Ion currents and molecules involved in oocyte maturation, fertilization and embryo development

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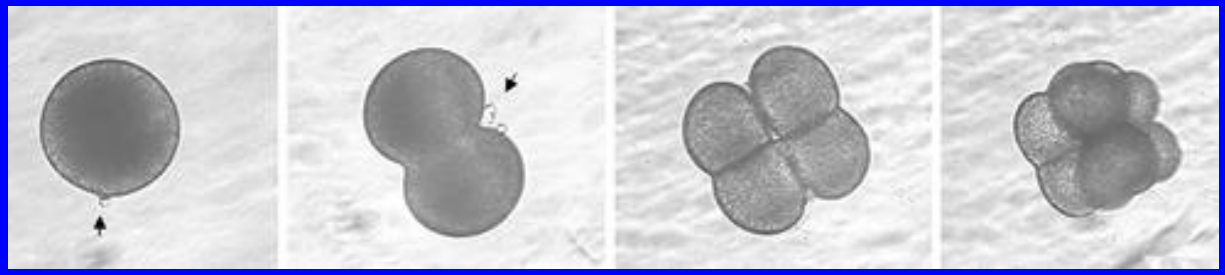


Main steps of the reproductive process

production and maturation of gametes

- oogenesis, spermatogenesis

gametes interaction

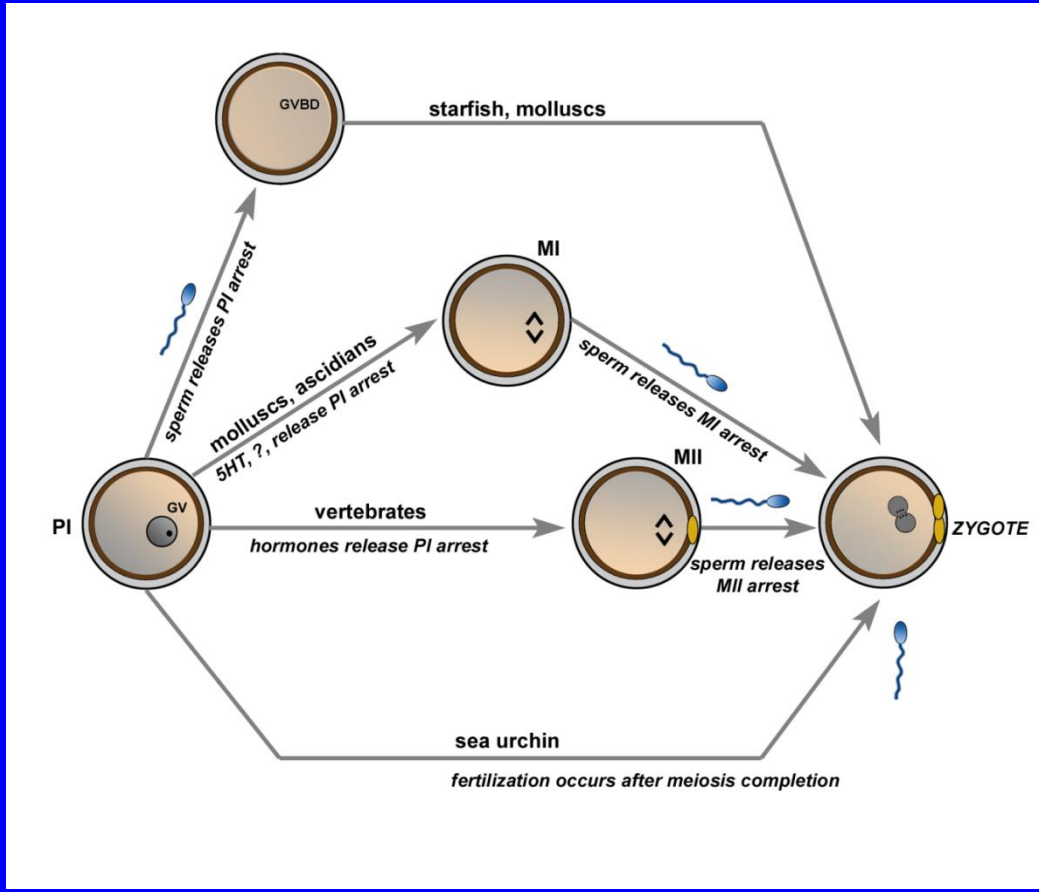
- sperm activation
- oocyte activation

fertilization

embryo development

The oocyte maturation process

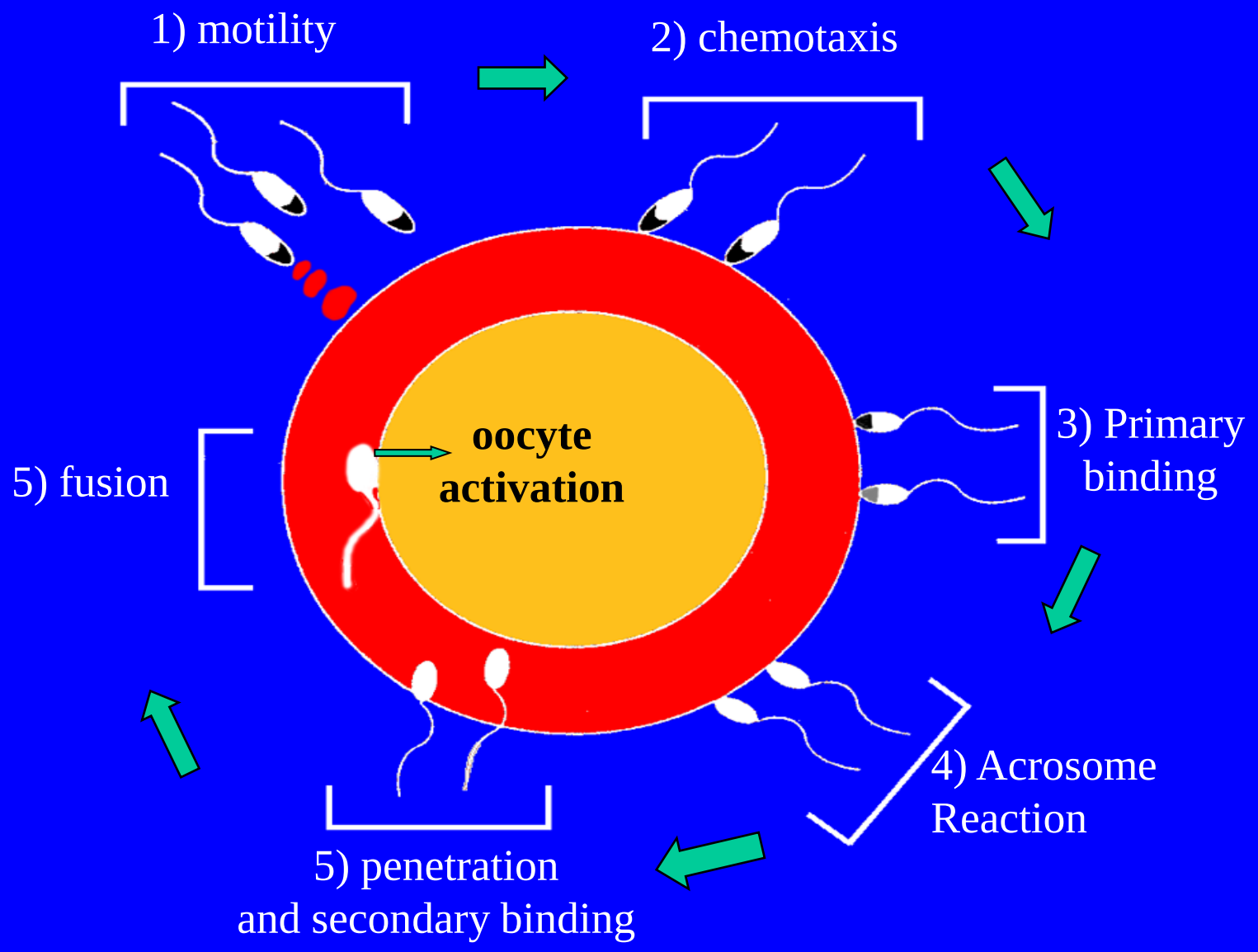
The immature oocyte is arrested at the I prophase (PI) of meiosis marked by the germinal vesicle (GV). At the PI, meiosis resumption starts with the germinal vesicle breakdown (GVBD) leading to a second meiotic block which occurs at the metaphase I (MI) or metaphase II (MII) or may be fertilized, depending on the species. MII is marked by one polar body (yellow). Resumption from the second meiotic block occurs upon sperm penetration leading to meiosis completion and zygote formation (Zy) marked by the two inner pronuclei and two polar bodies.



