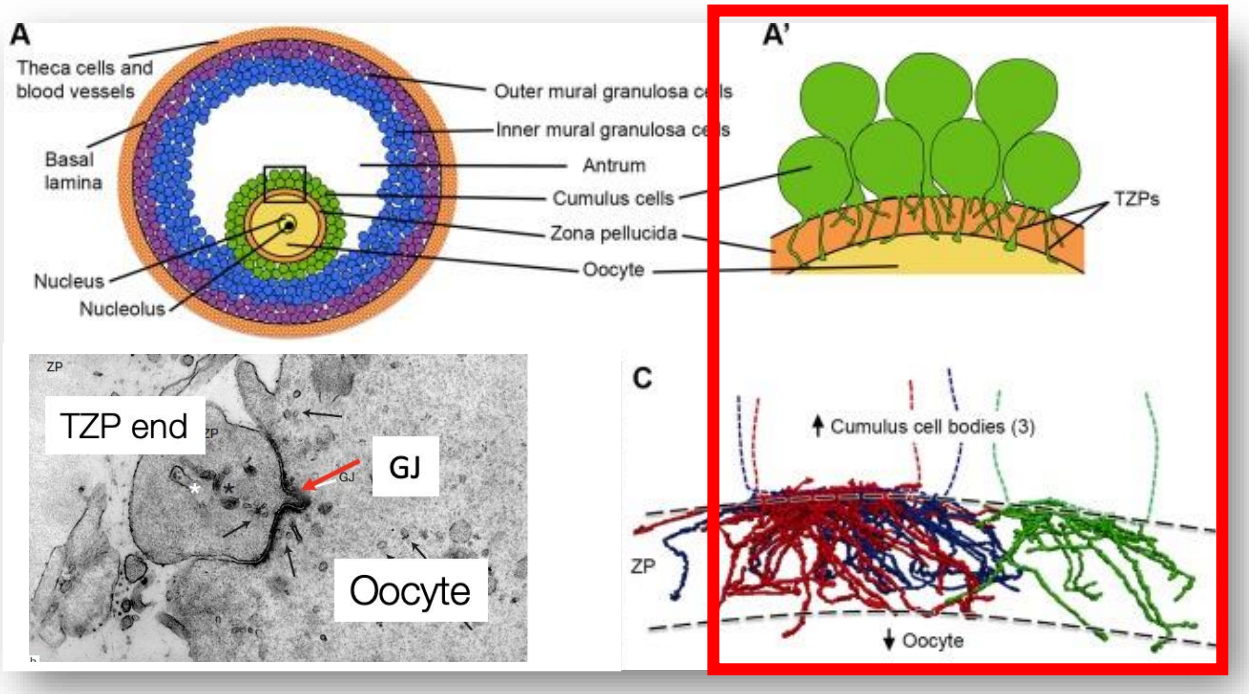






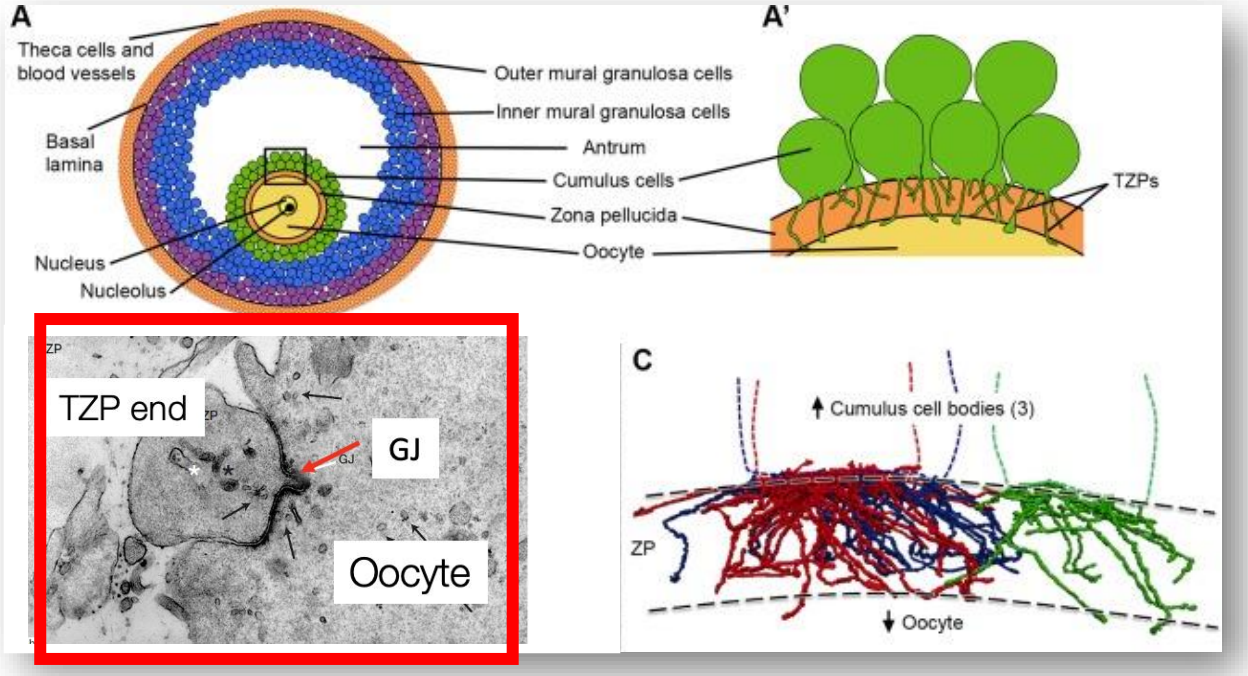
Oocyte–cumulus communication via Transzonal Projections (TZPs). TZPs connect cumulus cells and oolemma via actin and microtubules. They facilitate nutrient, ion, and signaling exchange.

Coticchio et al., 2015b



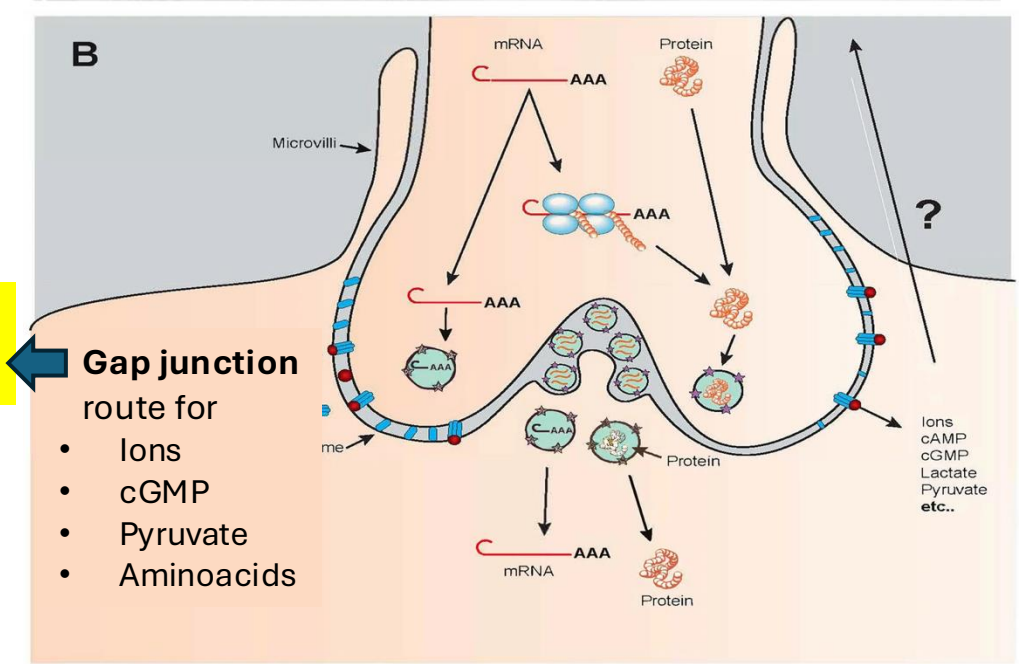
Oocyte–cumulus communication via Transzonal Projections (TZPs). TZPs connect cumulus cells and oolemma via actin and microtubules. They facilitate nutrient, ion, and signaling exchange.

Coticchio et al., 2015b



Oocyte–cumulus communication via Transzonal Projections (TZPs). TZPs connect cumulus cells and oolemma via actin and microtubules. They facilitate nutrient, ion, and signaling exchange.

Controlled by cortex

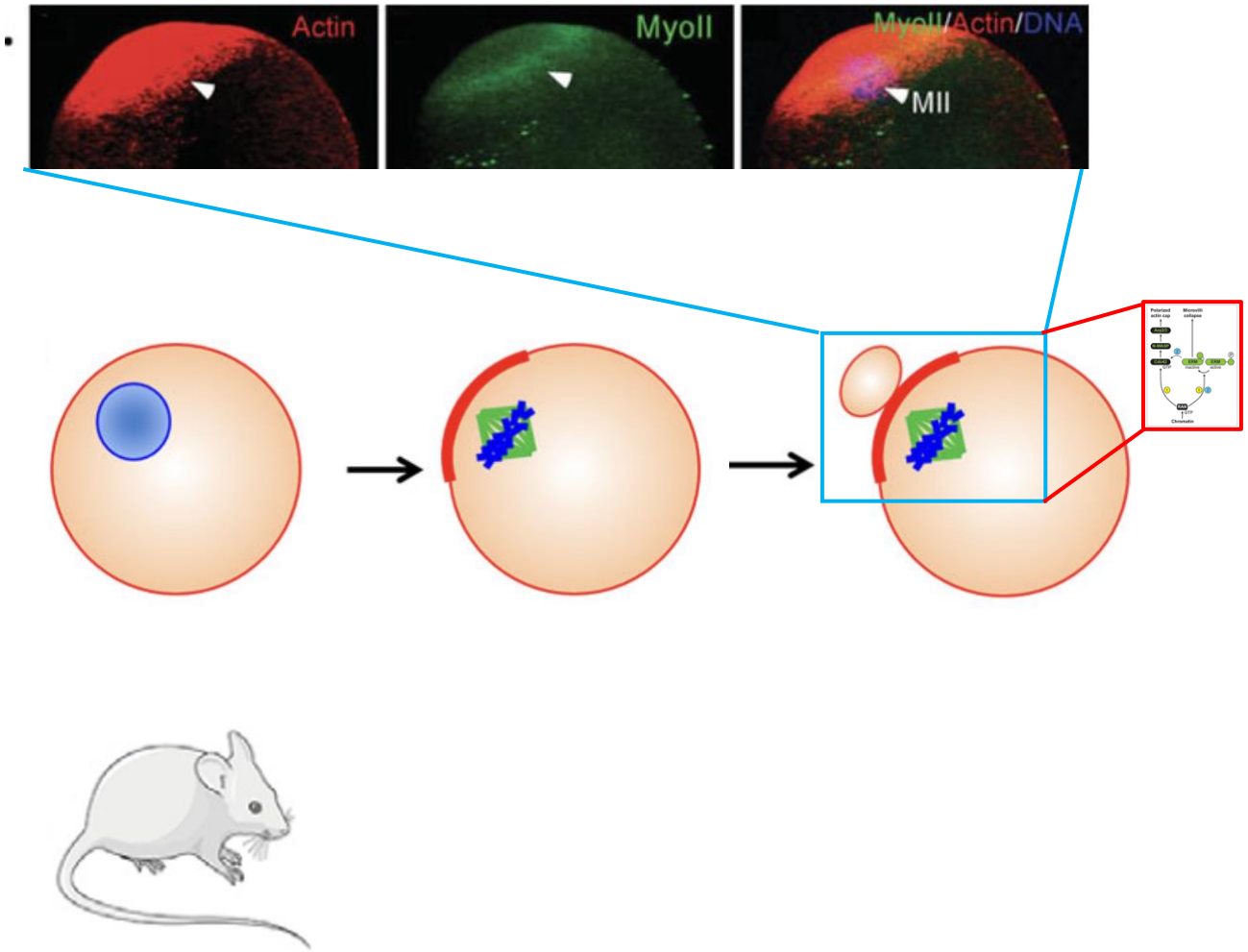


Marchais M, Gilbert I, Bastien A, Macaulay A, Robert C. Mammalian cumulus-oocyte complex communication: a dialog through long and short distance messaging.

