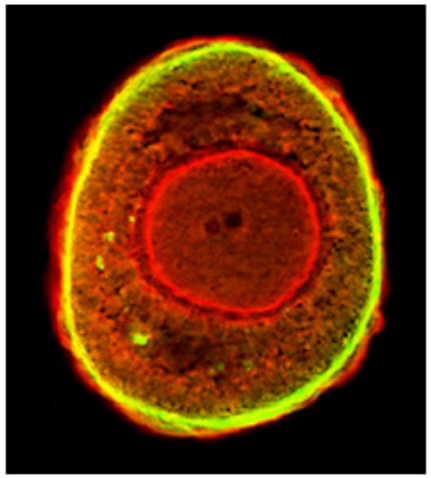
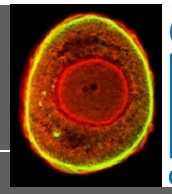


Oogenesis as acquisition of competences

Carlos E. Plancha[1,2]

[1] *Unidade de Biologia da Reprodução, Inst. Histologia e Biologia do Desenvolvimento, Faculdade de Medicina de Lisboa, Portugal*

[2] *CEMEARE – Centro Médico de Assistência à Reprodução, Lisboa, Portugal*



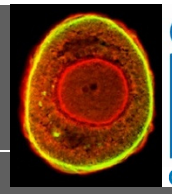
Presentation outline and learning objectives:

1. Germ cell lineage establishment

2. Structural features of Oocyte Growth and Maturation

3. Functional features of Oogenesis: acquisition of competences

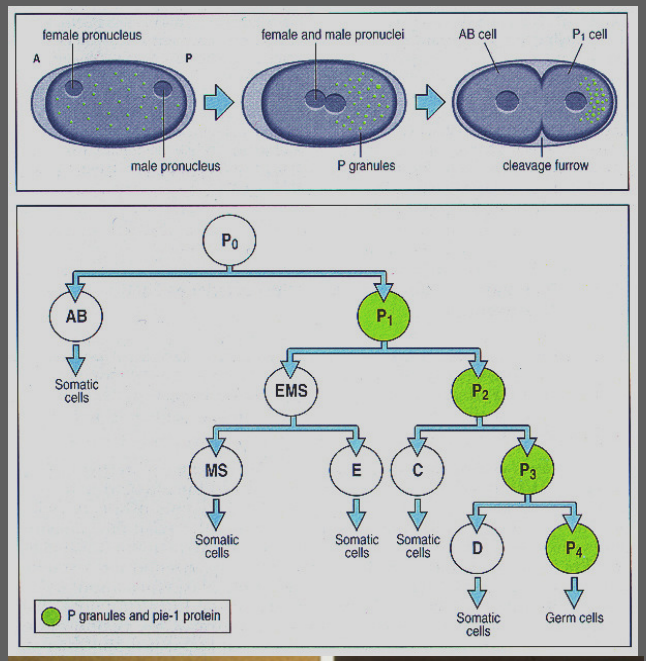
4. Future chalenges in this area of Clinical Embryology