Tosti E. Repr. Biol . Endocr. 2006

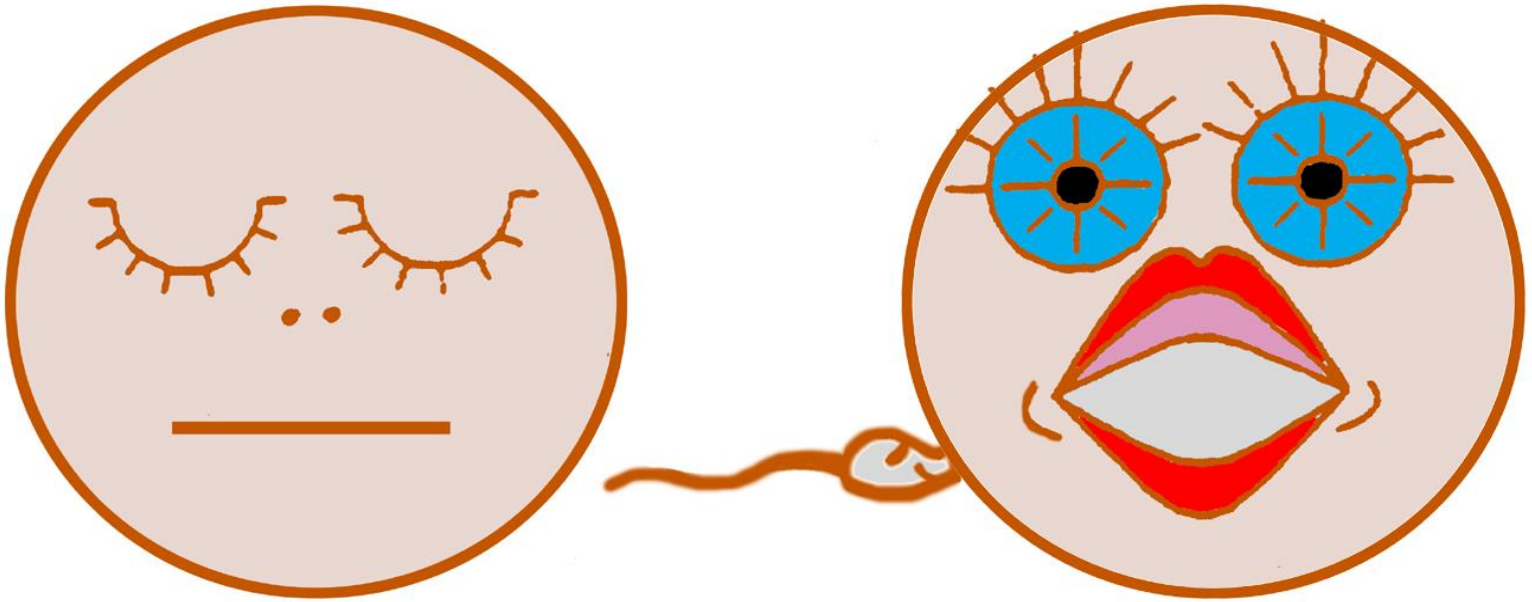
Gametes interaction:

step by step the reciprocal gametes activation



The oocyte activation

sleeping beauty and the prince



SPERM-INDUCED OOCYTE ACTIVATION

Electrical modifications:

fertilization current, depolarization, hyperpolarization;

Morphological modifications:

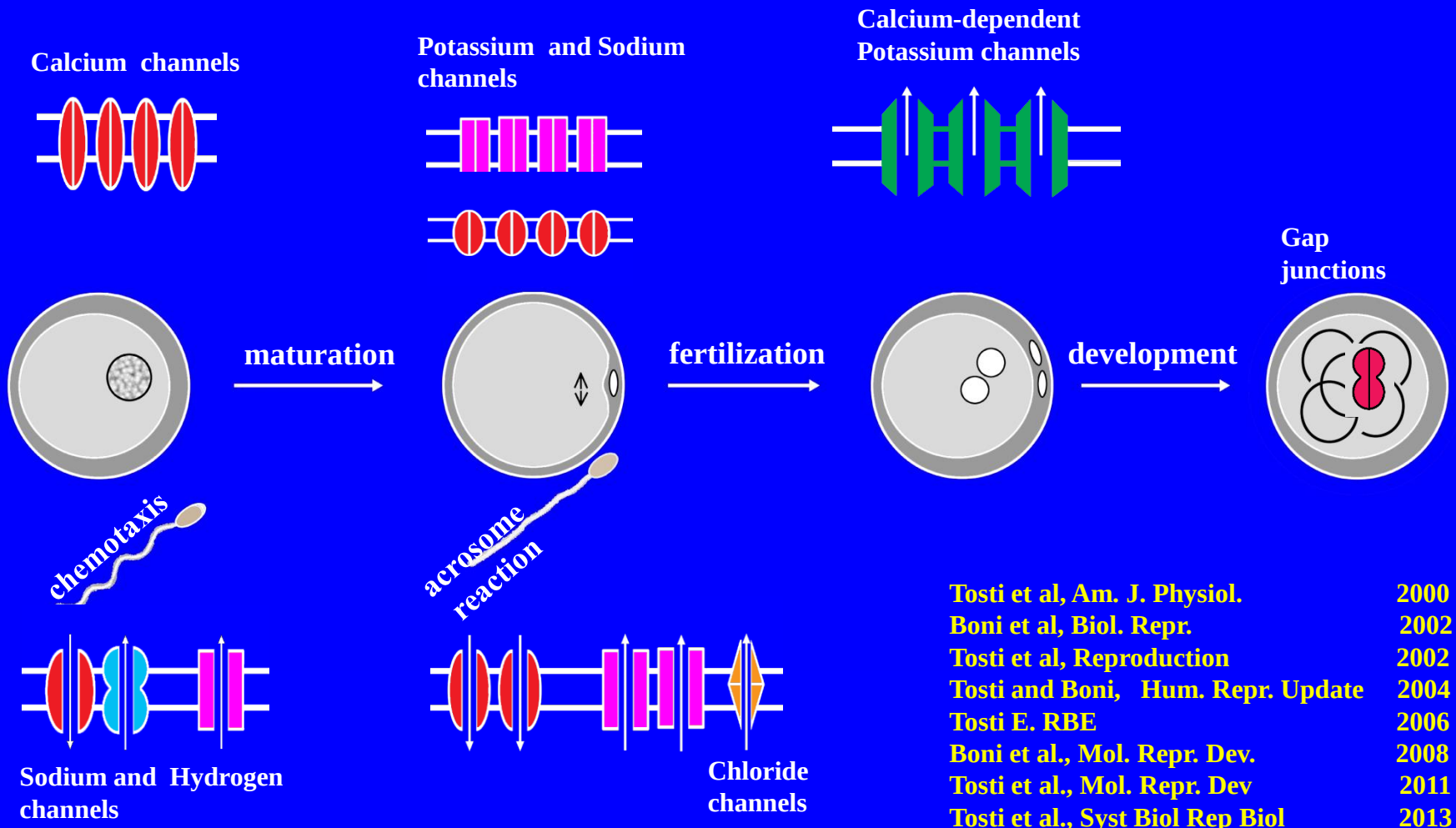
cortical reaction, zona hardening, oocyte contraction;

Metabolical modifications:

calcium increase, pH increase, PI hydrolysis, MPF inactivation

Why study the plasma membrane?