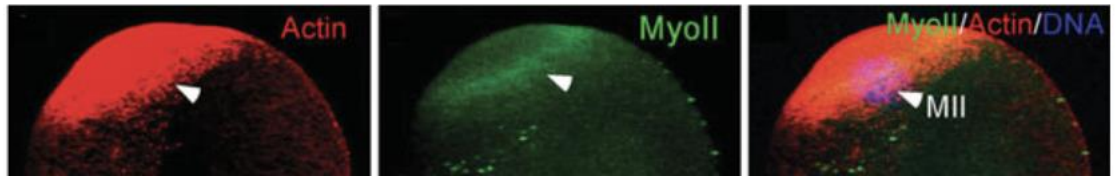
Coticchio et al., 2015b

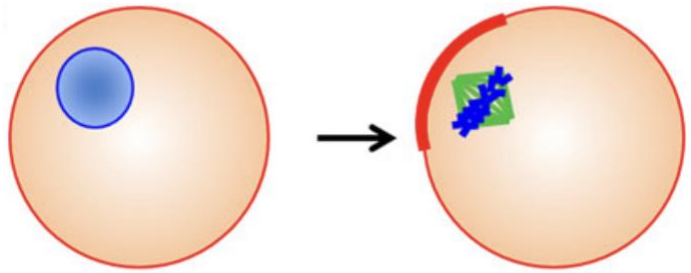
IVIRMA Global Research Alliance

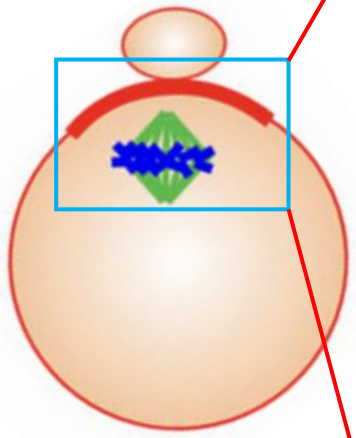
Chromatin activates two distinct pathways to regulate actin cap formation and microvilli disassembly in the polarized cortex

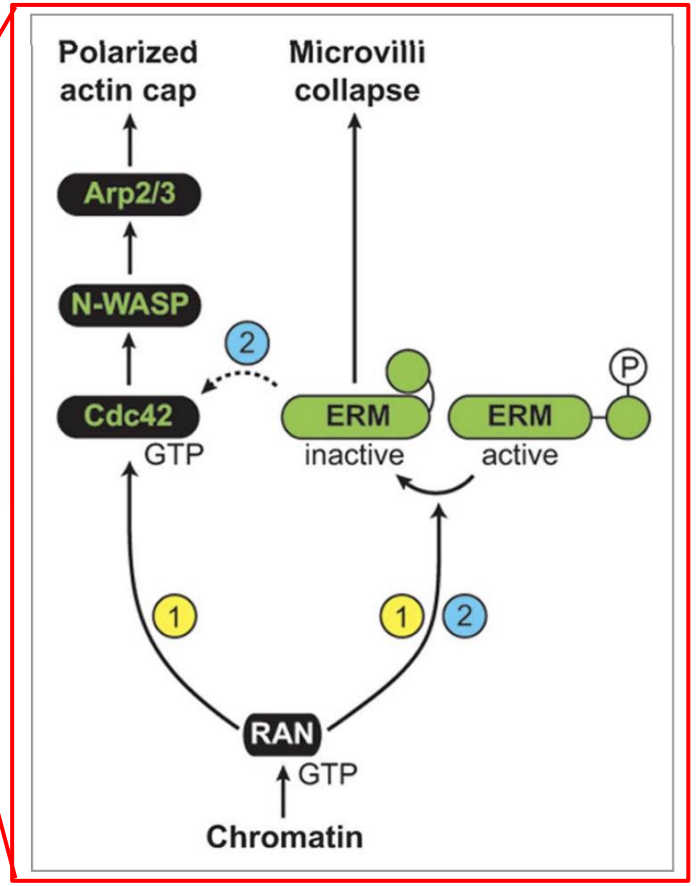


Chromatin activates two distinct pathways to regulate actin cap formation and microvilli disassembly in the polarized cortex



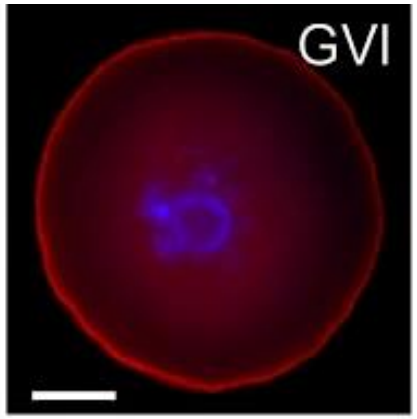




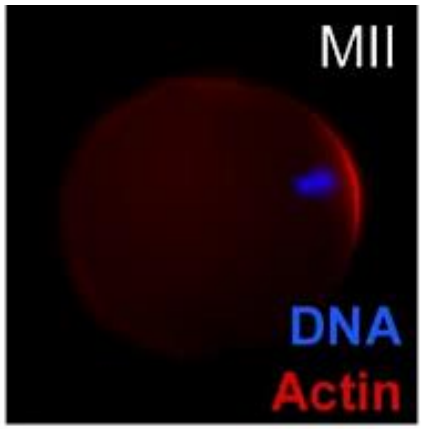


Dehaplot et al., 2013

The thread linking polarization, microvilli and fertilization



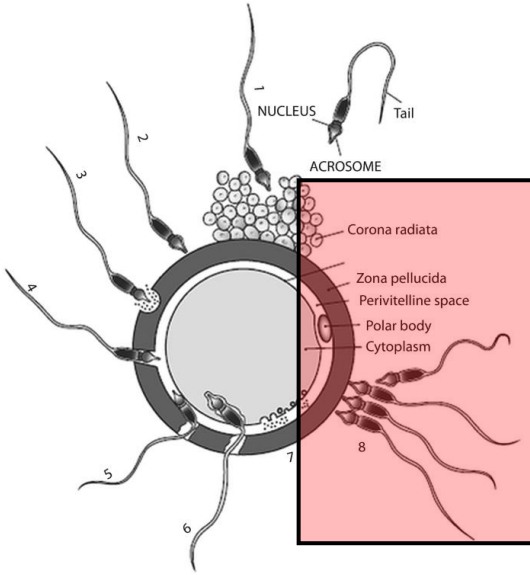
Symmetric



Asymmetric

Polarized domains:

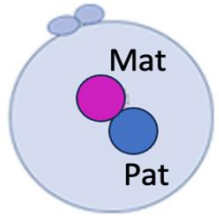
- **amicrovillar (spindle and chromosome)**
- **microvillar (gamete fusion)**



No sperm-oocyte fusion at the microvilli-free oocyte cortex, near the maternal chromatin

Maternal and paternal chromasomes are kept apart (mouse)

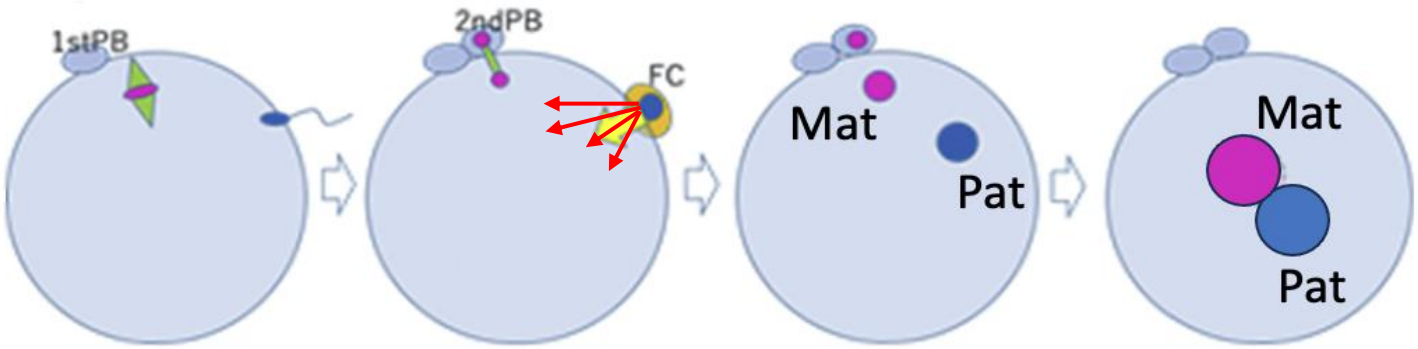
Normal 2PN fertilization



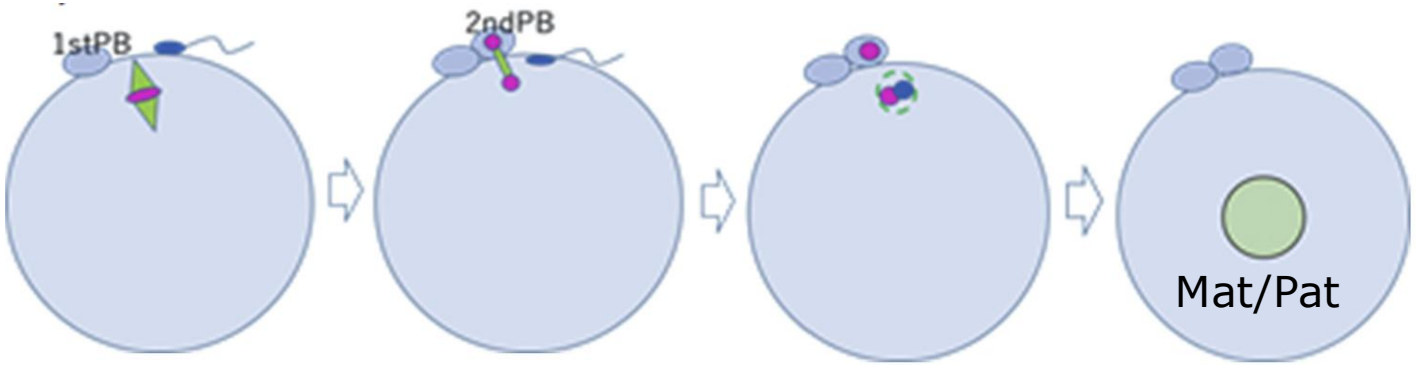
Absence of a microvilli-free area in the human oocyte cortex determines one of the possible origins of 1PN fertilization

Sperm oocyte fusion can occur anywhere on the oocyte surface

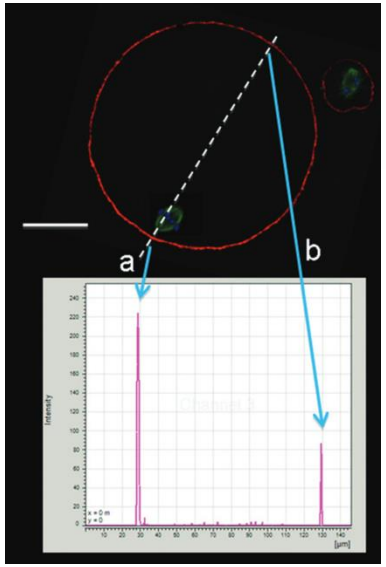
Option 1
Most frequent:
Gamete fusion away from the spindle



Option 2
Rearer:
Gamete fusion near the spindle



Images and graph from Wei et al., 2022



The human oocyte cortex has limited actin polarization and not an overlapping microvilli-free area

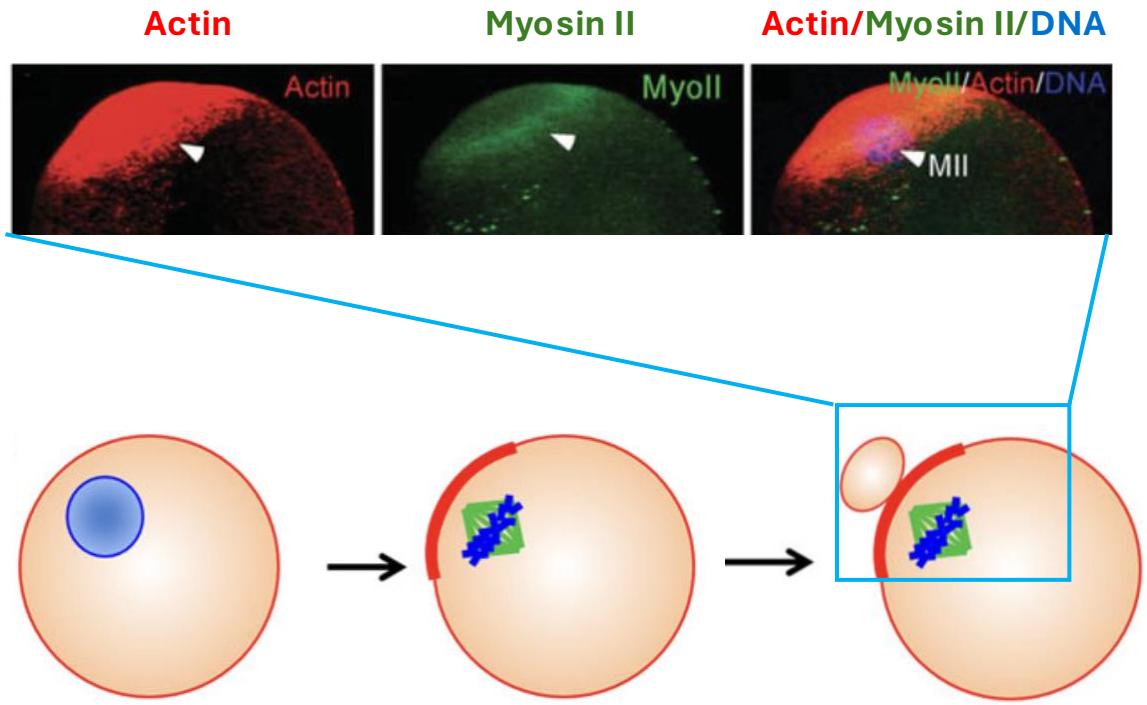
Coticchio et al., 2014

IVIRMA Global Research Alliance

PB: polar body; FC: fertilisation cone; Mat: maternal; Pat: paternal

Wei et al., Fertil Steril. 2022

Actin-myosin cap (inducing focal increase in cortical tension)

