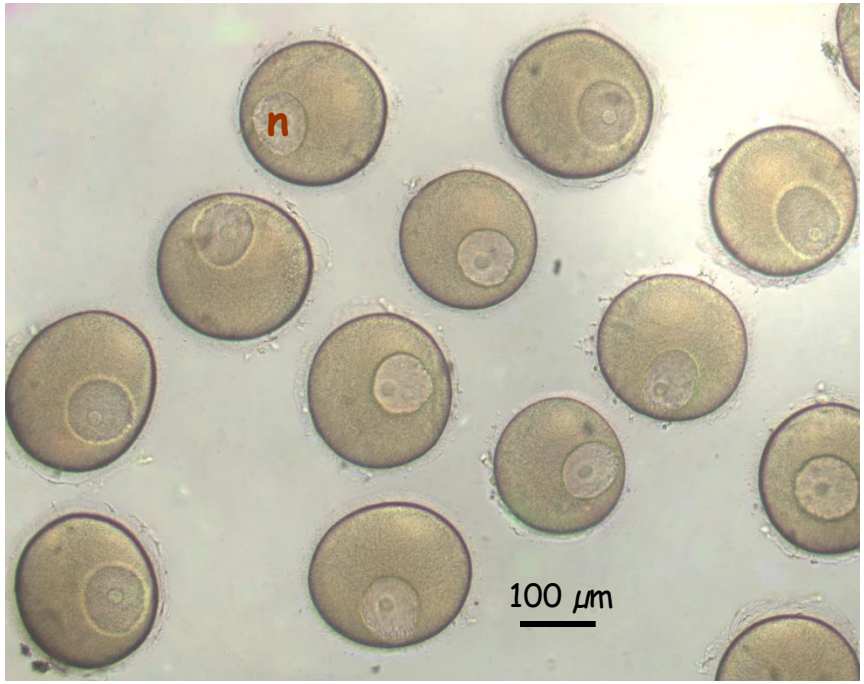
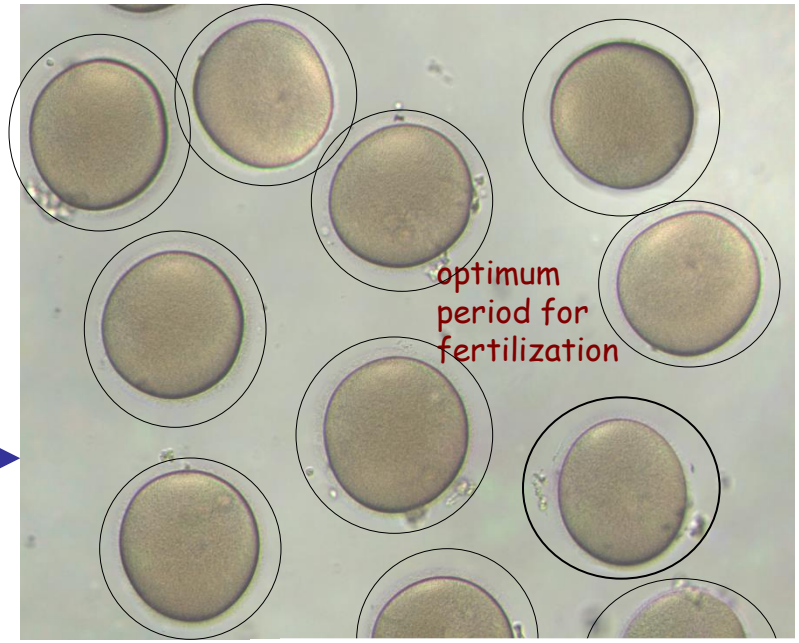


GV stage oocytes arrested
at the I meiotic division

1-MA

50 min after 1-MA addition



*synchronized population of large and transparent cells
* suitable for microinjection and imaging

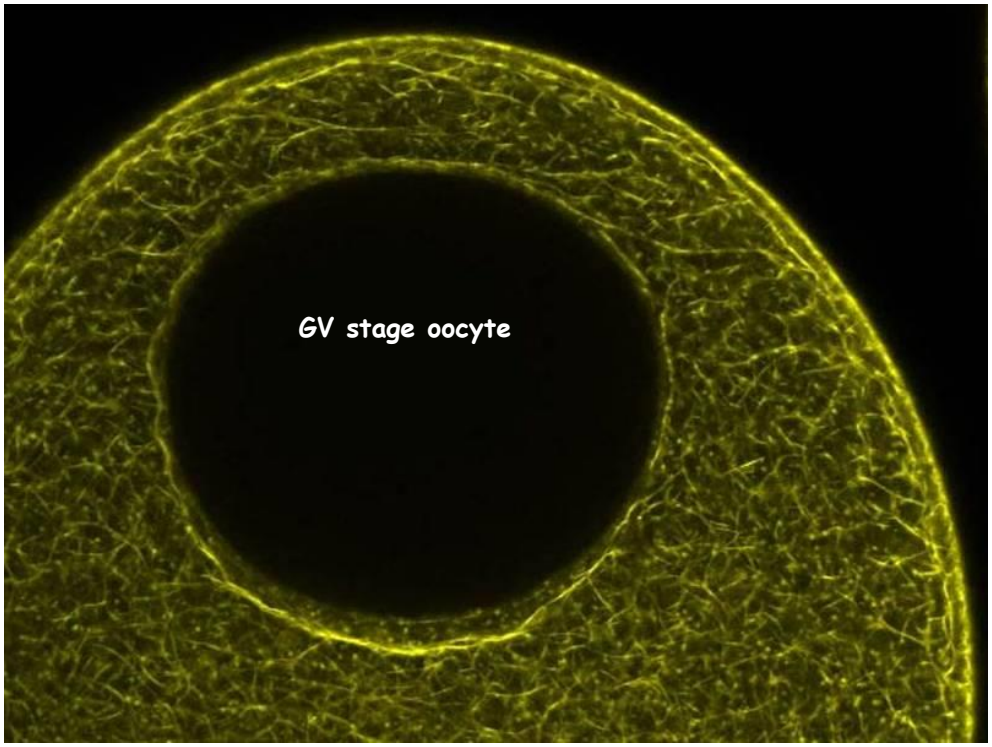
During the maturation process, in addition to the changes of the egg surface, the major cytoplasmic Ca^{2+} store (endoplasmic reticulum) undergoes extensive restructuring. This rearrangement increases the sensitivity of the channel that releases Ca^{2+} to the second messenger inositol trisphosphate ($InsP_3$)

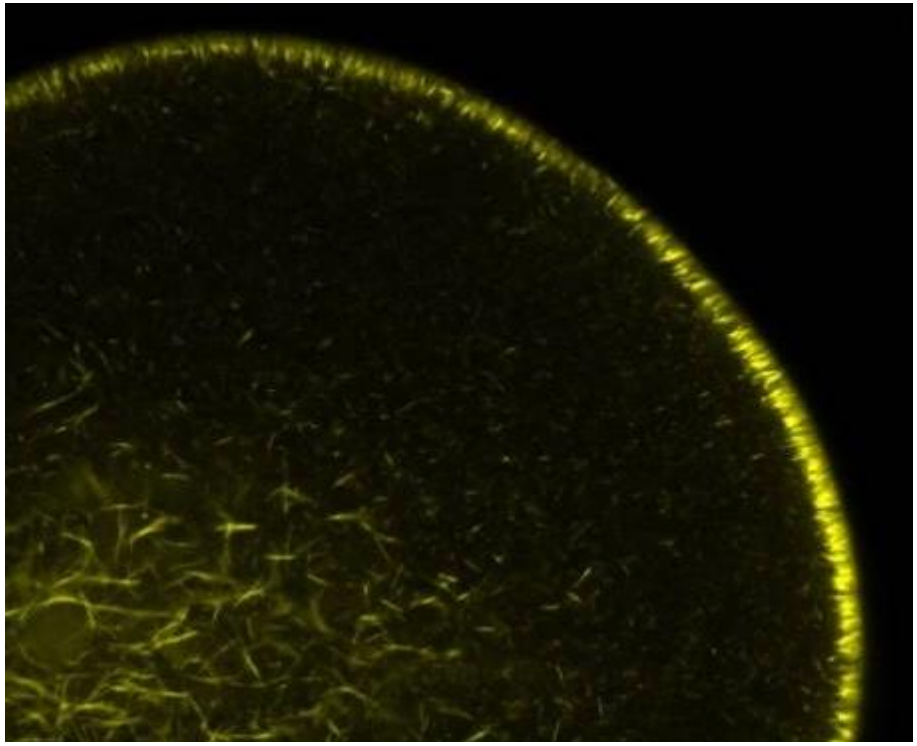
Chiba, Kado and Jaffe, Dev Biol (1990)