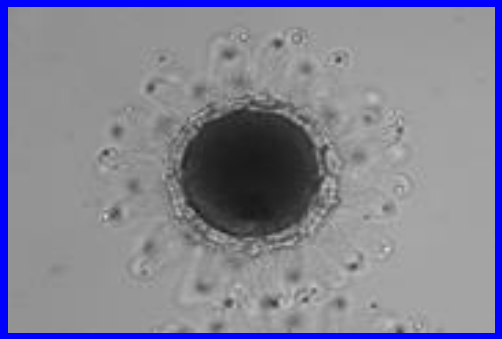
On the plasma membrane are located the ligands and receptors involved in the cell to cell interaction processes. Many of these events involve ion currents activity.



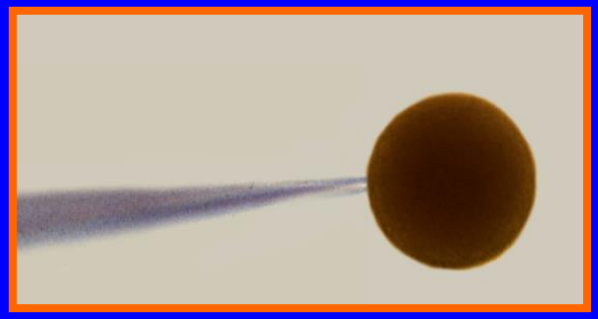
Tosti et al, Am. J. Physiol. 2000
Boni et al, Biol. Repr. 2002
Tosti et al, Reproduction 2002
Tosti and Boni, Hum. Repr. Update 2004
Tosti E. RBE 2006
Boni et al., Mol. Repr. Dev. 2008
Tosti et al., Mol. Repr. Dev 2011
Tosti et al., Syst Biol Rep Biol 2013

Electrophysiological techniques

Set up for electrophysiological recordings



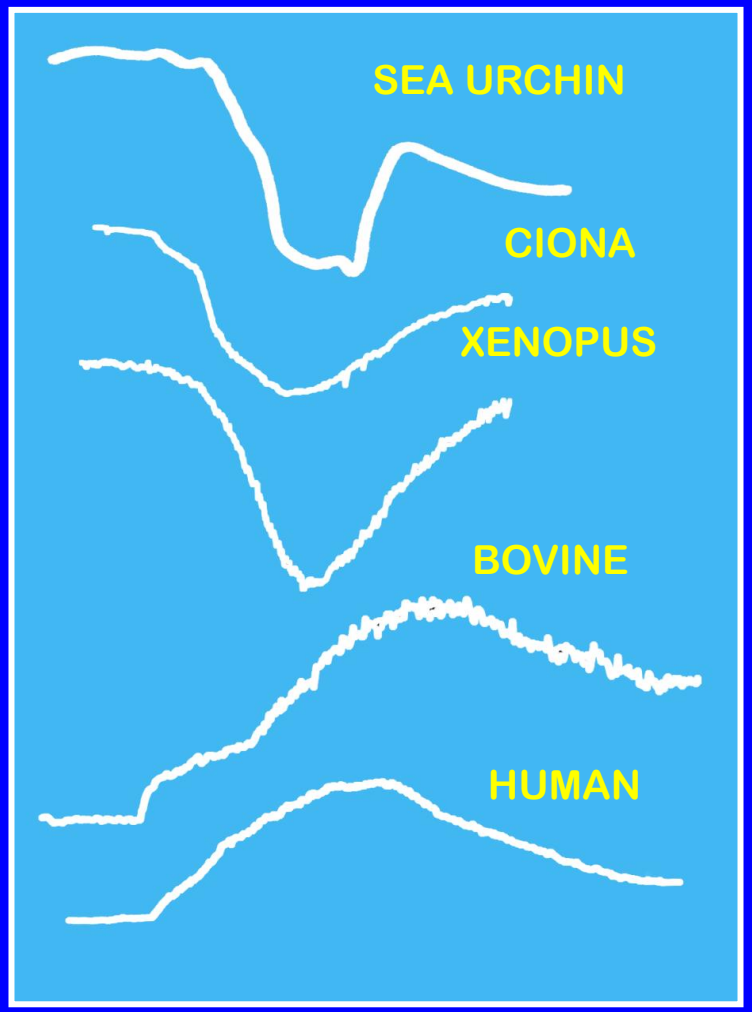
Ciona intestinalis mature oocyte at MI stage



Ciona intestinalis denuded oocyte under the whole - cell clamp configuration

The fertilization current

The first event of fertilization is the activation of a population of ion channels named fertilization channels. This current depolarizes the membrane potential in marine invertebrates and amphibians and hyperpolarizes in mammals.