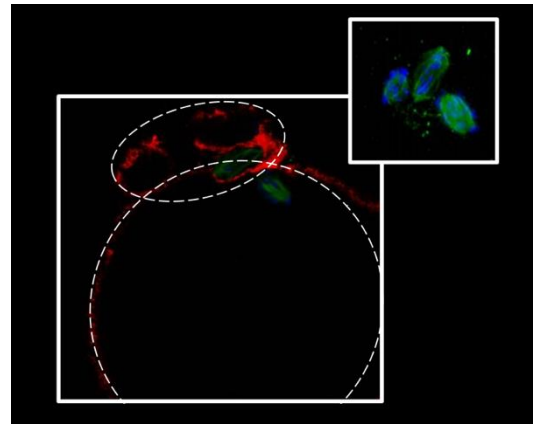


Inadequate cortical tension:
Higher or lower near the spindle

Dysfunctional effects

Large cap

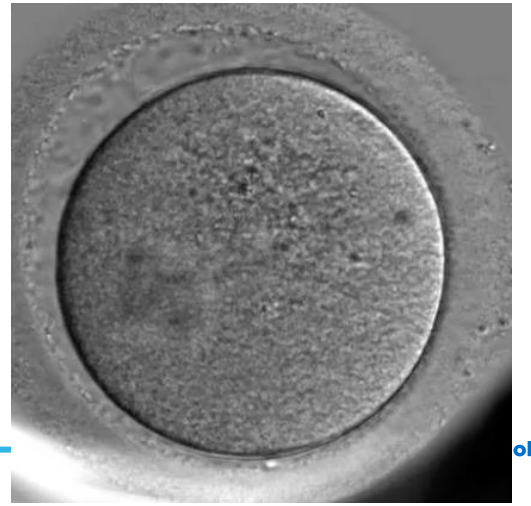
- Polar body I emission during maturation
  - Very large polar body I
  - Chromosome segregation abnormalities



Dal Canto et al. 2014

Reduced PBII cortex tension

- Polar body II emission during fertilization
  - Abnormal fertilization

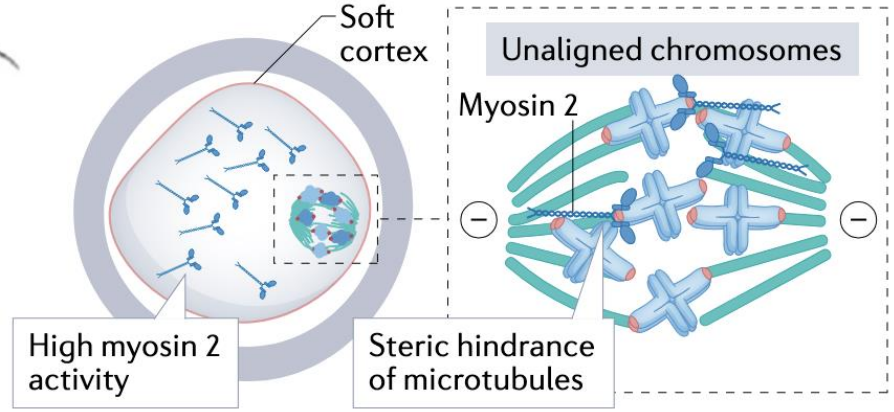


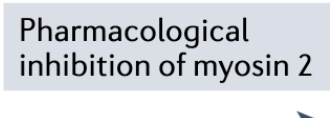
Ezoe et al. 2022

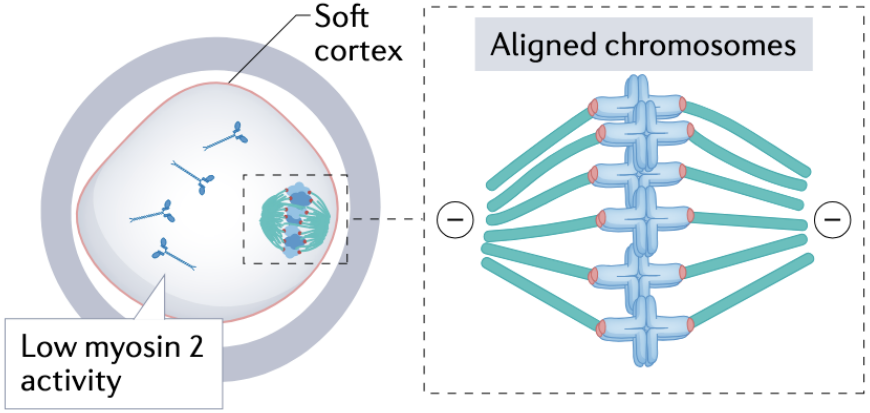
Cortical Tension and Aneuploidy

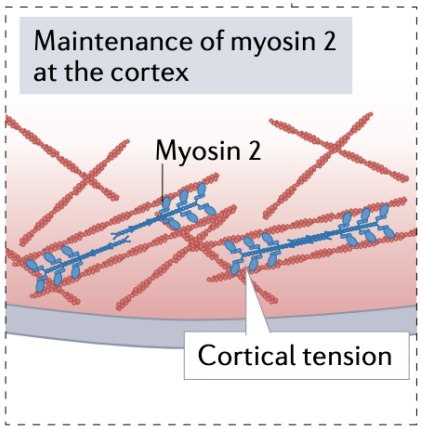
Experimentally-induced soft cortex in mouse oocytes

Bennabi et al., 2016.









Chromosome capture is less efficient in extra-soft oocytes.

Misalignment & segregation errors

Charalambous etal., 2022

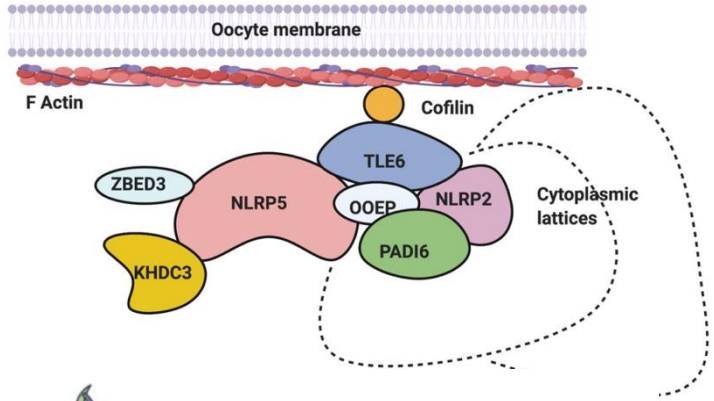
A major player:
The subcortical maternal complex

The Subcortical Maternal Complex (SCMC)

The SCMC is a multi-protein complex located beneath the oocyte cortex, in the subcortical cytoplasm

Main components:

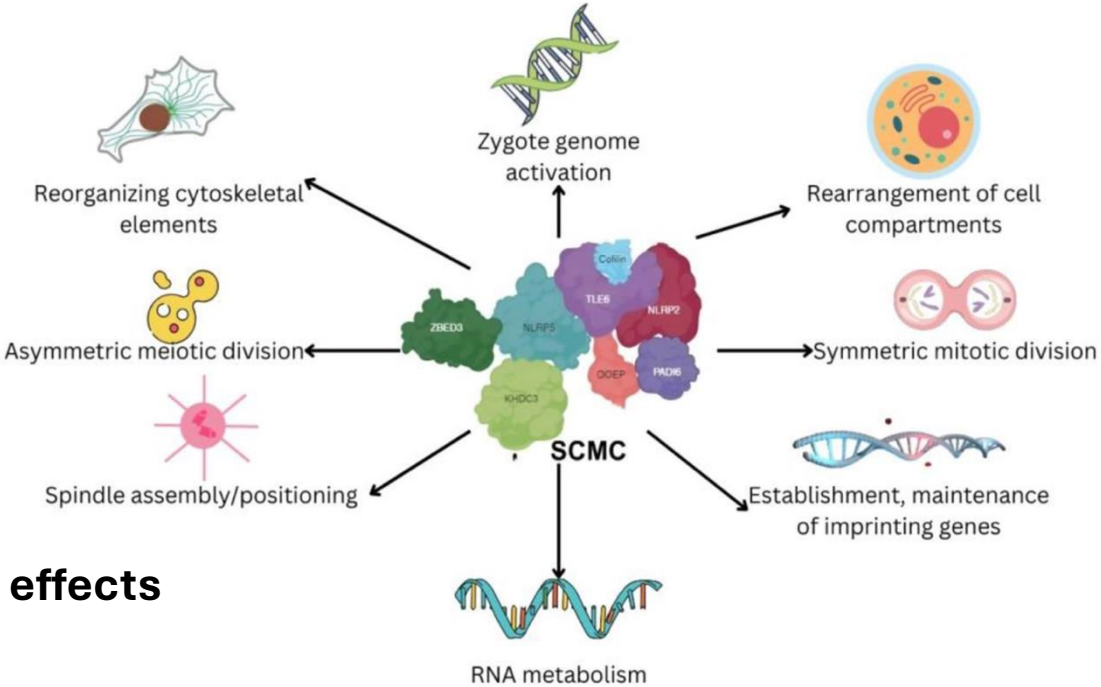
- **OOEP**
- **NLRP5**
- **TLE6**
- **KHDC3L**



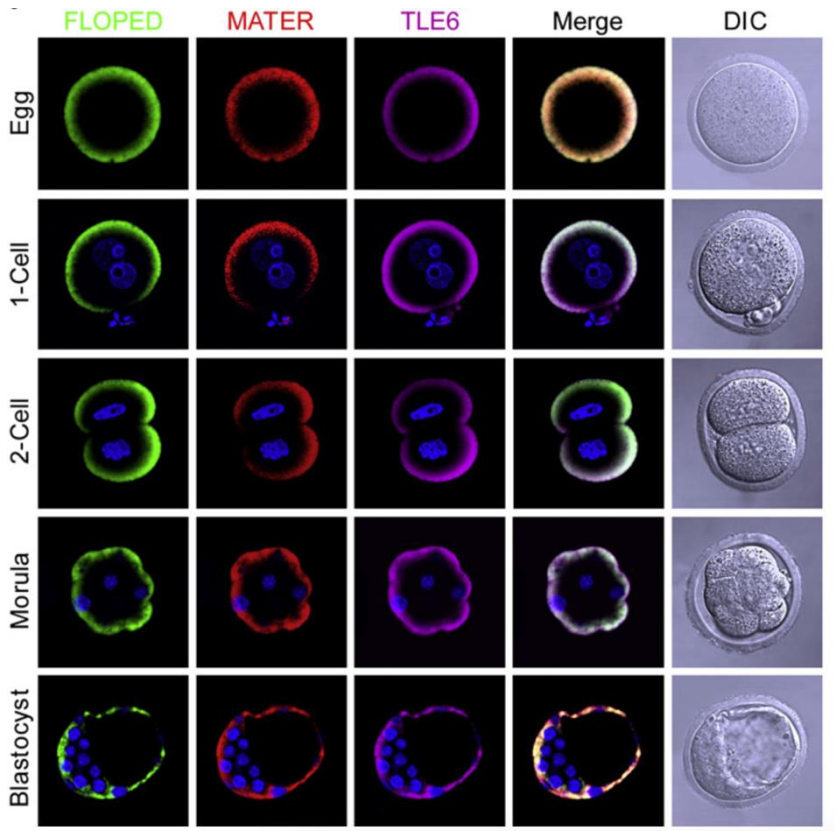
..and many other maternal-effect proteins

First identified in mouse oocytes (Li et al., 2008) later confirmed in other mammals including humans (Bebbere et al., 2021).