In starfish, the increased $InsP_3$-dependent Ca^{2+} release and the sperm-induced Ca^{2+} response after maturation may be correlated with the dynamic rearrangement of the actin cytoskeleton

Chiba, et al (1990) Dev Biol; Lim et al (2002) FASEB J; Lim et al (2003) JBC

F-actin visualization before and after starfish oocyte maturation



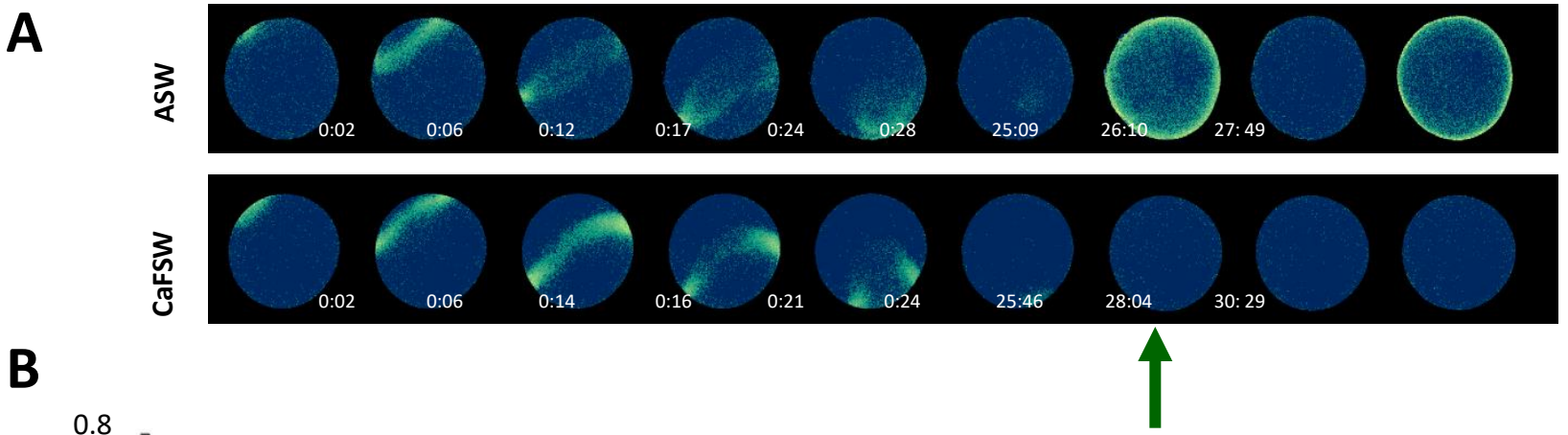


before 1-MA addition

1 h after 1-MA addition

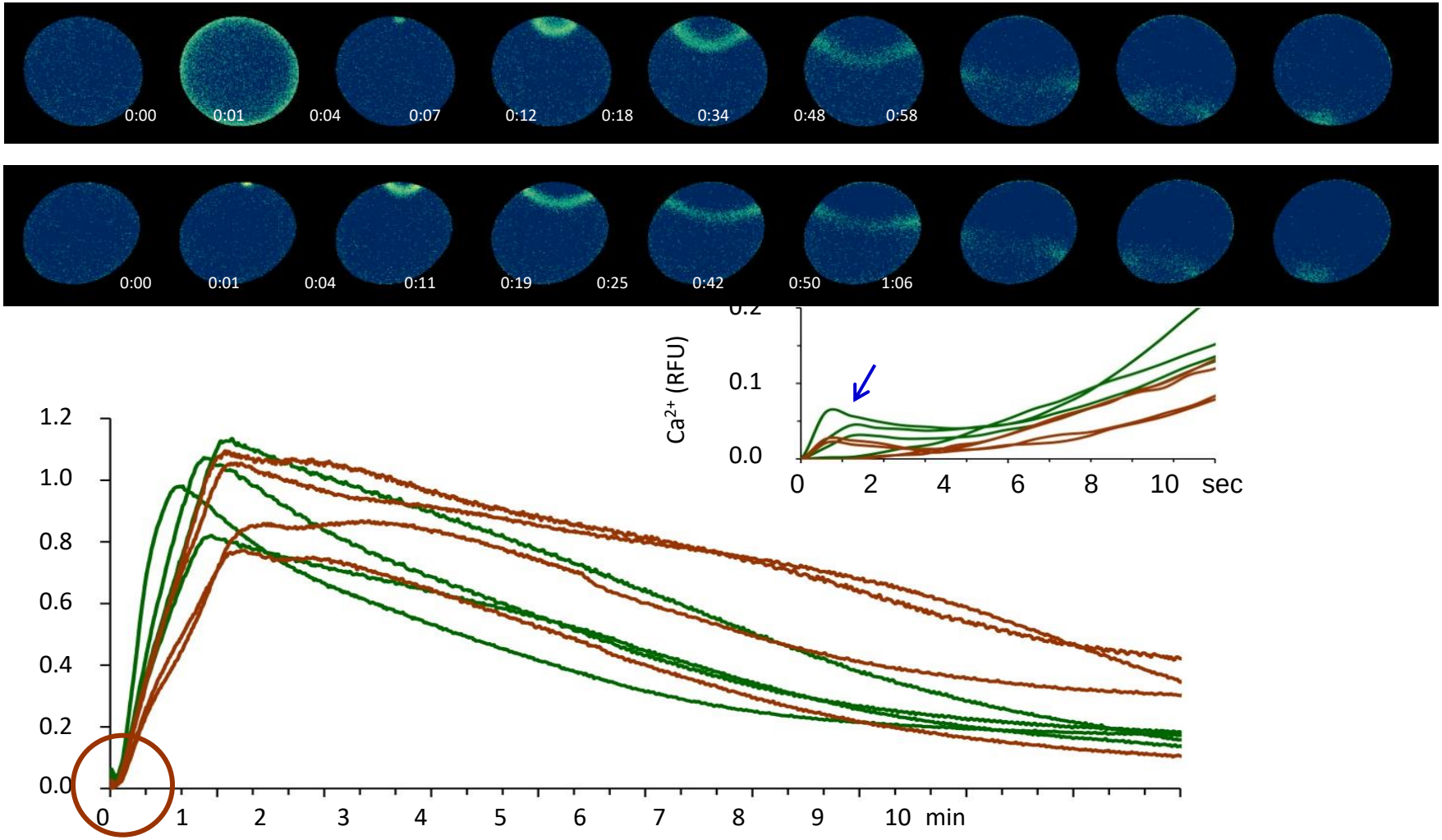
Santella et al 2012; 2014; 2015; 2016