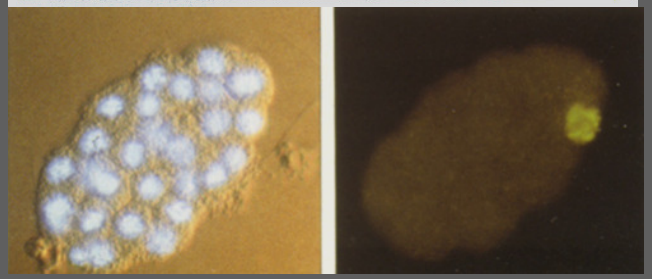


DISCLOSURE

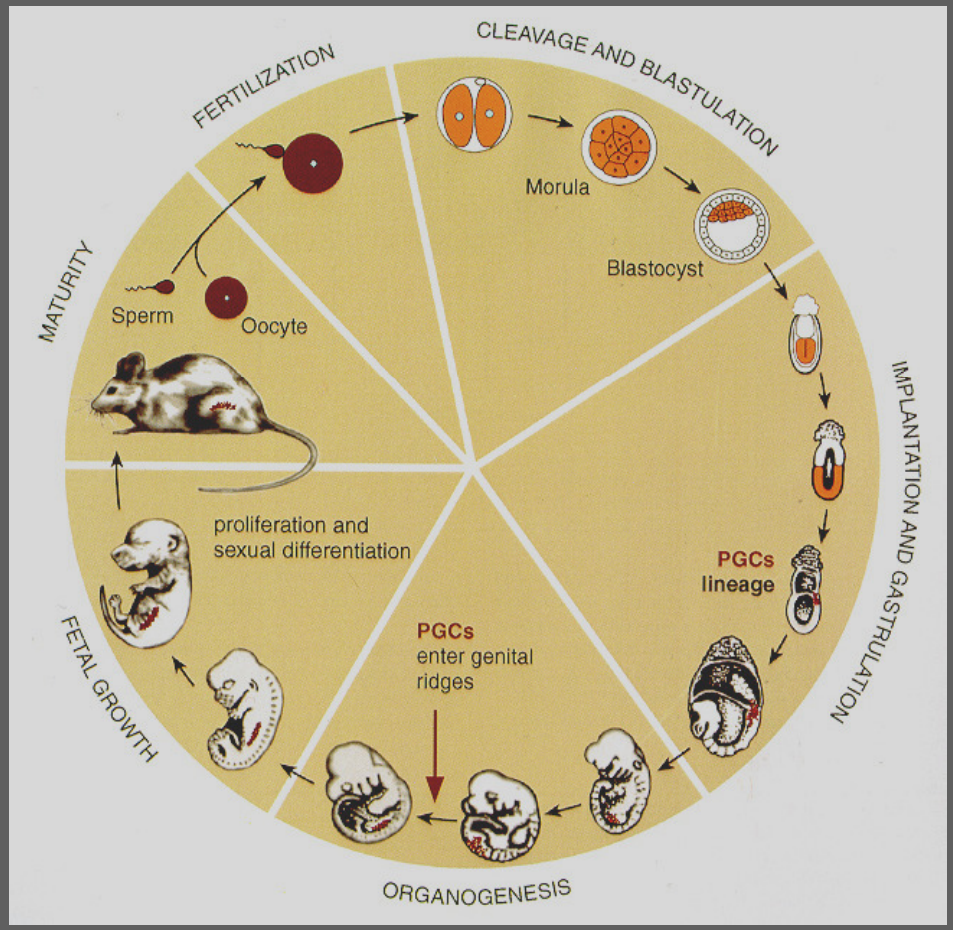
CE Plancha does not have any commercial and/or financial relationship with manufacturers of pharmaceuticals, laboratory supplies and/or medical devices.