Main downstream effects



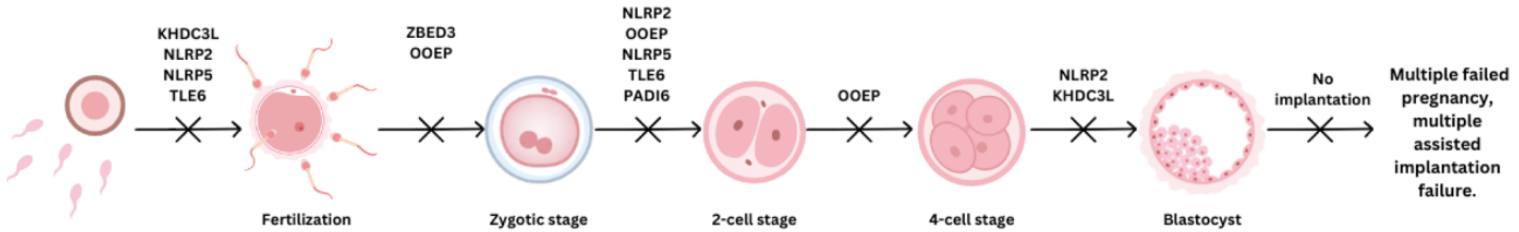
Impact of SCMC dysfunction



Li et al., 2008

SCMS components persist throughout preimplantation development

Hassan et al., 2025



SCMC protein		
Humans	**Mouse**	**Role in female fertility and embryogenesis**
PADI6	PADI6	Regulates ZGA and prevents imprinting dysfunction in the offspring
TLE6	TLE6	Facilitates symmetrical cleavage of the zygote
NLRP5	NLRP5	Prevents early embryonic arrest and imprinting dysfunction
NLRP2	NLRP2	Prevents early embryonic arrest and imprinting dysfunction
KHDC3L	KHDC3	Prevents aneuploidy, micronuclei formation, early embryonic arrest, or delayed embryogenesis
OOEP	OOEP	Prevents early embryonic arrest and imprinting dysfunction
ZBED3	ZBED3	Facilitates proper mitotic division through distribution of organelles and cytoskeletal elements
NLRP7	-	Regulates inflammasome assembly and cytokine secretion
-	Nlrp4f	Regulates cytoplasmic lattice assembly with no role in SCMC interactions

Mutations affecting SCMC components are cause of infertility

RESEARCH ARTICLE

Human Mutation | HGVS HUMAN GENOME VARIATION SOCIETY | WILEY

Mutations in *OOEP* and *NLRP5* identified in infertile patients with early embryonic arrest

Xiaomei Tong[1,2] | Jiamin Jin[1,2] | Zhanhong Hu[1,2] | Yingyi Zhang[1,2]
Heng-Yu Fan[2,3] | Yin-Li Zhang[1,2] | Songying Zhang[1,2]

Novel mutations in genes encoding subcortical maternal complex proteins may cause human embryonic developmental arrest

Xueqian Wang [a,b,1], Di Song [c,1], Dmytro Mykytenko [d,1], Yanping Kuang [e,1], Qifeng Lv [e], Bin Li [e], Biaobang Chen [a], Xiaoyan Mao [e], Yao Xu [a], Valery Zukin [d], Pavlo Mazur [d], Jian Mu [a], Zheng Yan [e], Zhou Zhou [a], ying Liu [f], Li Jin [a], Lin He [g], Qing Sang [a,b], Zhaogui Sun [h,], i Wang [a,b,*]*

Identification of Novel SCMC Gene Variants Associated With Early Embryonic Arrest

Changlong Zhang[1,2,3,4,5,6,7] | Shuai Zhao[1,2,3,4,5,6,7] | Honghui Zhang[1,2,3,4,5,6,7] | Wei Su[1,2,3,4,5,6,7] | Yang Wang[1,2,3,4,5,6,7] | Ying Cui[1,2,3,4,5,6,7] | Bohan Yang[1,2,3,4,5,6,7] | Yikun Wang[1,2,3,4,5,6,7] | Han Zhao[1,2,3,4,5,6,7]

OXFORD | molecular human reproduction

Molecular Human Reproduction, 2024, **30(9)**, gaae031
https://doi.org/10.1093/molehr/gaae031
Advance Access Publication Date: August 23, 2024
Original Research

Variants in NLRP2 and ZFP36L2, non-core components of the human subcortical maternal complex, cause female infertility with embryonic development arrest

Ximan Rui[1,†], Xiaolan Zhang[2,†], Xinru Jia[1,†], Jian Han[1,†], Congjing Wang[1], Qiqi Cao[1], Ou Zhong[1], Jie Ding[1,3], Chun Zhao[2], Junqiang Zhang[2], Xiufeng Ling[2], Hong Li[1,3], Xiang Ma[4,*], Qingxia Meng[1,3,*], and Ran Huo[1,5,*]

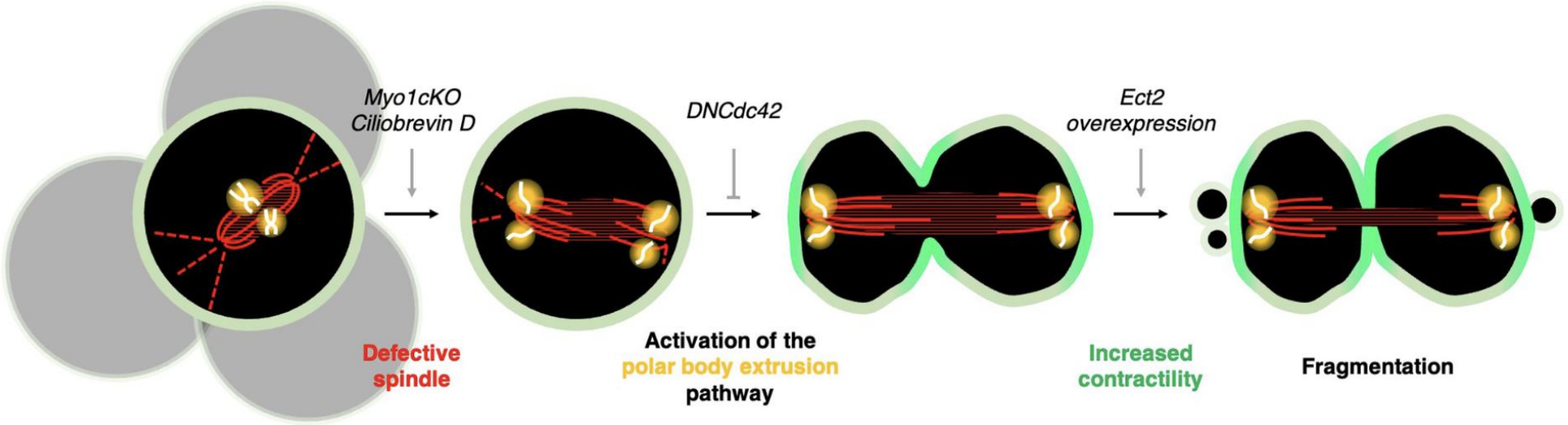
Novel Homozygous *PADI6* Variants in Infertile Females with Early Embryonic Arrest

Yao Xu[1†‡], Rongxiang Wang[2‡], Zhi Pang[3‡], Zhiyun Wei[1], Lihua Sun[2], Sa Li[4], Guanghua Wang[1], Yu Liu[2], Yiwen Zhou[2], Hongjuan Ye[2], Liping Jin[1*] and Songguo Xue[2*]*

IVIRMA Global Research Alliance

Cortical Dysregulation and Embryo Fragmentation

Myo1C KO mice: fragmentation from chromatin–cortex proximity



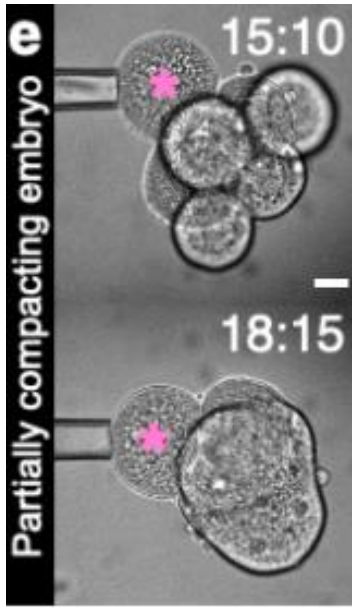
Cell fragmentation in mouse preimplantation embryos induced by ectopic activation of the polar body extrusion pathway

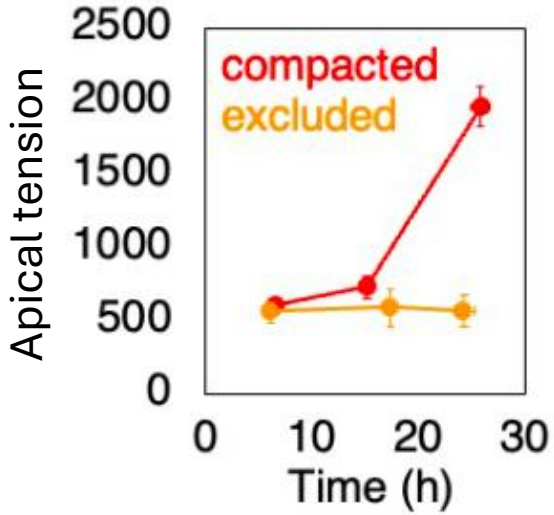
Diane Pelzer[1], Ludmilla de Plater[1], Peta Bradbury[1], Adrien Eichmuller[1], Anne Bourdais[2], Guillaume Halet[2] & Jean-Léon Maître[1,*]

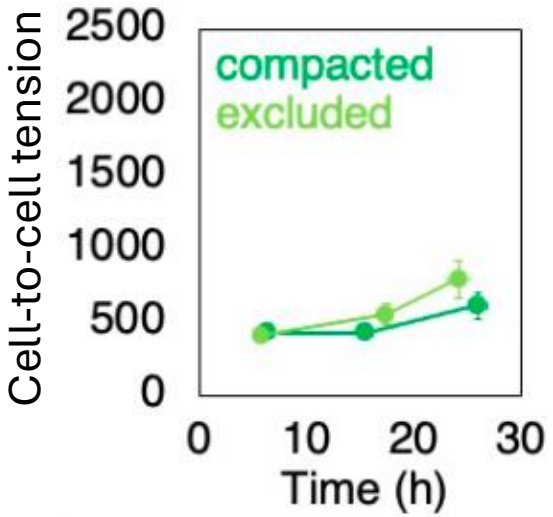
The inefficient chromosome separation due to spindle defects, caused by dysfunctional molecular motors Myo1c or dynein, leads to fragmentation during mitosis.

IVIRMA Global Research Alliance

Cell compaction at the morula stage relies on cortical tension regulation



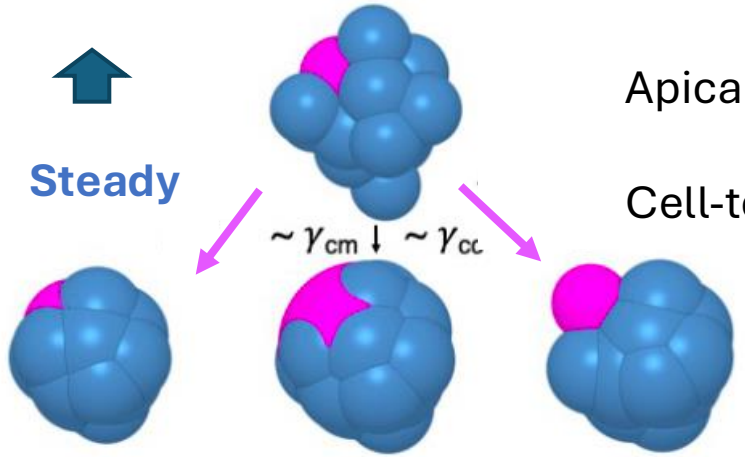




Firmin et al., 2022

Apical tension ↑

Cell-to-cell tension **Steady**

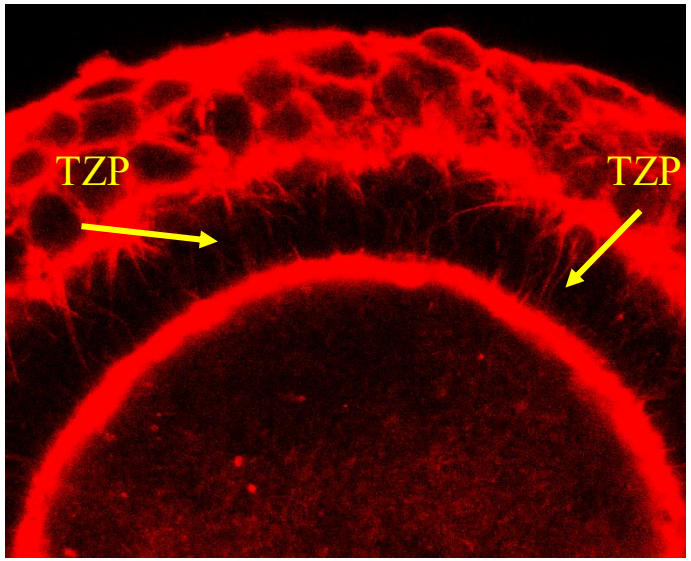


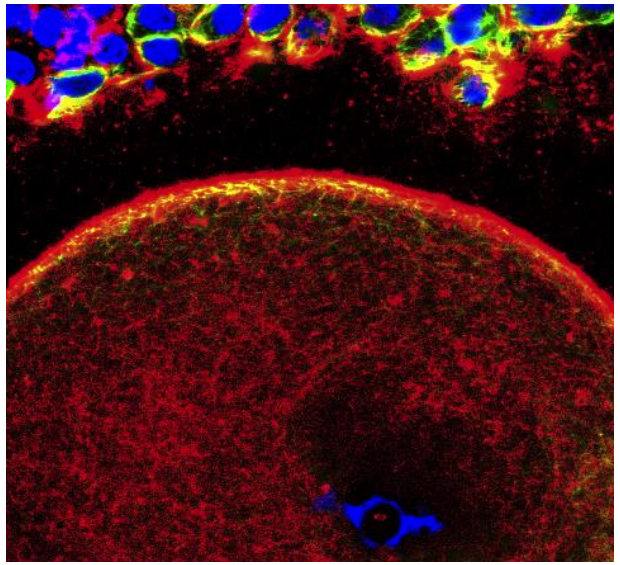
Apical tension **Steady**

Cell-to-cell tension ↑

Practical implications for the IVF lab

Vitrification disrupts trans-zonal processes of human cumulus cell-oocyte complexes