Ca^{2+} increases during maturation of starfish oocytes: early and late signals

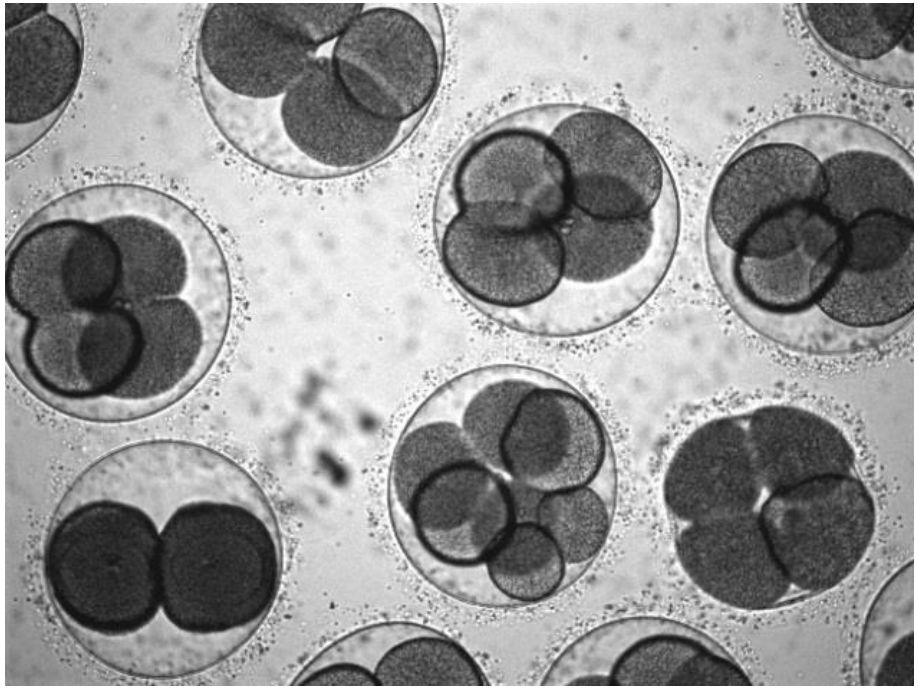


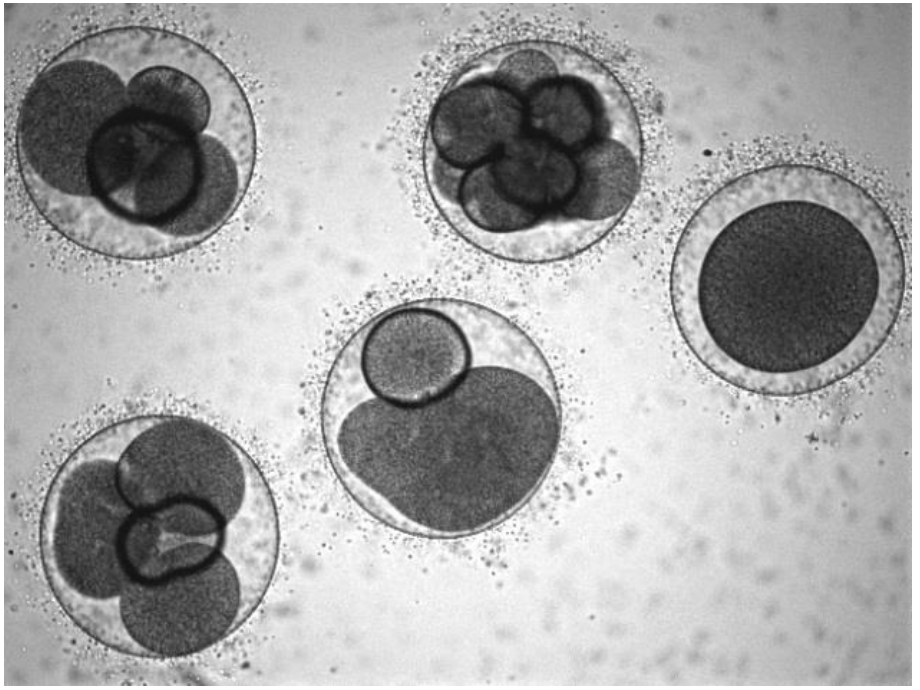
Santella and Kyozuka, BBRC (1994); Santella et a al BBRC (1998); Kyozuka et al (2008) Dev Biol; Limatola et al (2015) Cell Calcium

Sperm-induced Ca^{2+} changes in control and oocytes matured in CFSW



Early starfish embryos (2 hour after insemination)



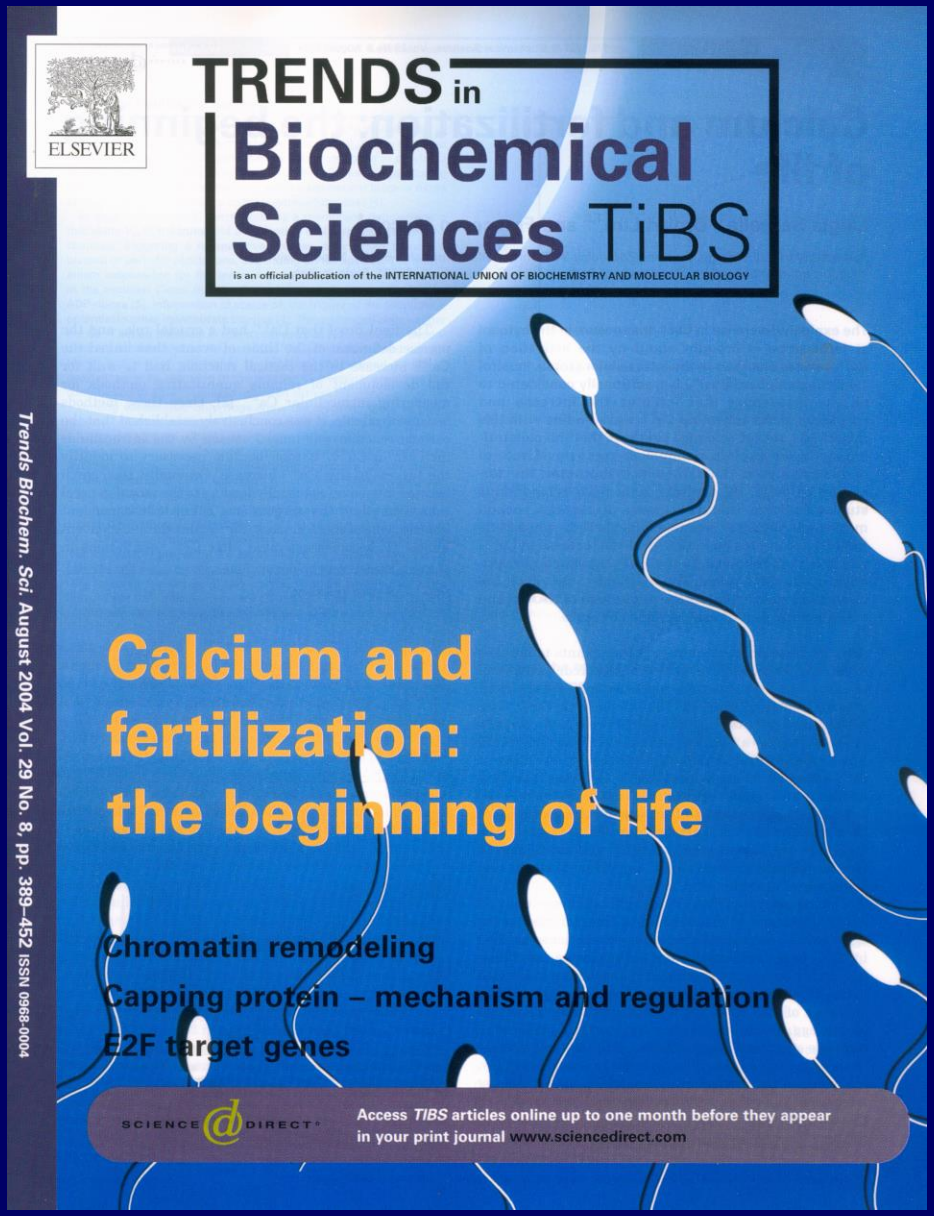


100 µm

matured in ASW and fertilized in ASW (control)