De Simone et al., Zygote 1994

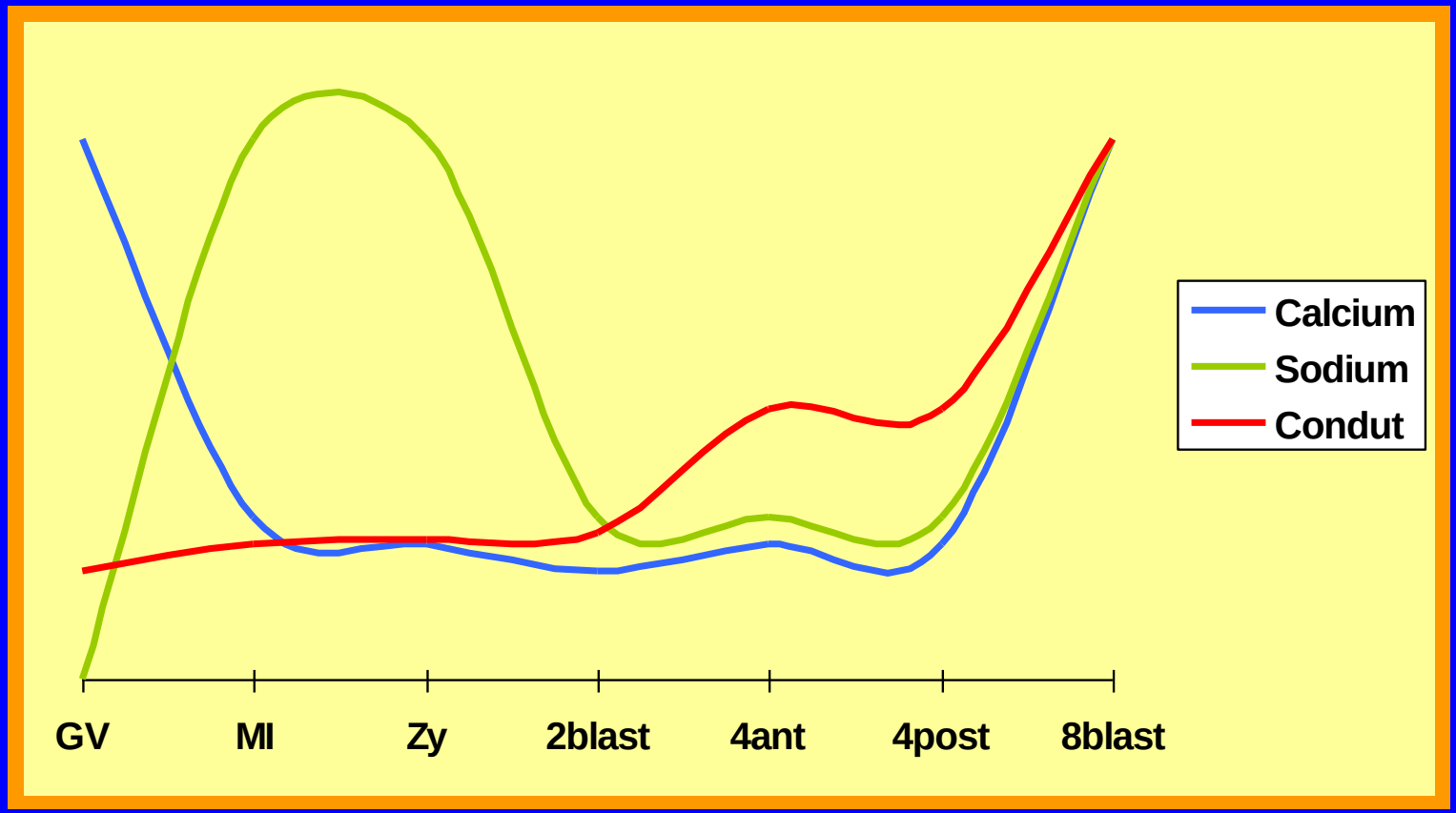
Dale and De Felice, Dev Biol. 1984

Glahn and Nuccitelli , DGD. 2003

Tosti et al., Reproduction, 2002

Gianaroli et al., MRD, 1994

Pattern of ion currents activity in *C. intestinalis* gametes and embryo



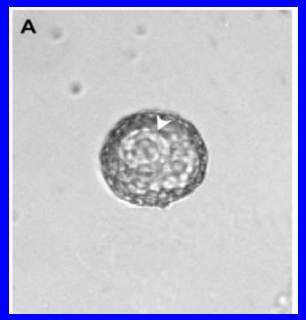
Cuomo et al., MRD 2006

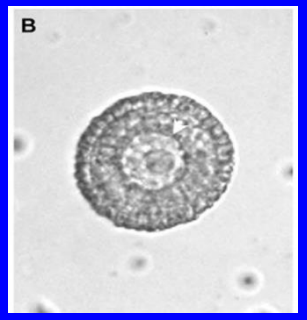
Silvestre et al., MRD 2009

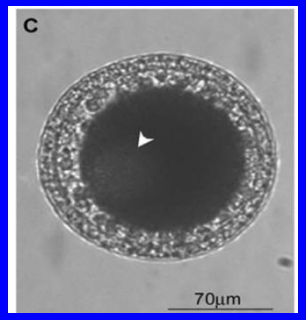
Tosti et al., MRD, 2011

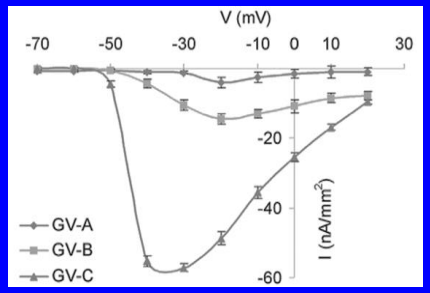
Tosti et al., Syst Biol Repr Med 2013

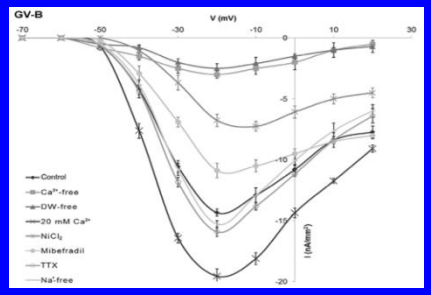
T-type Ca^{2+} current activity and mitochondria distribution during oocyte growth and maturation in the ascidian *Styela plicata*

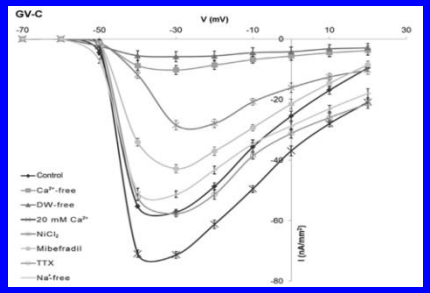


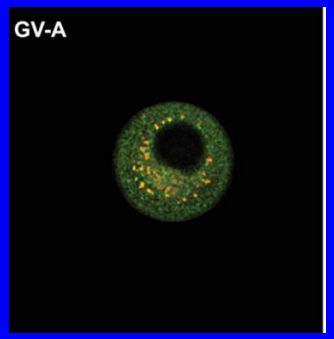


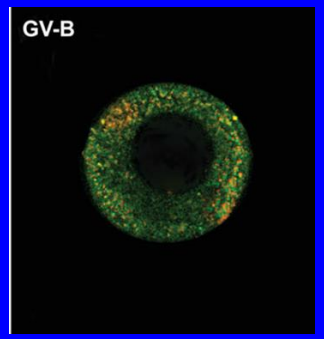


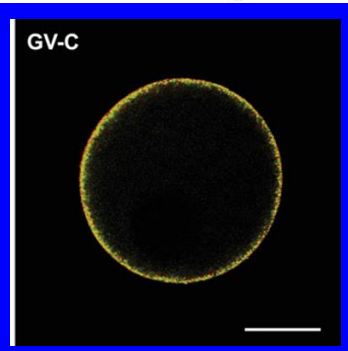












Gallo et al., PlosOne 2013
Bezzaouia et al., Zygote, 2013

Conclusions

involvement of L and T- type Ca^{2+} currents in meiosis progression and in charging intracellular stores to support Ca^{2+} release at fertilization;

Role for Na^{+} currents during electrical events at fertilization and subsequent development;

Mitochondria distribution and activity during the oocyte growth may correlate with oocyte competence acquisition.

Application outcomes

Ion currents are present from gametogenesis to development and may be a good marker to study the physiology of these processes ;

Ion currents manipulation may be essential for improving IVM and IVF techniques in humans, animals of zoo-technical and aquacultural interest.;

Ion currents may targets for modern protocols non involving hormonal contraception.

IMPACT OF XENOBIOTICS ON REPRODUCTION OF MARINE INVERTEBRATES

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Reproduction of marine animals occurs in the sea water

Consequently, gametes and embryo may be influenced by substances that can alter their physiological functions and in turn may affect fertilization success, embryo development, larval viability and fitness/ survival of the species.

Marine pollution

is defined as the introduction in marine environment of substances (Xenobiotics) able to produce negative effects on the biological resources, human health, marine activities and water quality. (United Nations)

Xenobiotics

are substances foreign to an entire biological system:

- **heavy metals**
- **herbicides, pesticides, fungicides, plasticizers, phenols, dioxins**
- **organotins, and more specifically tributyltins (TBT), used for its biocide properties as the active agent in antifouling paints**