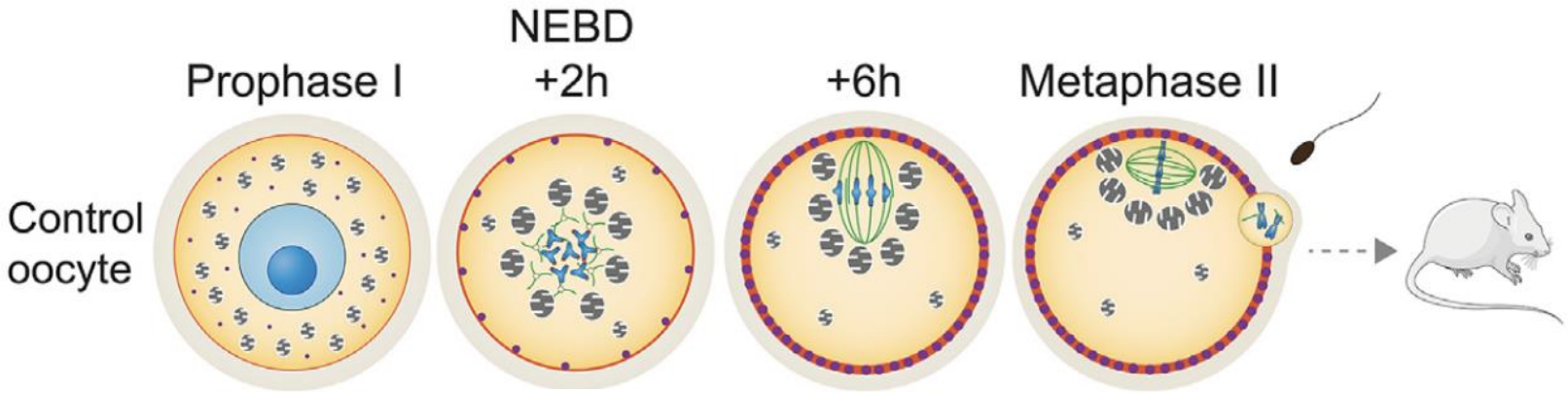


Fresh control

Vitrified / warmed

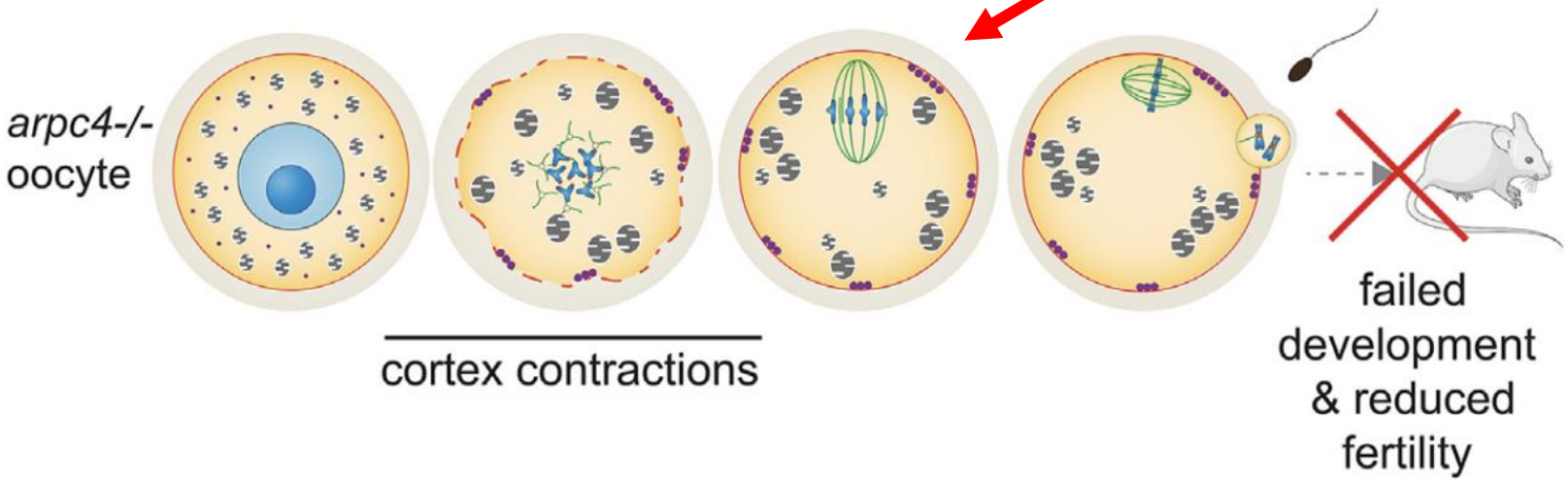
Brambillasca et al., 2013

Aberrant cortex contractions impact mammalian oocyte quality



- Mouse oocytes depleted of Arpc4 complex re-organize their cortex upon division
- F-actin cortex re-organization induces oocyte contractions lasting several hours
- Cortex contractions impact organelle distribution and activity
- Cortex contractions impact mouse oocytes' developmental potential

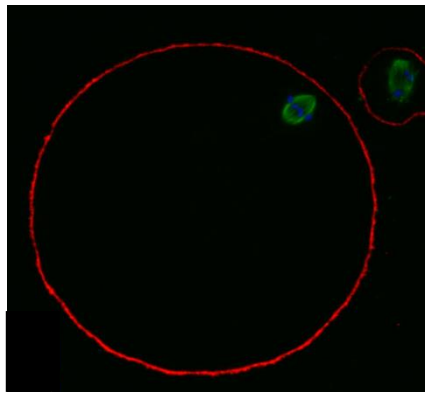
Reminiscent of abnormal human phenotype



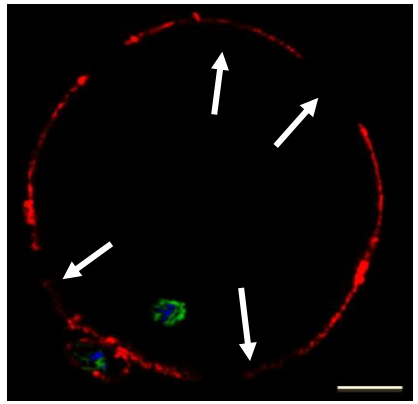
Nikalayevich et al., 2024

Sub-oolemmal actin organization is disrupted in oocytes displaying smooth endoplasmic reticulum aggregates (SERa) and cytoplasmic granularity (CG)

Sub-oolemmal actin pattern



Normal (continuous)



Dashed

Actin intensity signal/patter	Control	SER	GC	*P*
Intensity in domain proximal to the spindle	200 (100 – 260)	130 (100 – 250)	150 (50 – 255)	Control vs. SER = 0.057 Control vs. GC = 0.112
Intensity in domain distal to the spindle	150 (40 – 230)	115 (50 – 250)	100 (50 – 250)	Control vs. SER = 0.940 Control vs. GC = 0.370
Continuous, non-dashed (%)	33/33 (100)	18/27 (66.6)	15/35 (42.9)	Control vs. SER < 0.0001 Control vs. GC < 0.0001

Dal Canto et al., 2017

Possible reason for subperformance of dysmorphic oocytes?

Cortex and ICSI

Injection funnel persistence linked to embryo quality

Oolemma response patterns:

- **minimal resistance**
- **elastic**
- **sudden breakage**

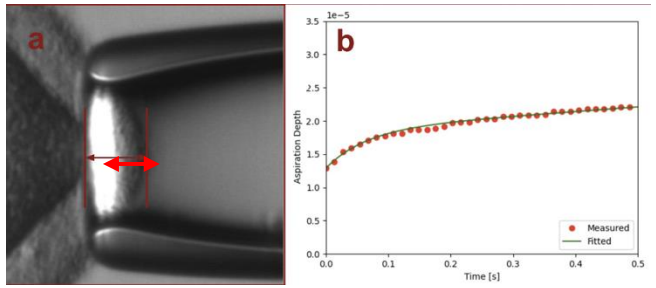
Large funnel = better fertilization outcomes

Metaphase II oocyte immediately after withdrawal of the injection pipette. The corresponding funnel volume is 25.2 pL; x is one-half of the funnel extension at the site of injection; y is the depth of injection.

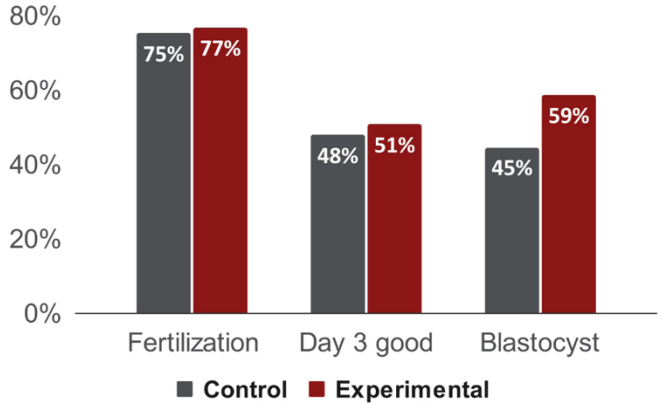
Krause I, Pohler U, Grosse S, Shebl O, Petek E, Chandra A, Ebner T. Characterization of the injection funnel during intracytoplasmic sperm injection reflects cytoplasmic maturity of the oocyte. Fertil Steril. 2016

Development and evaluation of a usable blastocyst predictive model using the biomechanical properties of human oocytes

Daniel Meyer[1]*, Jonathan Kort[2], Ching Hung Chen[3,4,5], Huan Zhao[6], Xiaoling Yi[6], Shin-Yu Lai[5], Farn Lu[4,5], Wen Jui Yang[4,5], I-Chiao Hsieh[7], Chung-Li Chiang[7], Wei-Ming Chen[7], Jack Yu Jen Huang[2,4,5], David Camarillo[1], Barry Behr[2]



Mechanical oocyte assessment



Safety

Prediction results of the usable blastocysts predictive classifier with biomechanical properties compared to maternal factors and morphological information by four embryologists

	Biomechanical properties [b]	Maternal factors [c]	Mixed features [d]	Emb1 [e]	Emb2 [e]	Emb3 [e]	Emb4 [e]
ACC [a] [%]	71	57	52	38	42	50	44
PPV [a] [%]	68	55	51	40	39	47	33
NPV [a] [%]	77	61	75	48	43	48	47
SEN [a] [%]	81	71	97	19	35	58	13
SPE [a] [%]	63	44	9	72	47	38	75

[a] Accuracy (ACC), Positive Predictive Value (PPV), Negative Predictive Value (NPV), Sensitivity (SEN), Specificity (SPE).

[b] Predictive classifier was trained using 209 measured oocytes, which included four optimal biomechanical features.

[c] Predictive classifier was trained using 209 measured oocytes, which included maternal factors of patient age and number of MII.

[d] Predictive classifier was trained using 209 measured oocytes, which included four optimal biomechanical features, patient age, and number of MII.

[e] Prediction results predicted by experienced embryologists.