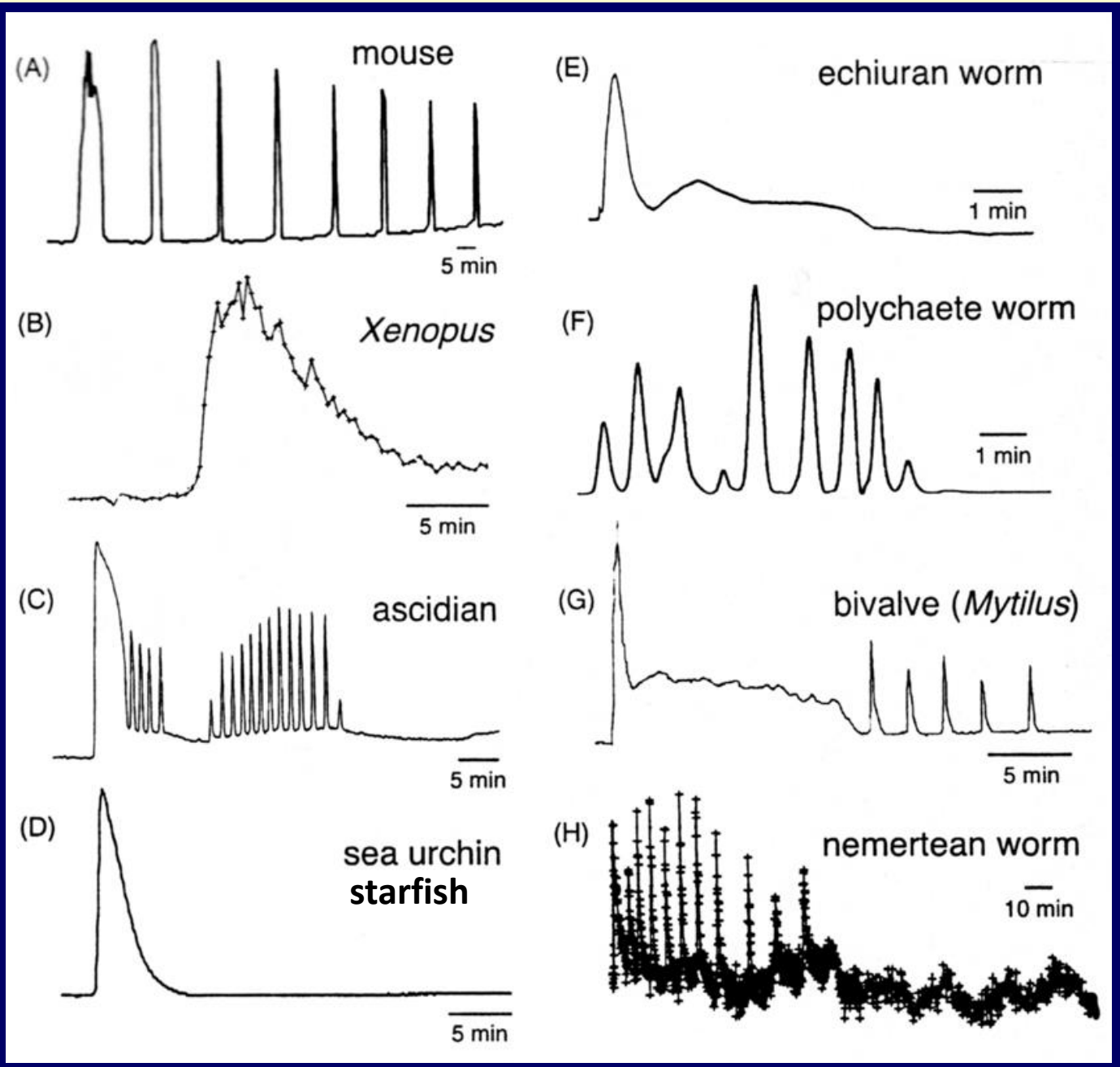
matured in CaFSW and fertilized in ASW

Ca^{2+} and fertilization

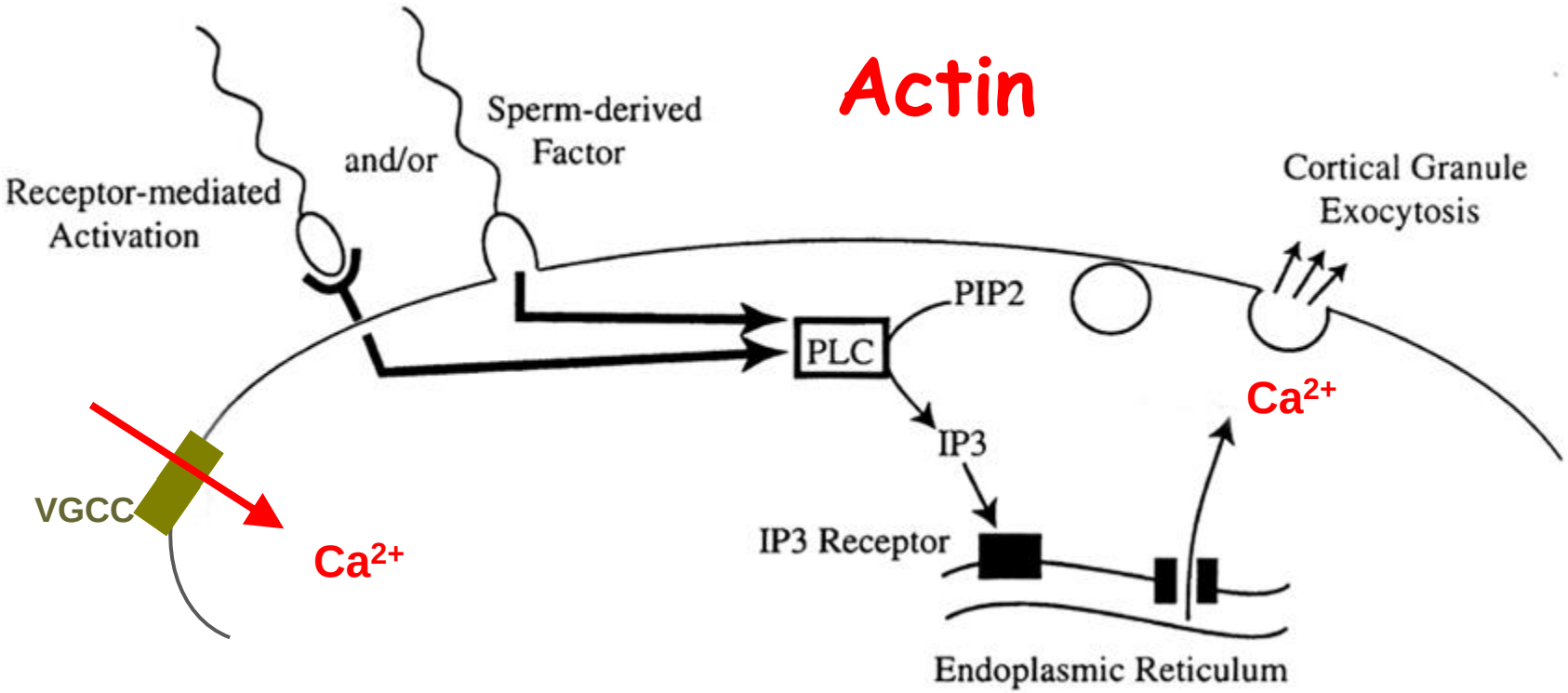


Santella et al. (2004) Trends Biochem Sci. 29:400-8

Ca^{2+} responses at fertilization in eggs

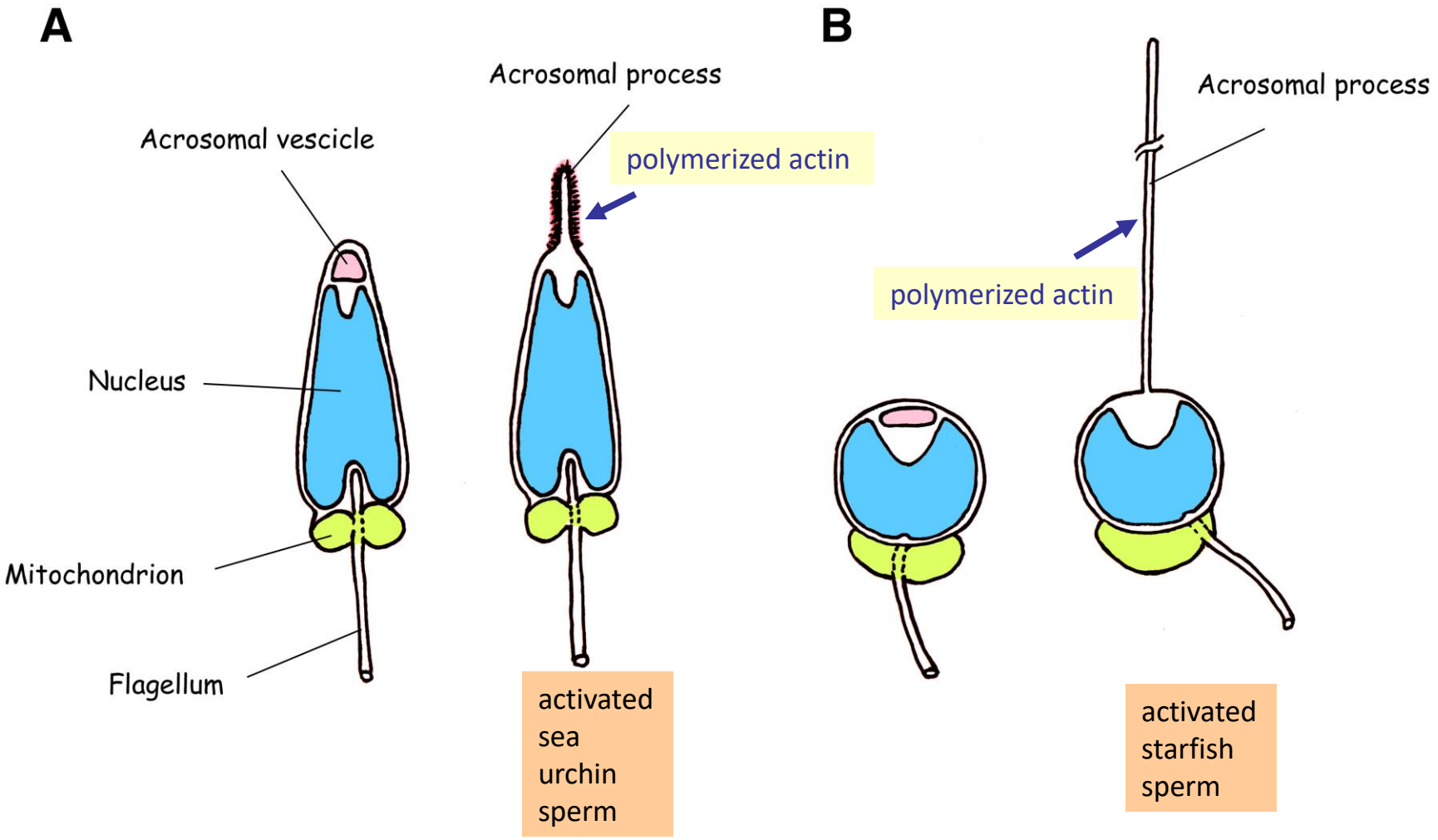


Fertilized eggs provide an excellent opportunity to study cell interaction, intracellular Ca^{2+} signaling, and cortical granules exocytosis