Germ cell lineage



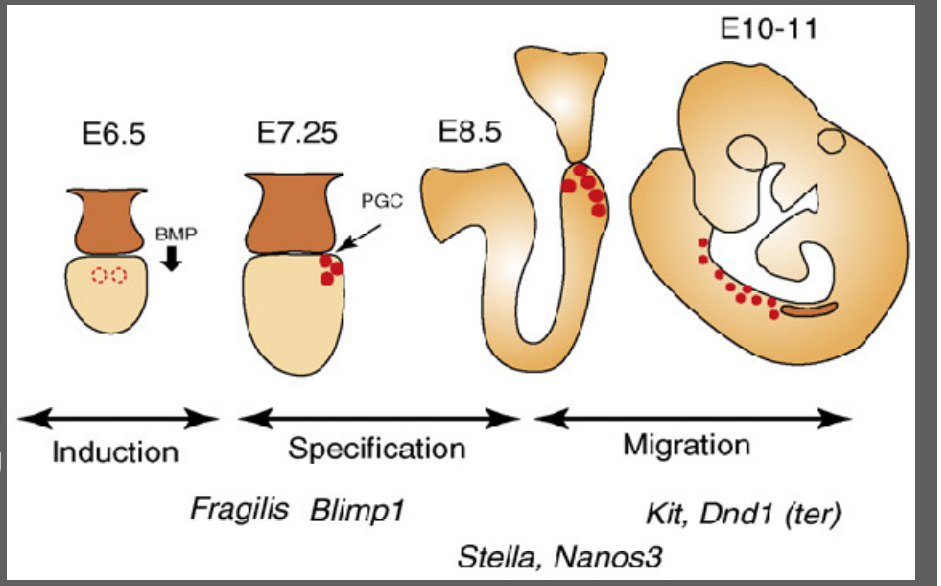


C. elegans - one cell as founder

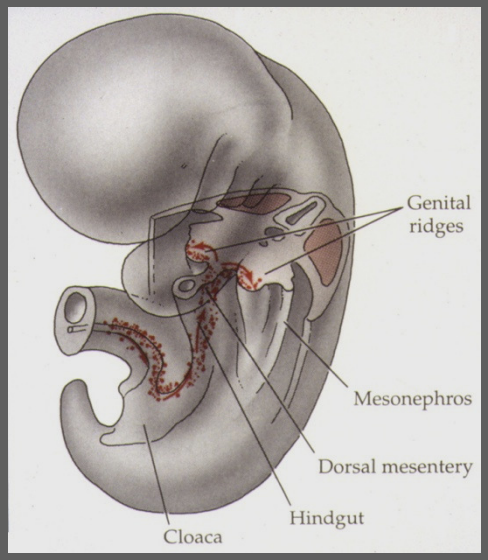


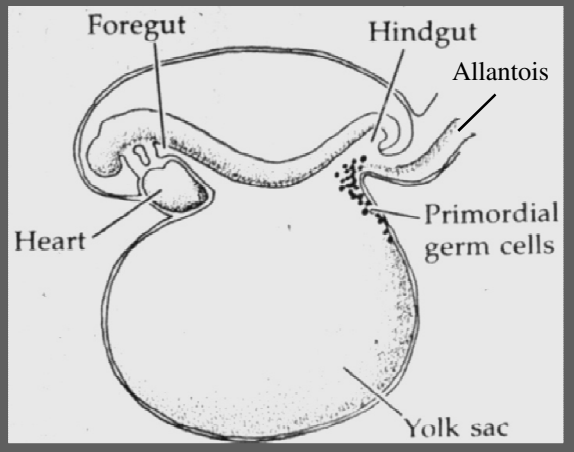
Mouse - a cell population as founder

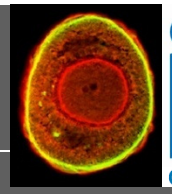
Signalling pathways involved in **Induction, Specification and Migration** of Primordial Germ Cells in mammals



Y Saga (**2008**) Mouse germ cell development during embryogenesis.Curr Opin Genet & Develop 18:1–5.



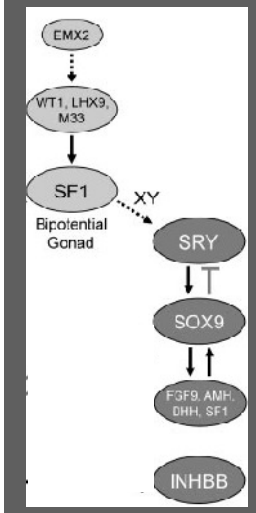




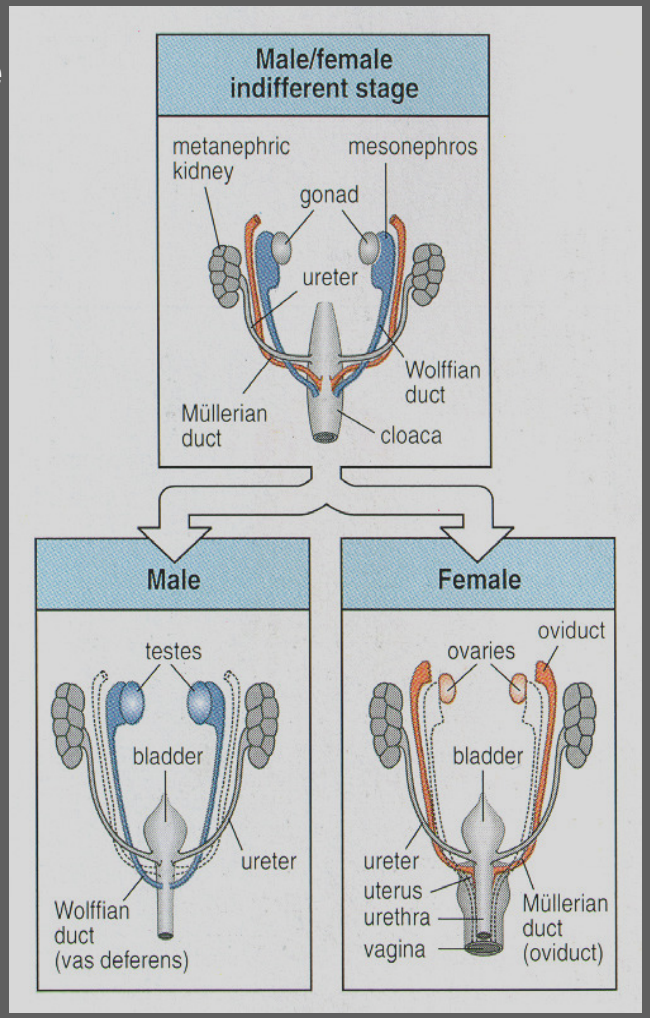
Development of the **Somatic Sexual Phenotype** in mammals