Take home messages

- **Cortex integrates signaling, mechanics, and developmental regulation**
- **Key for oocyte maturation, fertilization, embryo quality**
- **Potential prognostic tool in assisted reproduction** → Ongoing IVM studies of IVIRMA/ Kato Clinic
- **Future perspective: biomechanical biomarkers for non-invasive oocyte assessment**


OXFORD human reproduction

Human Reproduction, 2025, 00(00), 1–9
https://doi.org/10.1093/humrep/deaf208
Mini-Review

Developments in reproductive biology and medicine

The emerging role of the oocyte cortical domain in maturation, fertilization, and development

Giovanni Coticchio[1,*], Valentina Cecconi[2], Laura Rienzi[2,3], Danilo Cimadomo[2,4], Chiara Cosseddu[2], Marilena Tagg[2], Sergio Leddi[5], and Daniela Bebbere[5]

[1]IVIRMA Global Research Alliance, IVIRMA ITALIA, Rome, Italy
[2]IVIRMA Global Research Alliance, Genera, Clinica Valle Giulia, Rome, Italy
[3]Department of Biomolecular Sciences, University of Urbino "Carlo Bo", Urbino, Italy
[4]Department of Biology and Biotechnology "Lazzaro Spallanzani", University of Pavia, Pavia, Italy
[5]Department of Veterinary Medicine, University of Sassari, Sassari, Italy

Take home messages

OXFORD human reproduction

Human Reproduction, 2025, **00(00)**, 1–9
https://doi.org/10.1093/humrep/deaf208
Mini-Review

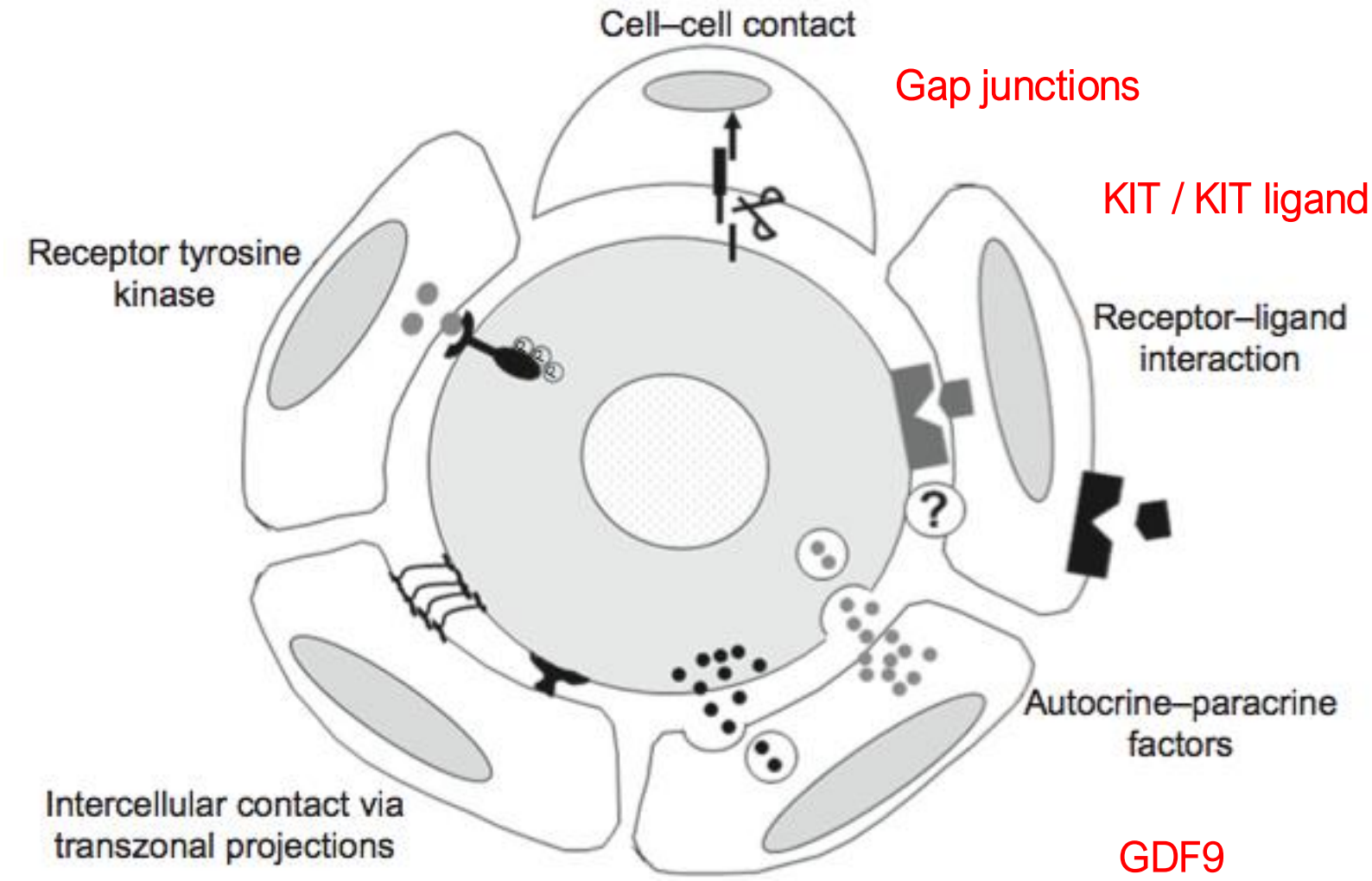
Developments in reproductive biology and medicine

The emerging role of the oocyte cortical domain in maturation, fertilization, and development

Giovanni Coticchio [1,*], Valentina Casciani [2], Laura Rienzi [2,3], Danilo Cimadomo [2,4], Chiara Cosseddu [5], Marilena Taggi [2], Sergio Ledda [5], and Daniela Bebbere [5]

[1]IVIRMA Global Research Alliance, IVIRMA ITALIA, Rome, Italy
[2]IVIRMA Global Research Alliance, Genera, Clinica Valle Giulia, Rome, Italy
[3]Department of Biomolecular Sciences, University of Urbino "Carlo Bo", Urbino, Italy
[4]Department of Biology and Biotechnology "Lazzaro Spallanzani", University of Pavia, Pavia, Italy
[5]Department of Veterinary Medicine, University of Sassari, Sassari, Italy

Multiple signalling modalities ensure somatic cell-oocyte communication



Zooming on trans-zonal processes

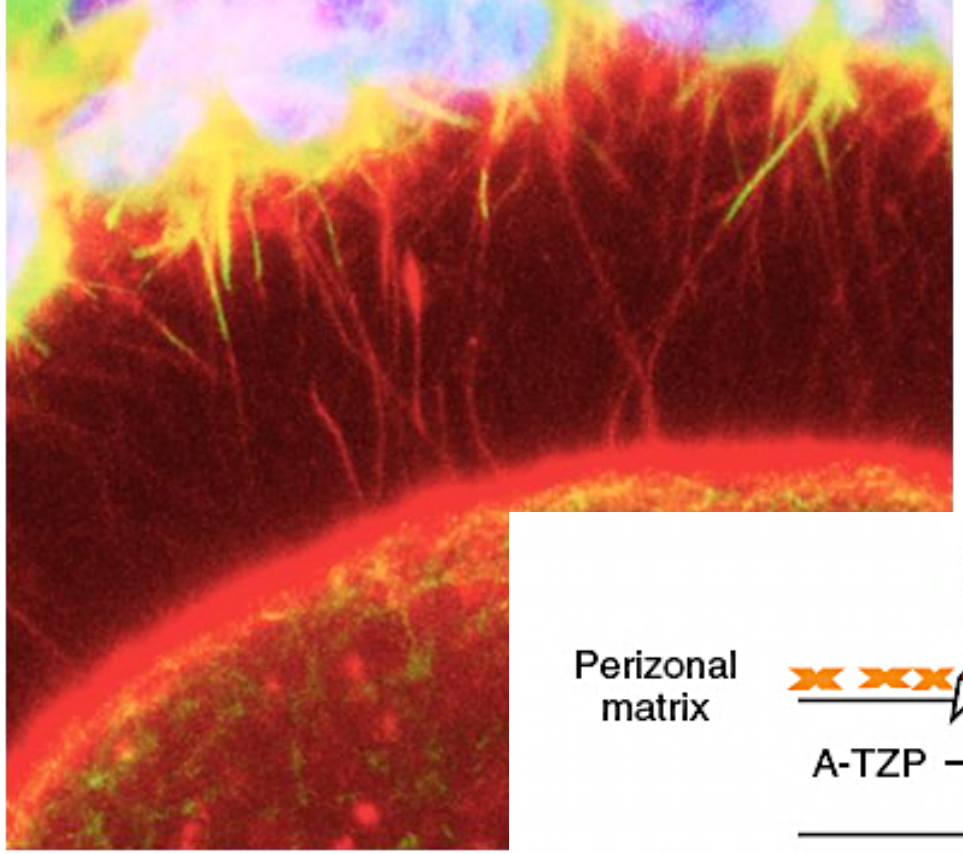
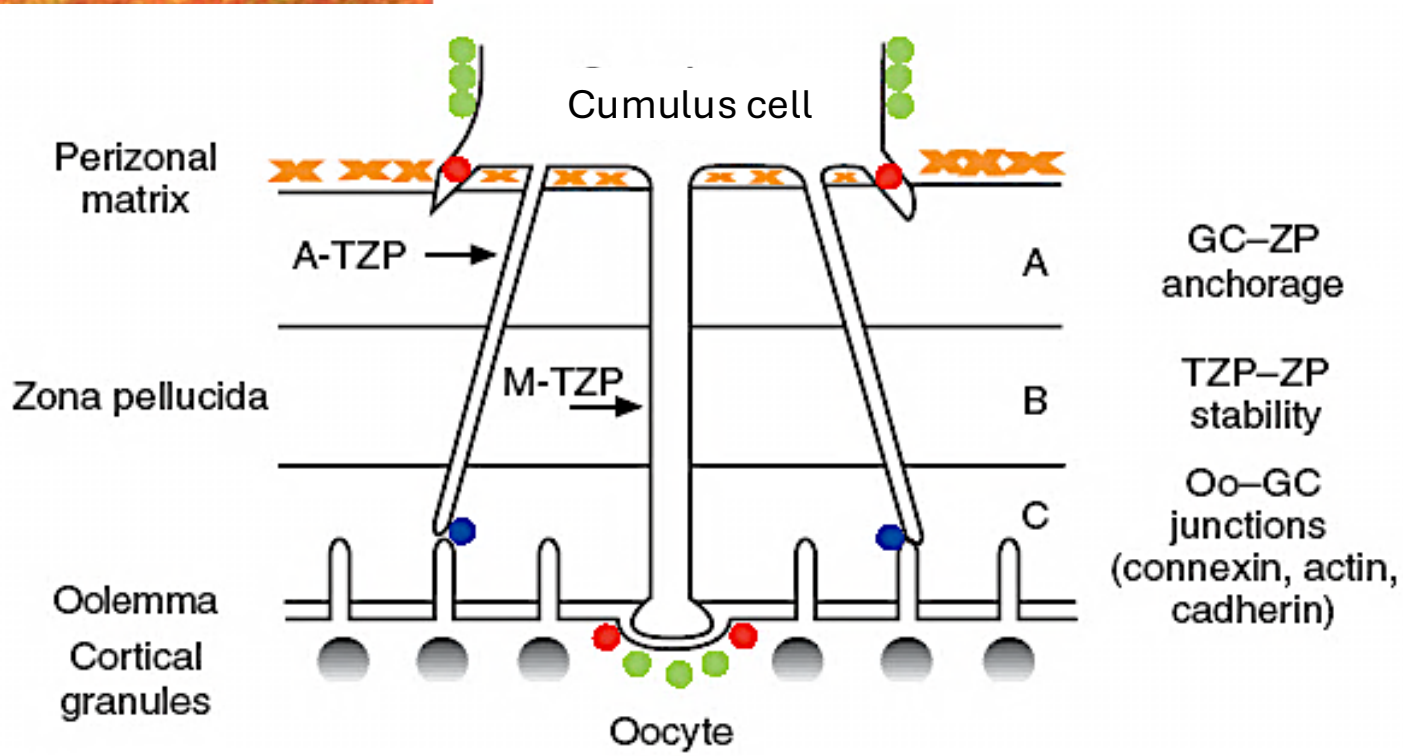
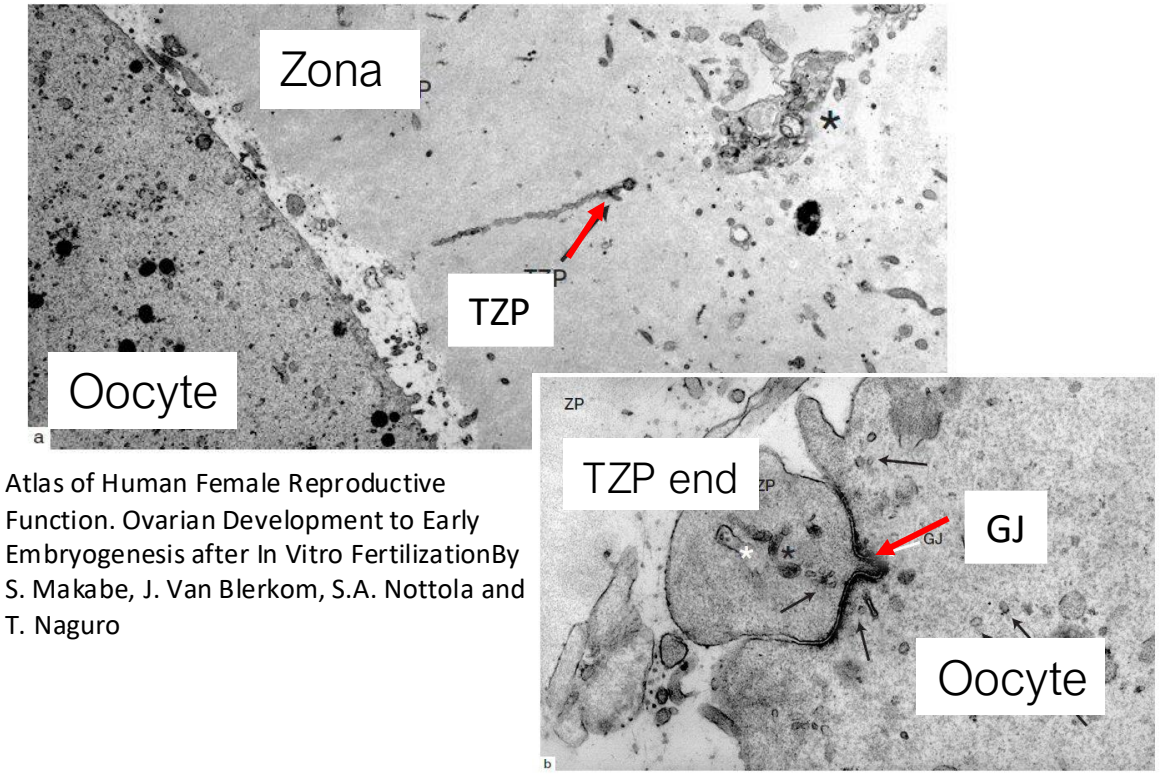