Xenobiotics may act as disruptors in many endocrine systems

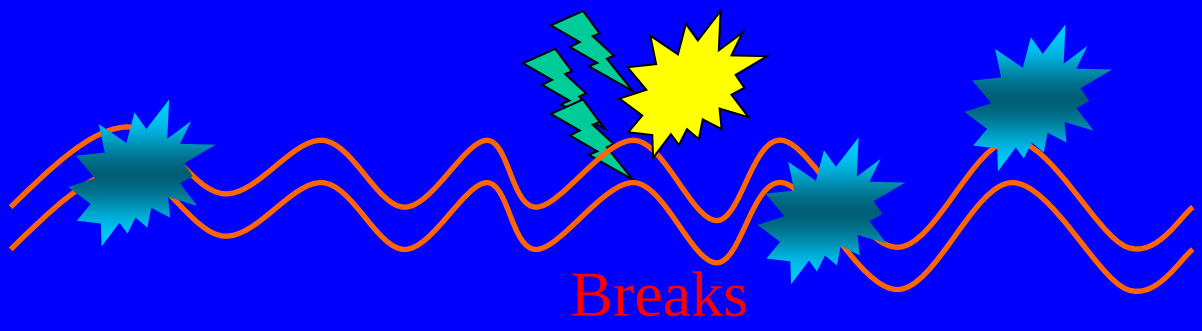
Action:

- **mimic the effects of hormones,**
- **Alter the pattern of synthesis and metabolism of hormones,**
- **antagonize the effects of hormones,**
- **modify hormone-receptor levels**
- **impair the plasma membrane**

Xenobiotics effects on the reproductive system

- **reproductive failure and abnormalities of male and female reproductive systems**
- **reproductive functions in adulthood**
- **imposex**

Xenobiotics may induce sperm DNA fragmentation



Environmental etiology:

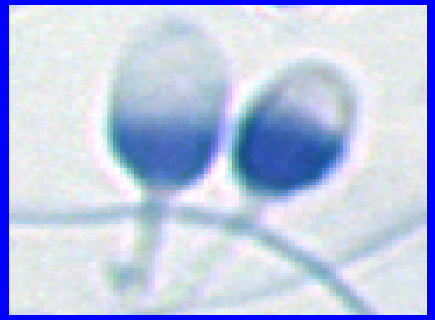
- **Pollution, Drugs, Pesticides, Chemicals, Heat**

Impact on reproductive outcome :

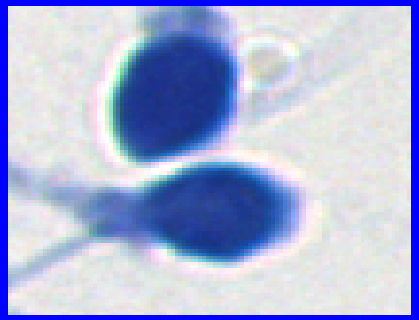
- **Abnormal embryo and fetal development, miscarriage**

Xenobiotics may induce sperm chromatin decondensation

Normal sperm

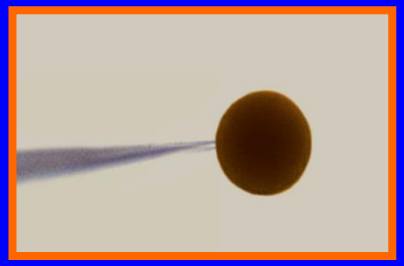


Middle decondensed sperms



High decondensed sperms

Impact of heavy metals on reproductive events of *Ciona intestinalis*



Zinc (essential) partecipates to:
sperm chromatin condensation, -production of vitellogenin at the oogenesis:

induces: reduction of Na^+ currents, reduction of plasma membrane conductance, inhibition of fertilization current, abnormal larval development .

Lead (non essential):
replaces zinc in protamines, replaces iron in haemoglobins, affects gonadotropins release;

induces: reduction of Na+ currents, reduction of plasma membrane conductance, inhibition of post- fertilization contraction.

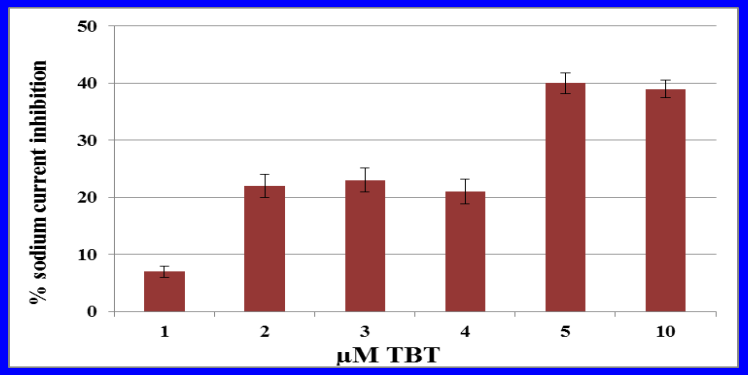
Gallo et al., Marine Ecology, 2011

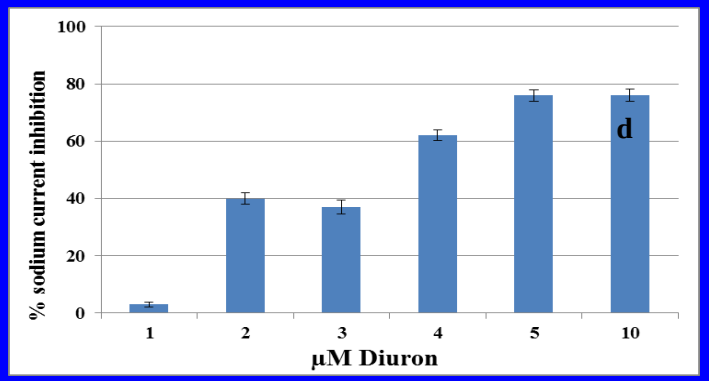
Toxic effects of the antifoulant compounds: TBT and diuron