Initial **indifferent** / **bipotential** stage

Germ cells arrive to genital ridge at this stage

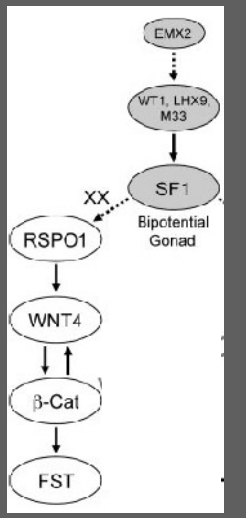


Subsequent differentiation of **gonads** and other **reproductive system** organs



Mouse:
12,5 days p.f.

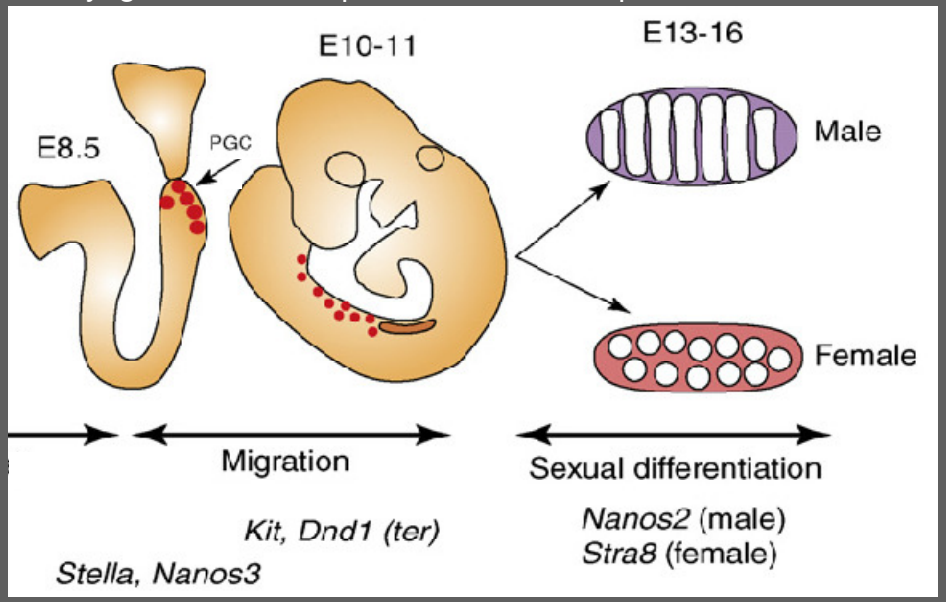
Human:
7th week p.f.

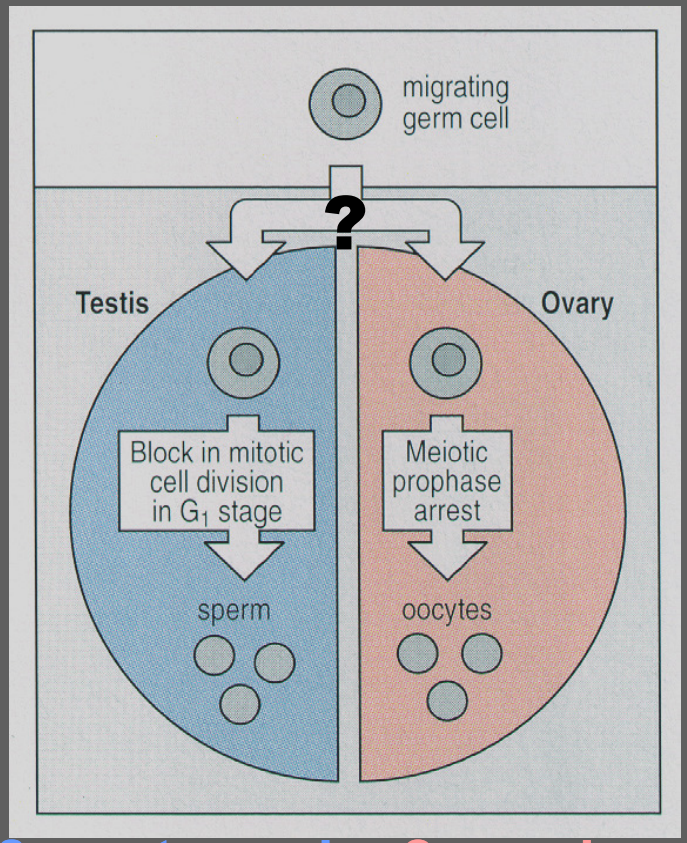


MA Edson et al.. (2009) The Mammalian Ovary from Genesis to Revelation. Endocrine Reviews, 30:624-712.