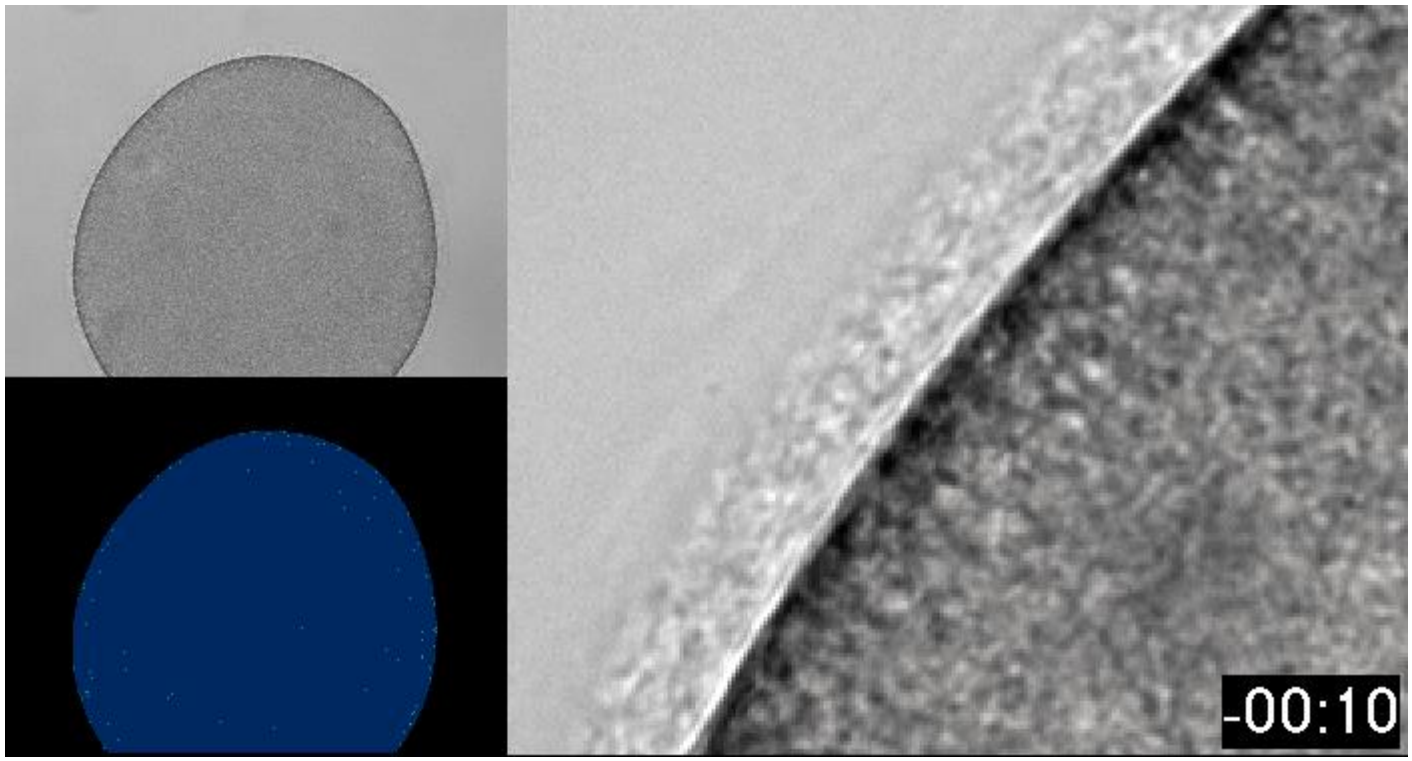
modified from Stricker 1999

The acrosomal process in the spermatozoa of the sea urchin and starfish



from Santella et al (2012) BBRC

Calcium signalling and sperm incorporation in a fertilized starfish egg



Honorable mention NiKon Competition on "Small world in motion" (2015)

What happens when the eggs are activated by ionomycin

Proc. Nat. Acad. Sci. USA
Vol. 71, No. 5, pp. 1915–1919, May 1974

Activation of Sea-Urchin Eggs by a Calcium Ionophore

(fertilization/membrane conductance/respiration/protein synthesis/DNA synthesis)

RICHARD A. STEINHARDT* AND DAVID EPEL†

* Department of Zoology, University of California, Berkeley, Calif. 94720; and † Scripps Institution of Oceanography, La Jolla, California 92037

Exposure of sea urchin eggs to micromolar amounts of A23187 resulted in rapid discharge of the cortical granules and elevation of vitelline envelopes.
The activation of the eggs were independent of the external calcium

Nature Vol. 252 November 1 1974

Is calcium ionophore a universal activator for unfertilised eggs?

RICHARD A. STEINHARDT

Department of Zoology,
University of California,
Berkeley, California 94720

DAVID EPEL
EDWARD J. CARROLL, JUN.

Marine Biology Research Division,
Scripps Institution of Oceanography,
University of California, San Diego,
La Jolla, California 92037

RYUZO YANAGIMACHI

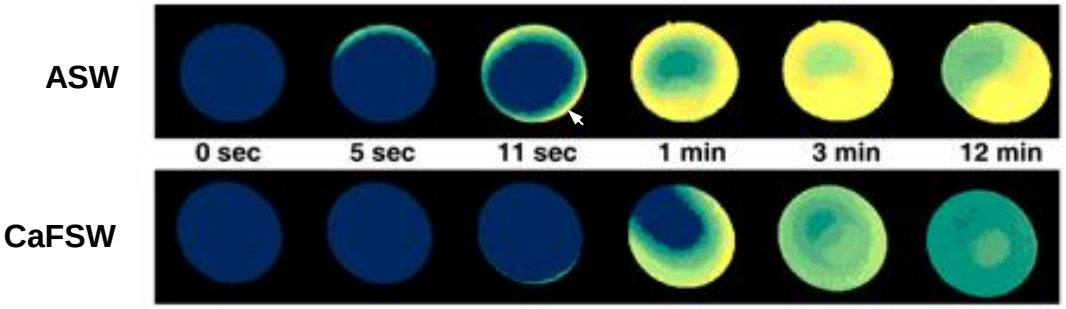
Department of Anatomy and Reproductive Biology,
University of Hawaii School of Medicine,
Honolulu, Hawaii 96822

The authors proposed that release of intracellular calcium ionic calcium may be the universal factor promoting activation of egg metabolism at fertilization

Determinations of the «free» and «bound» calcium were made from homogenates

Changes of intracellular Ca^{2+} levels in starfish *A. aranciacus* oocytes exposed to ionomycin in Ca^{2+} and Ca^{2+}-free SW