Image from
Biogenesi Research Lab



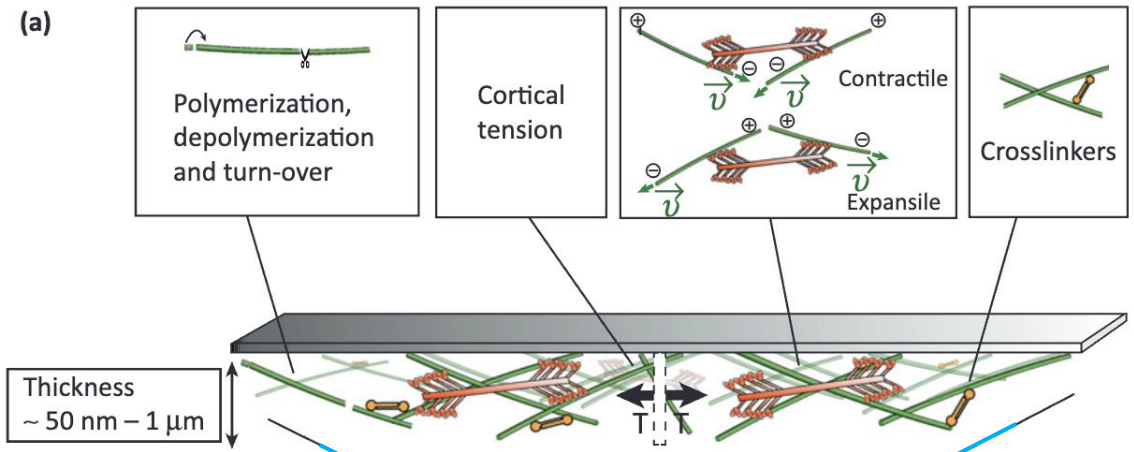
Gap-junction (GJ) communication is integral to TZP communication

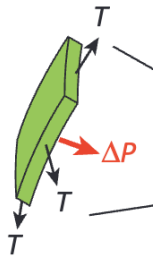


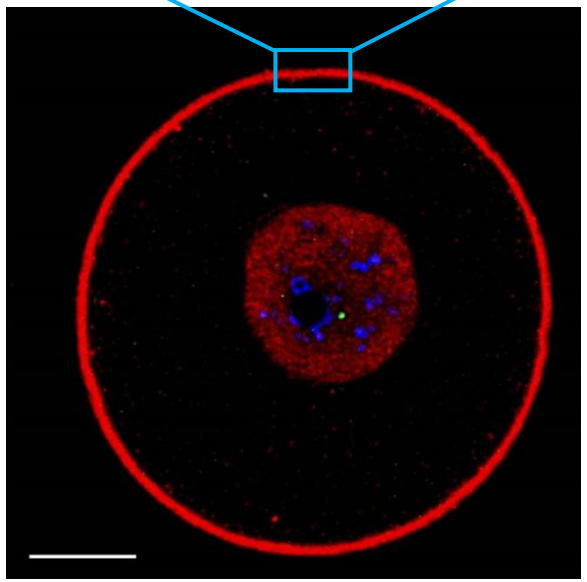
Atlas of Human Female Reproductive Function. Ovarian Development to Early Embryogenesis after In Vitro FertilizationBy S. Makabe, J. Van Blerkom, S.A. Nottola and T. Naguro

GJ opening is subject to regulation … and dysregulation

Salbreux et al., 2012

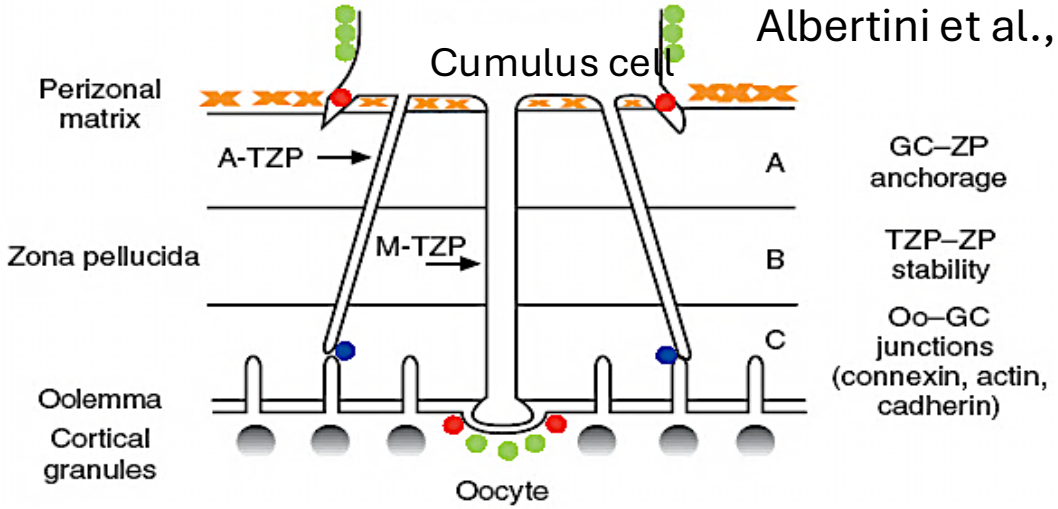


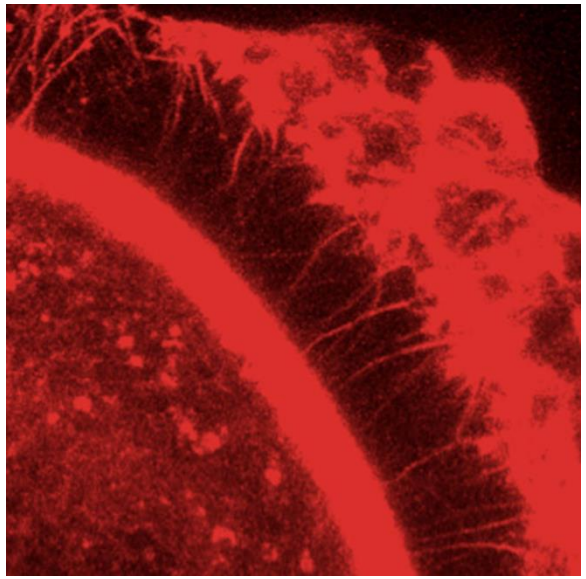




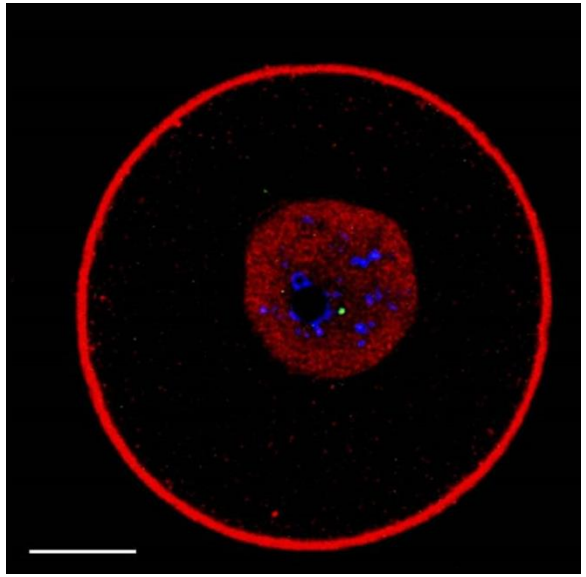
Coticchio et al., 2015a

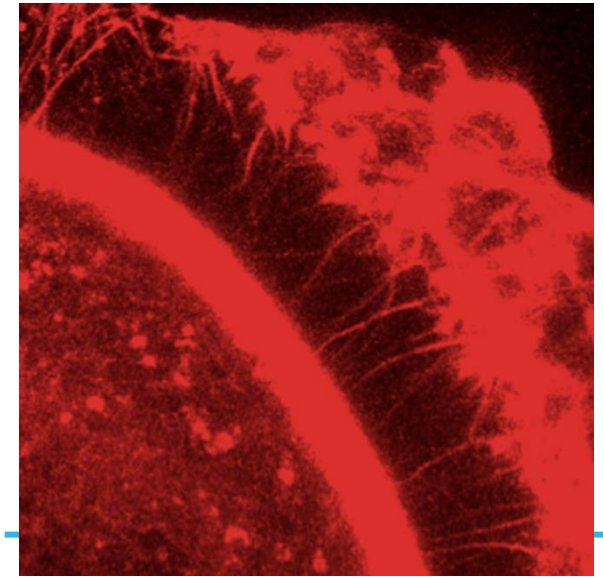
Albertini et al., 2013

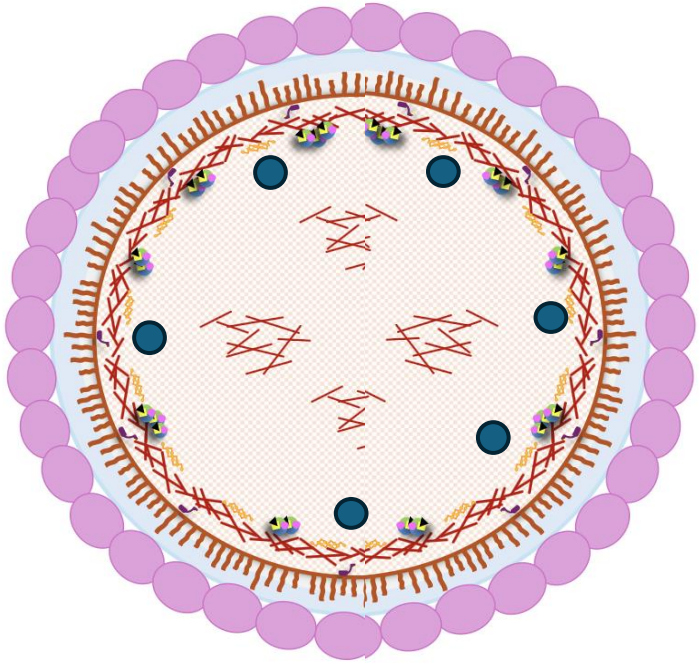




Coticchio et al., 2015b







F-actin

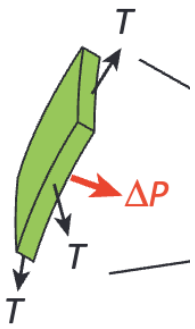
Oolemma

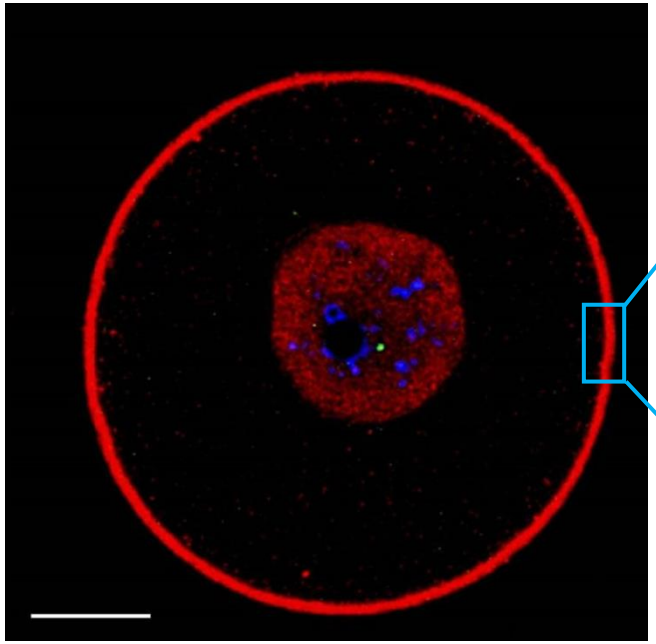
Fyn kinases

Subcortical Maternal Complex (SCMC)