prohibition of TBT use by January 2008

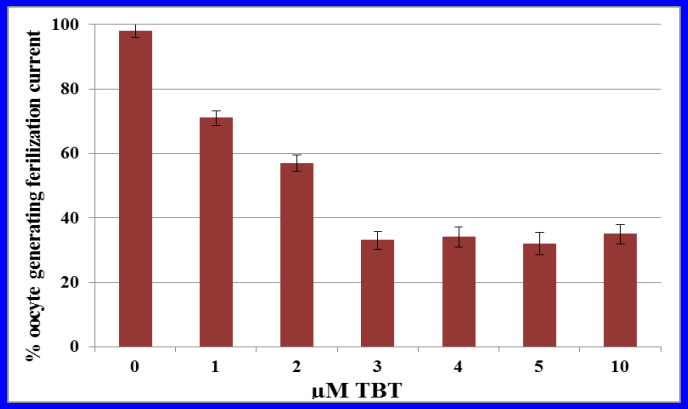
Formulation and production of new antifoulants compounds: e.g. Diuron

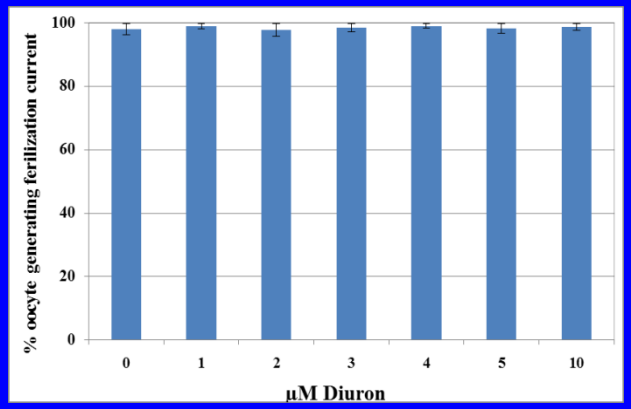
Effect of two of antifouling compounds (TBT and Diuron) on sodium currents in MI oocyte of *C. intestinalis*



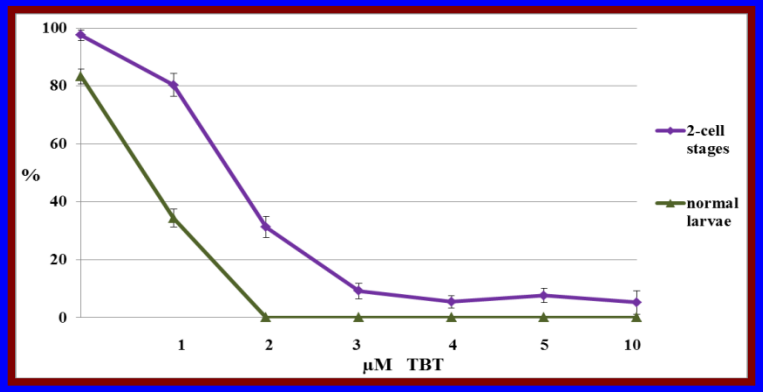


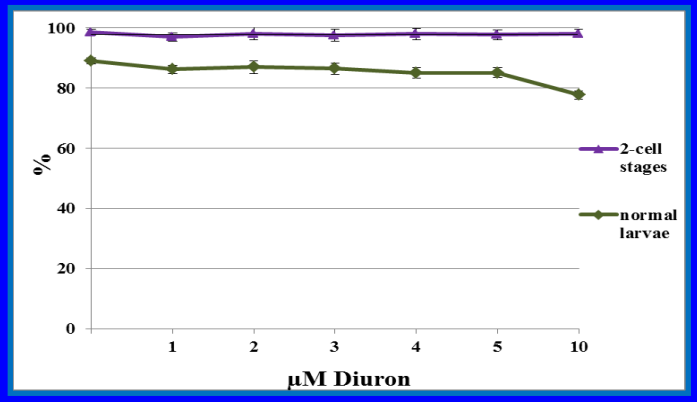
Effect of TBT and Diuron on the fertilization current





Embriotoxicity of two of antifouling compounds (TBT and Diuron) in *Ciona intestinalis*





TBT induces: reduction of Na+ currents, inhibition of fertilization current, inhibition of post- fertilization contraction, embryo development arrest;

Diuron induces: reduction of Na+ currents, abnormal larval development

Tosti and Gallo, J. Marine Sci. Res. Devel. 2012

Gallo and Tosti, Marine Drugs 2013

Conclusions

- Heavy metals and antifouling compounds do affect some steps of reproduction with a possible long-term effect on larval development;
- Evidence for a novel toxic activity of Zinc;
- Less toxicity of antifoulants of new generation

Application outcomes

- Ion currents may be a good tool for studying the influence of xenobiotics on reproductive processes since the plasma membrane is a target of xenobiotics-induced ROS formation
- The ascidian *Ciona intestinal*is may be considered a bio-indicator organism
- Reproduction may be a suitable bio-marker to asses ecological risk