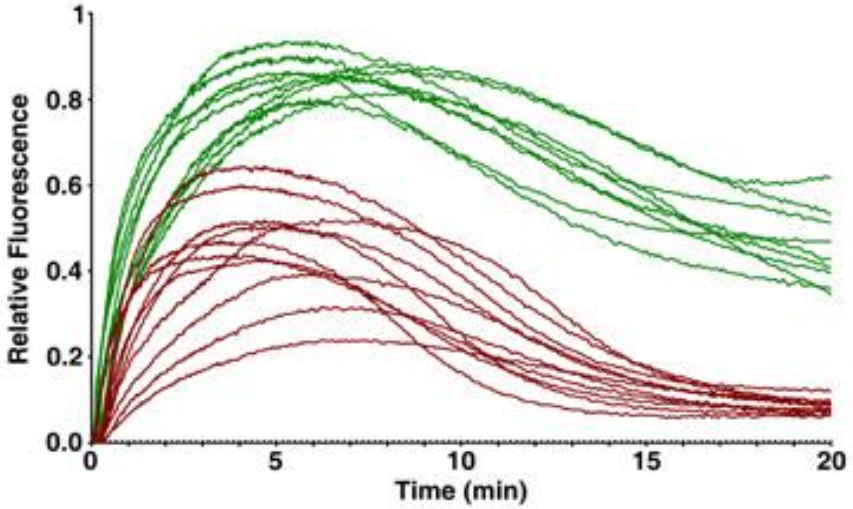
A



ASW-artificial sea water

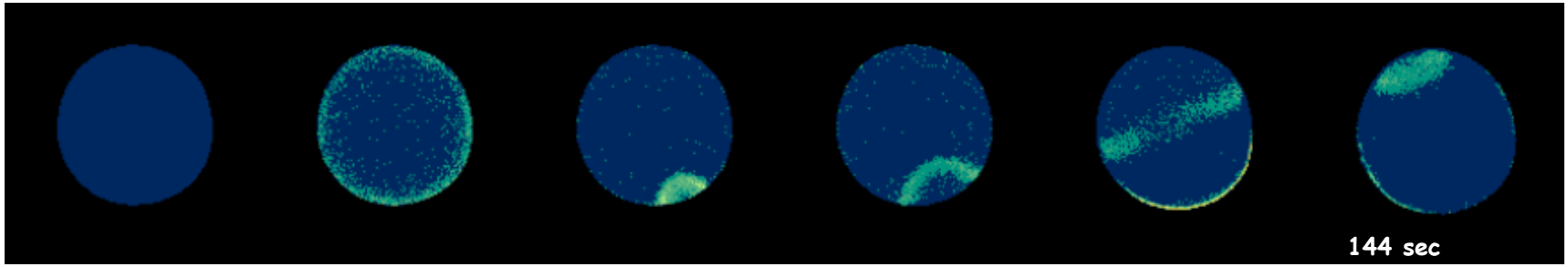
CaFSW- Ca^{2+}-free sea water

B

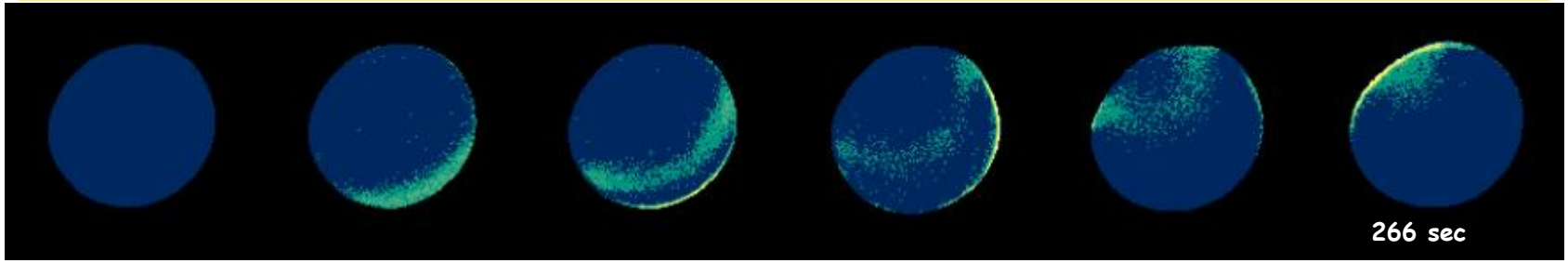


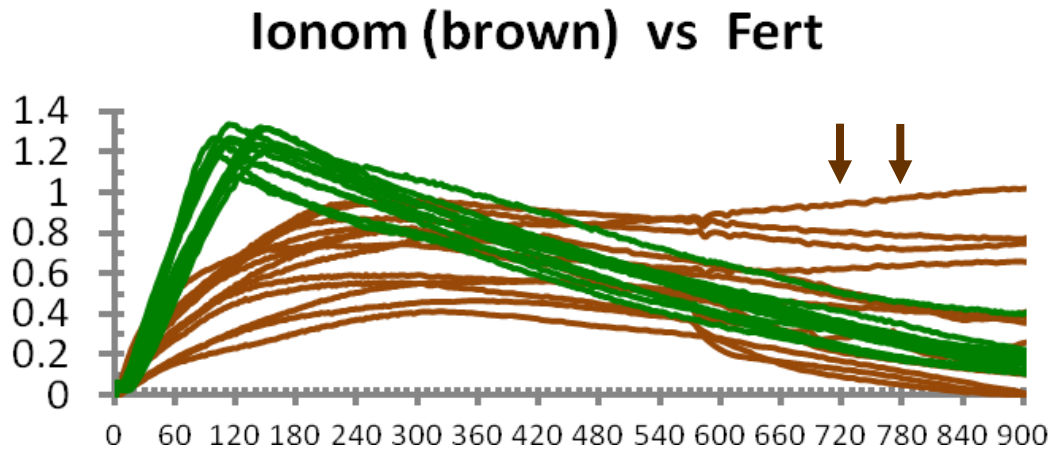
Vasilev et al. (2012) PLOsONE

Sperm-induced Ca^{2+} signal

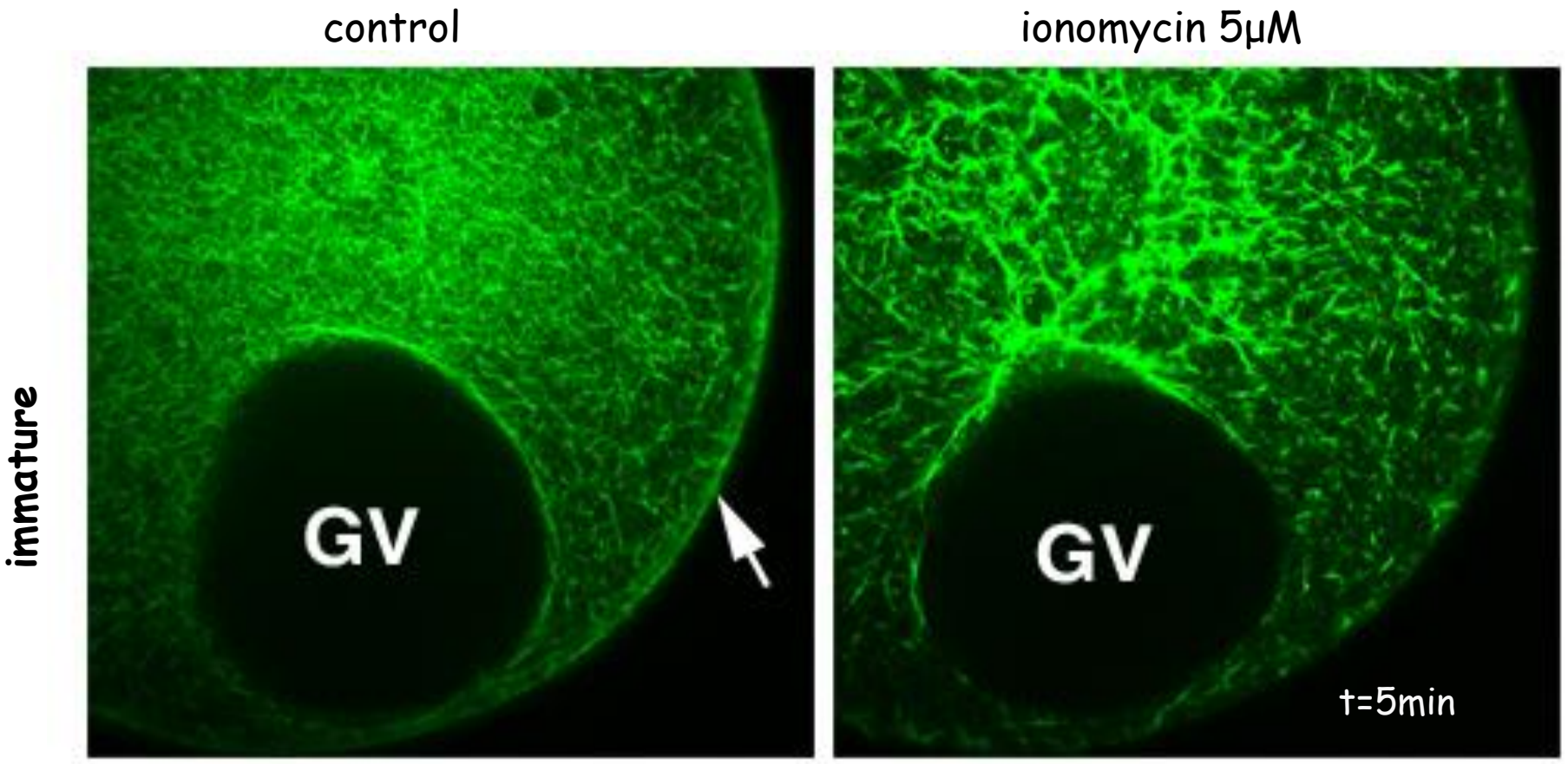


Ionomycin-induced Ca^{2+} signal





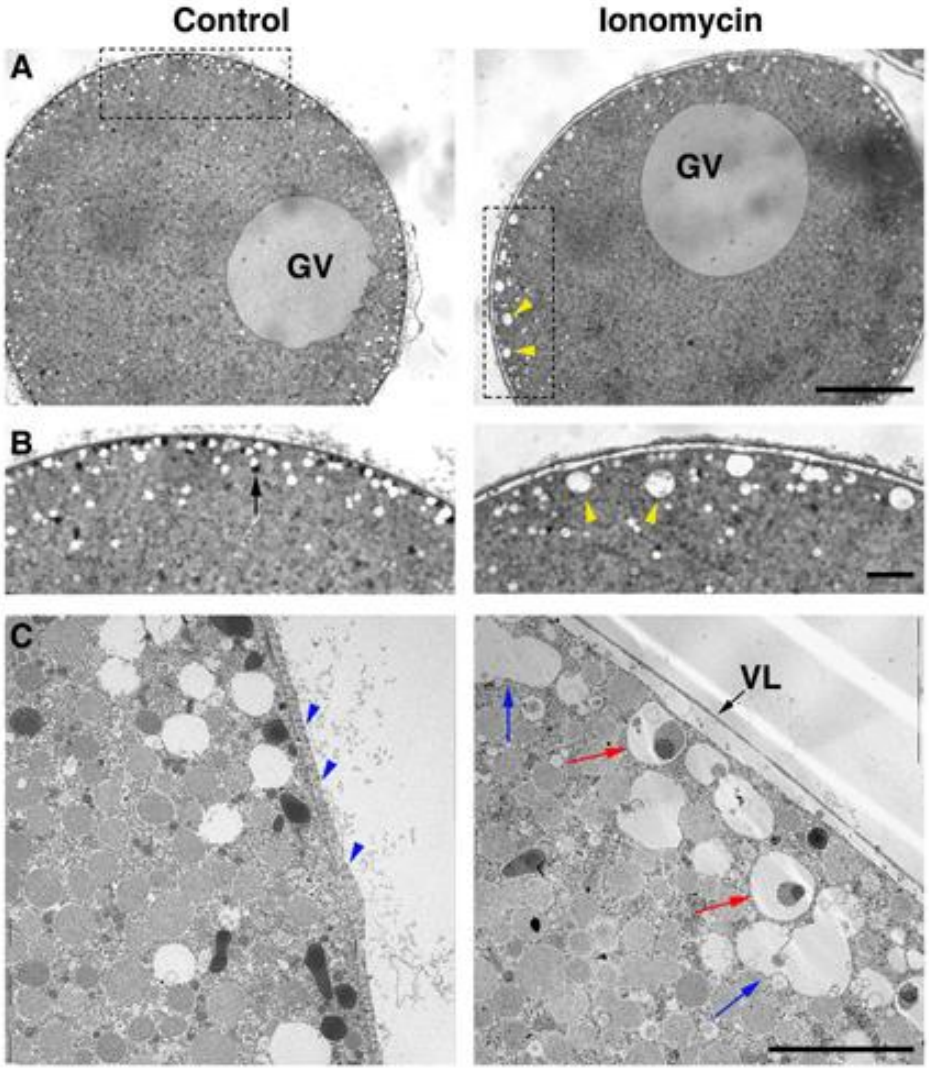
Ionomycin induces rapid rearrangement of the actin cytoskeleton in starfish oocytes