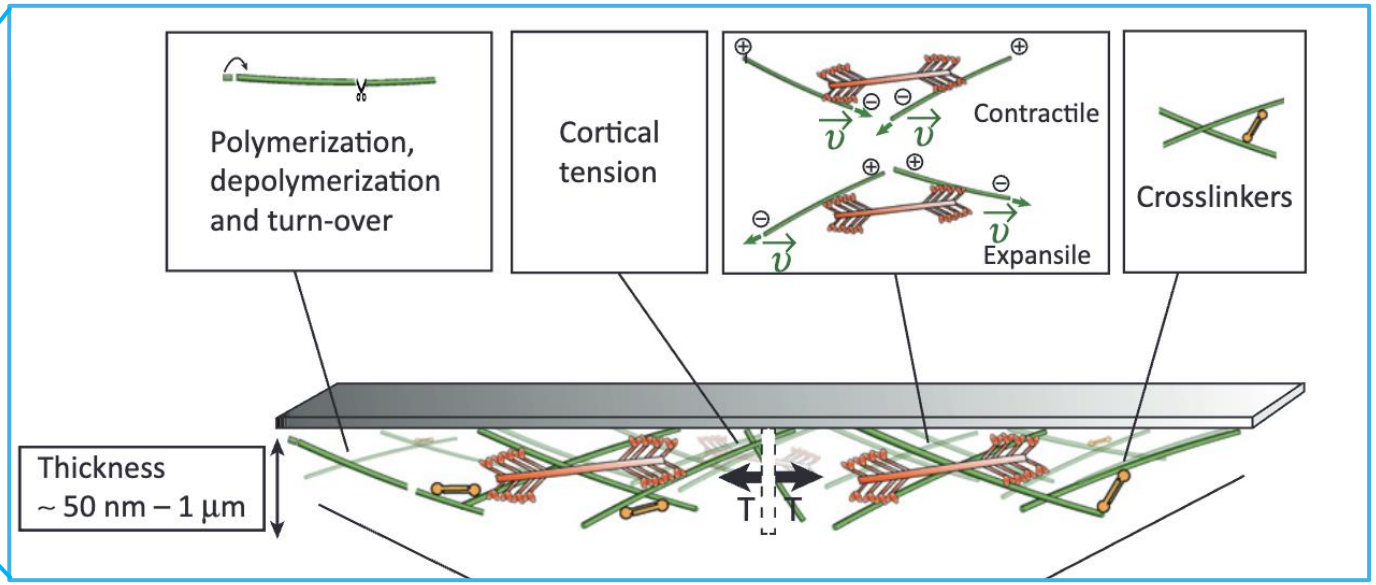
Oocyte microvilli

Actin-related protein 2/3 (Arp 2/3)

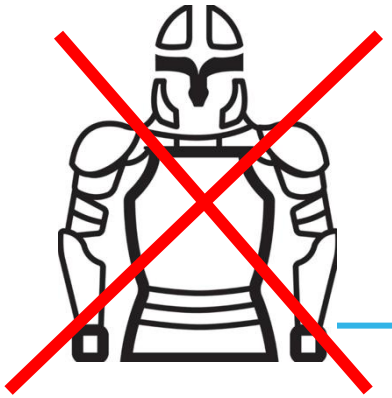




Coticchio et al., 2015a

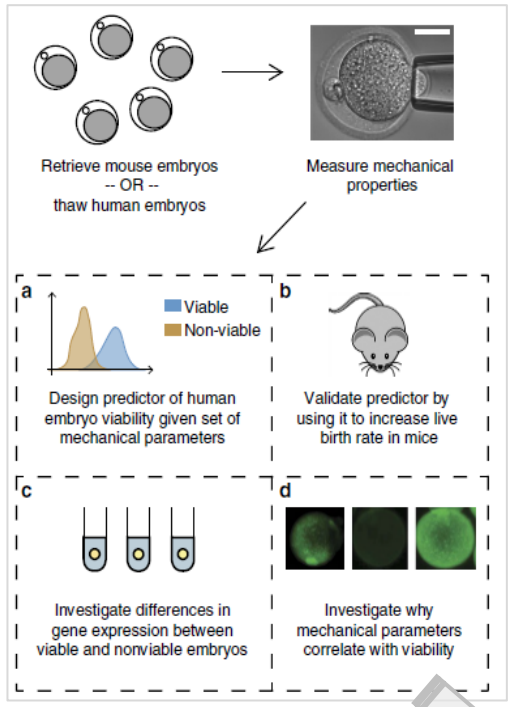


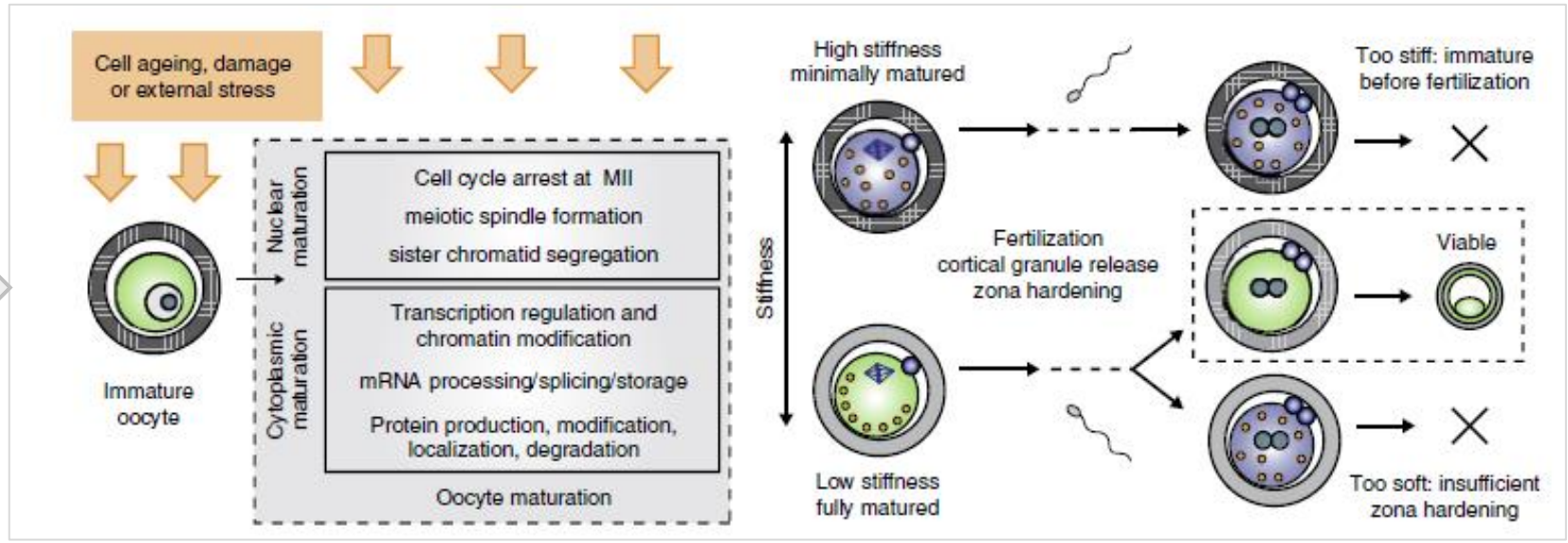
Salbreux et al., 2012



Once considered a passive structure, it is now recognized as a central determinant of oocyte quality and developmental competence

Measuring Cortex mechanics in IVF





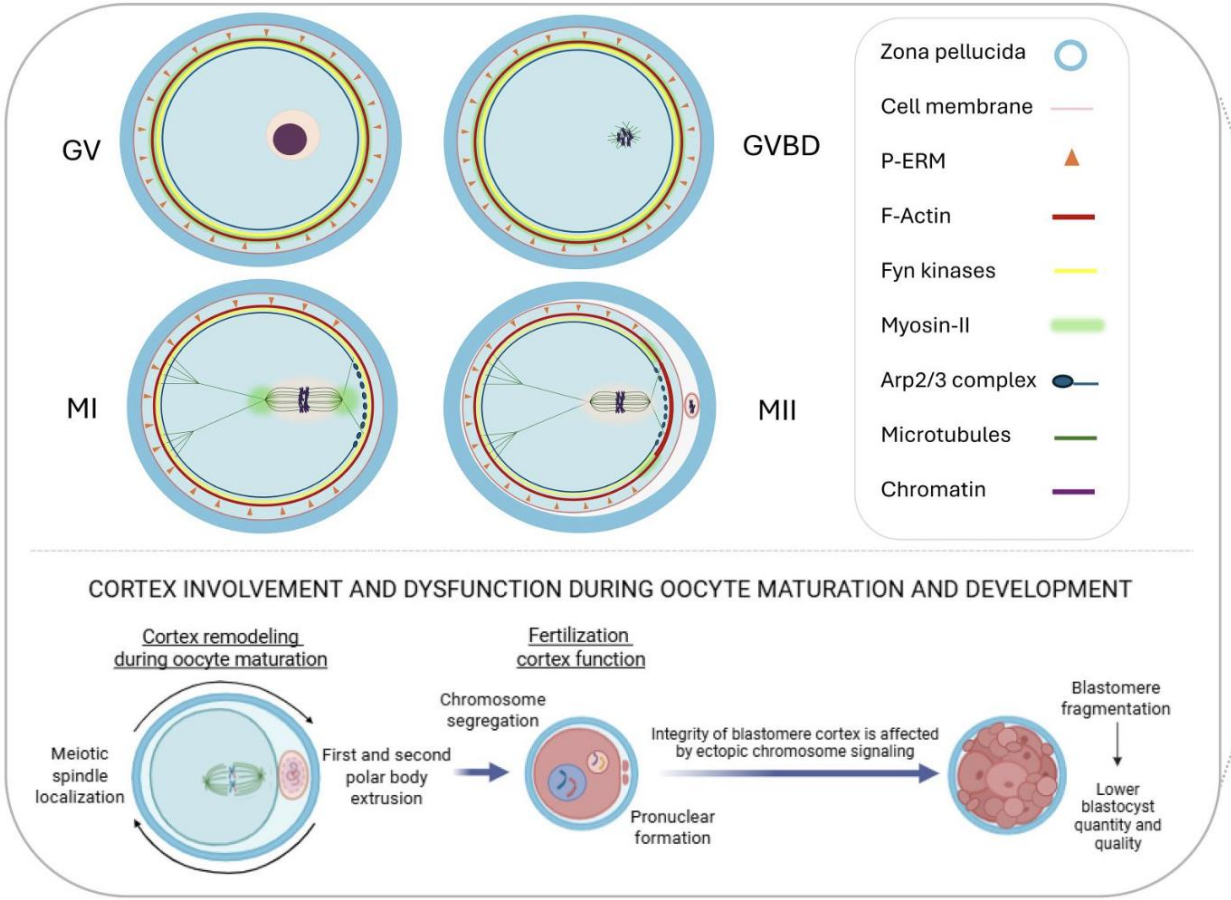
Model

Poorly matured oocytes show abnormal mechanical properties (too stiff or too soft) due to failed cortical granule dynamics. Optimally matured oocytes undergo correct mechanical changes after fertilization, enabling successful embryonic development.

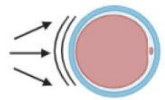
Yanez LZ, Han J, Behr BB, Pera RAR, Camarillo DB. Human oocyte developmental potential is predicted by mechanical properties within hours after fertilization. Nat Commun. 2016

IVIRMA Global Research Alliance

The emerging role of the oocyte cortical domain in maturation, fertilization, and development



Clinical relevance



Cortical mechanics as biomarkers of oocyte quality and developmental competence