Ionomycin treatement induced actin cytoskeleton depolymerization in the cortex and enhanced polymerization in the inner cytoplasm

Vasilev et al (2012) PLoS ONE

Morphological changes in the cortex of the *Astropecten aranciacus* oocytes exposed to ionomycin



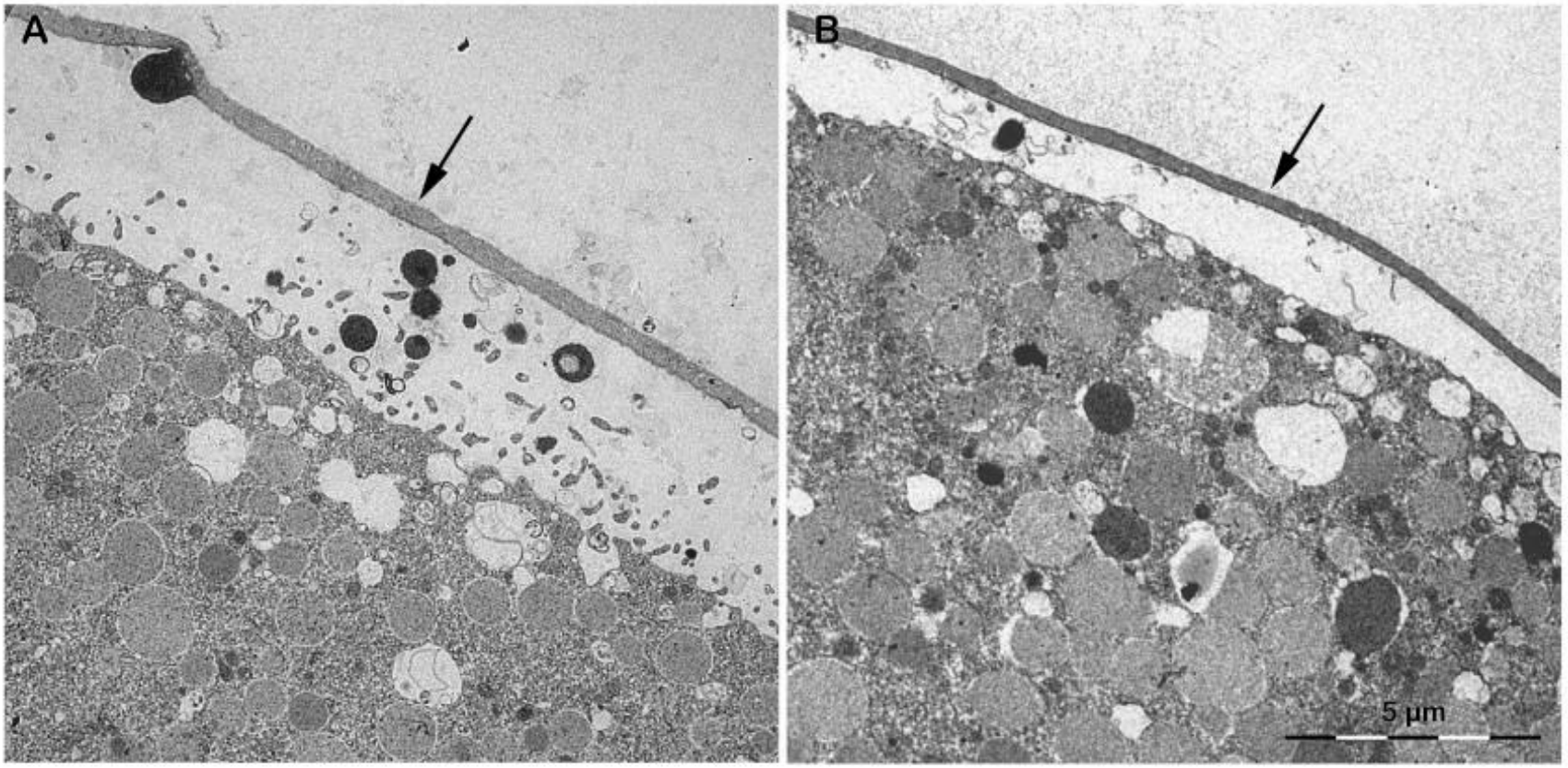
transmitted light microscopy

transmitted electron microscopy

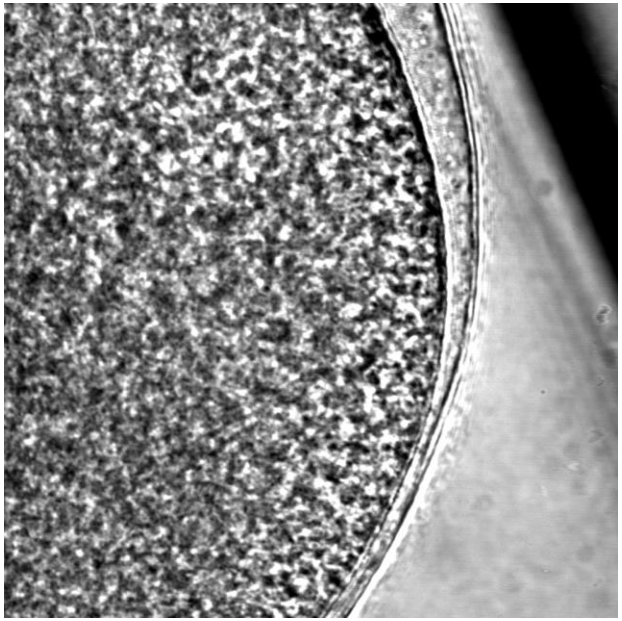
Ionomycin treatement (5μM, 3 min) of immature oocytes induces fusion of cortical granules with vesicles

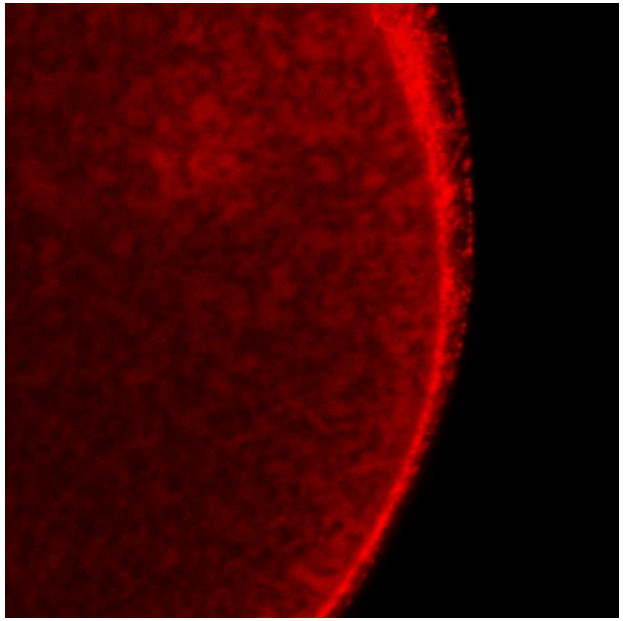
Vasilev et al (2012) PLoS ONE

Activation of starfish eggs by A) spermatozoon and
B) by ionomycin. Note that the elongation of microvilli in the perivitelline space beneath the fertilization envelope (arrow) of inseminated eggs is missing in those activated by ionomycin. The ionomycin-activated eggs show a surface without microvilli and a less elevated vitelline envelope

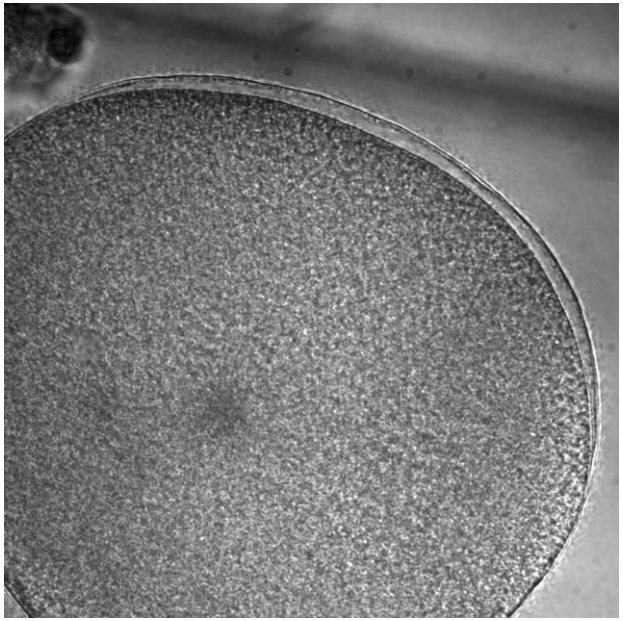


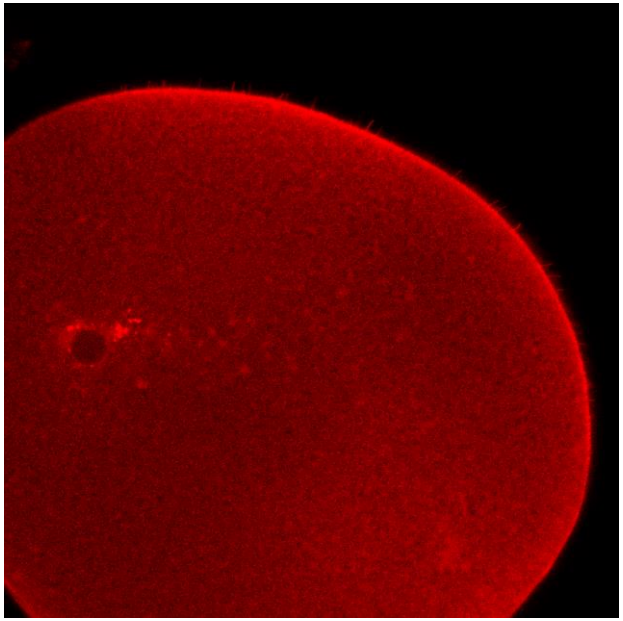
Partial cortical reaction induced by ionomycin and A23187





ionomycin

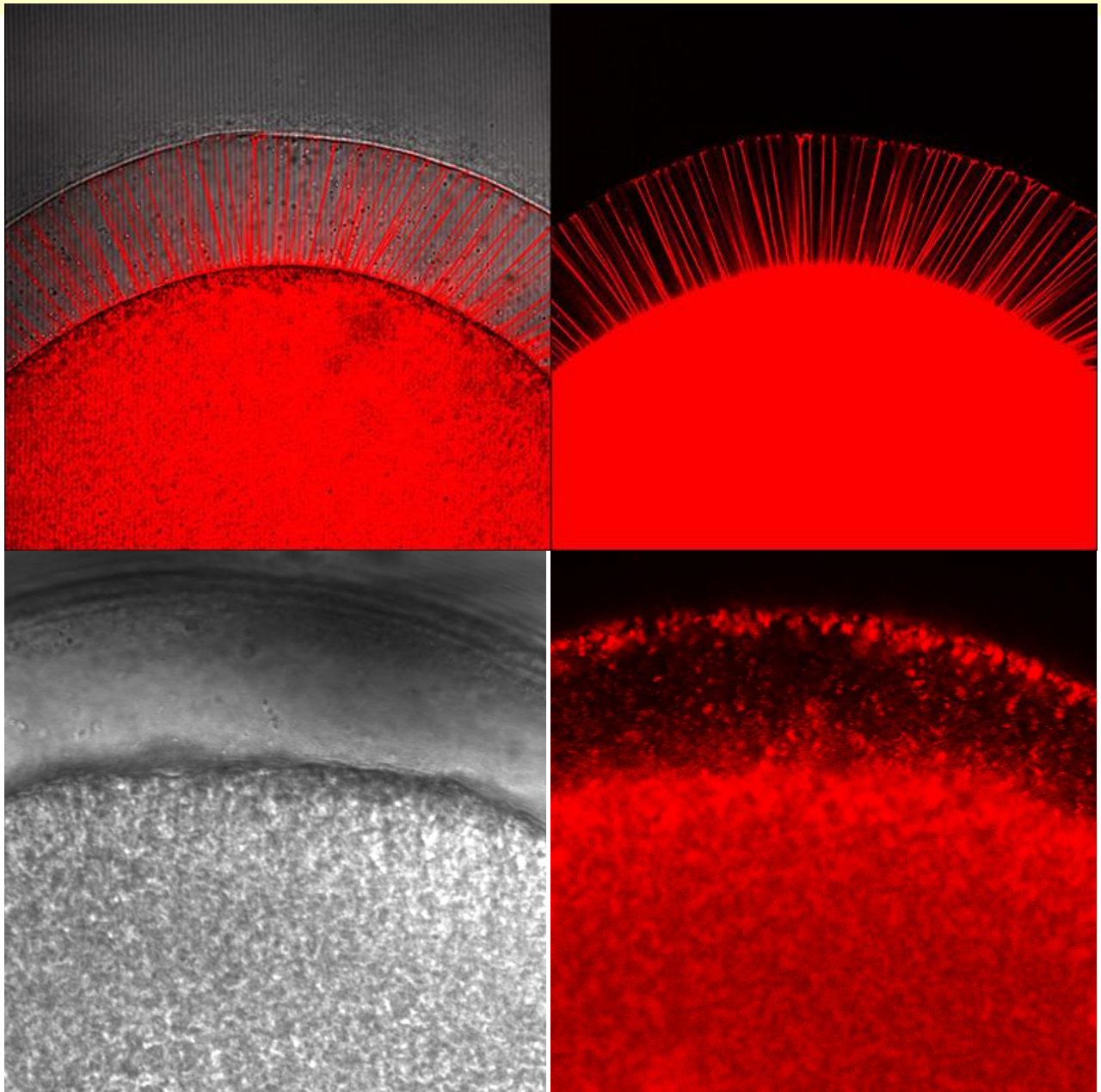




Ca ionophore
A23187

Cortical reaction induced by $InsP_3$ and by ionomycin

Spikes formation into the perivitelline space of a starfish egg visualized by a membrane dye



$InsP_3$

Ionomycin

Conclusions

The fundamental act in fertilization are the changes occurring at the egg surface (cortical reaction) following the attachment of the spermatozoon. All animal eggs show visible changes in the egg cortex in response to sperm-attachment.

The elevation of the fertilization envelope in starfish and sea urchin eggs constitutes an easily visible indicator of the underlying surface-changes initiated by the fertilization reaction and of the quality of the eggs.

The importance of intracytoplasmic sperm injection to permit almost any type of spermatozoa to fertilize oocytes has made it the most successful target in treatment of male infertility. However it should be kept in mind that the selection of the injected spermatozoon by the embryologist bypasses the natural selection process.

The role and duty of assisted reproductive technology is to achieve success by applying the least possible invasive technique. Resources should be invested to find ways, (1) of improving the quality of gametes *in vivo* by selecting the most appropriate protocol for ovarian stimulation, and, (2) by evaluating patient's gametes *in vitro* with efficient technologies rather than stimulating defective gametes.

Filip Vasilev Jong T. Chun Nunzia Limatola Gianni Gragnaniello Ezio Garante


ACQUARIO