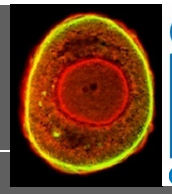
Signalling pathways involved in **Germ Cell Sex Differentiation** in mammals

Y Saga (**2008**) Mouse germ cell development during embryogenesis. Curr Opin Genet & Develop 18:1–5.

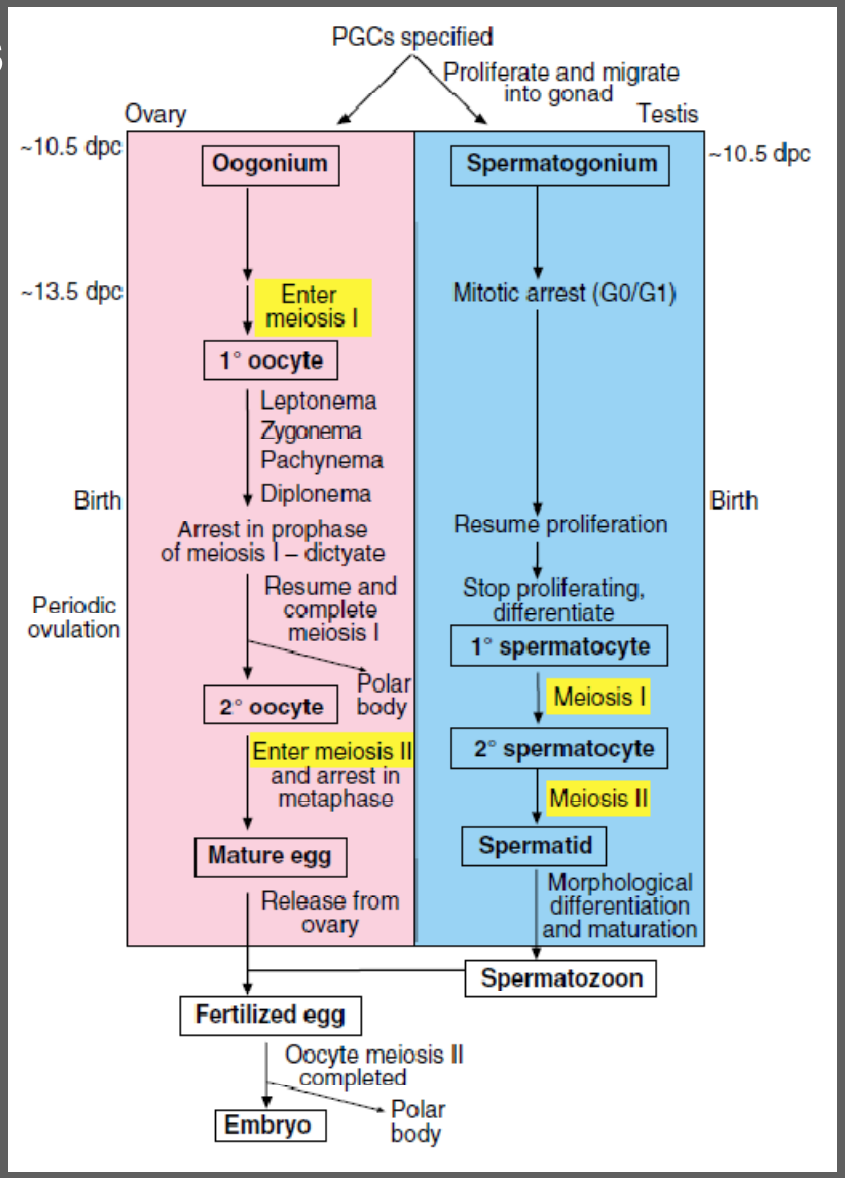




Spermatogenesis **Oogenesis**



Gametogenesis



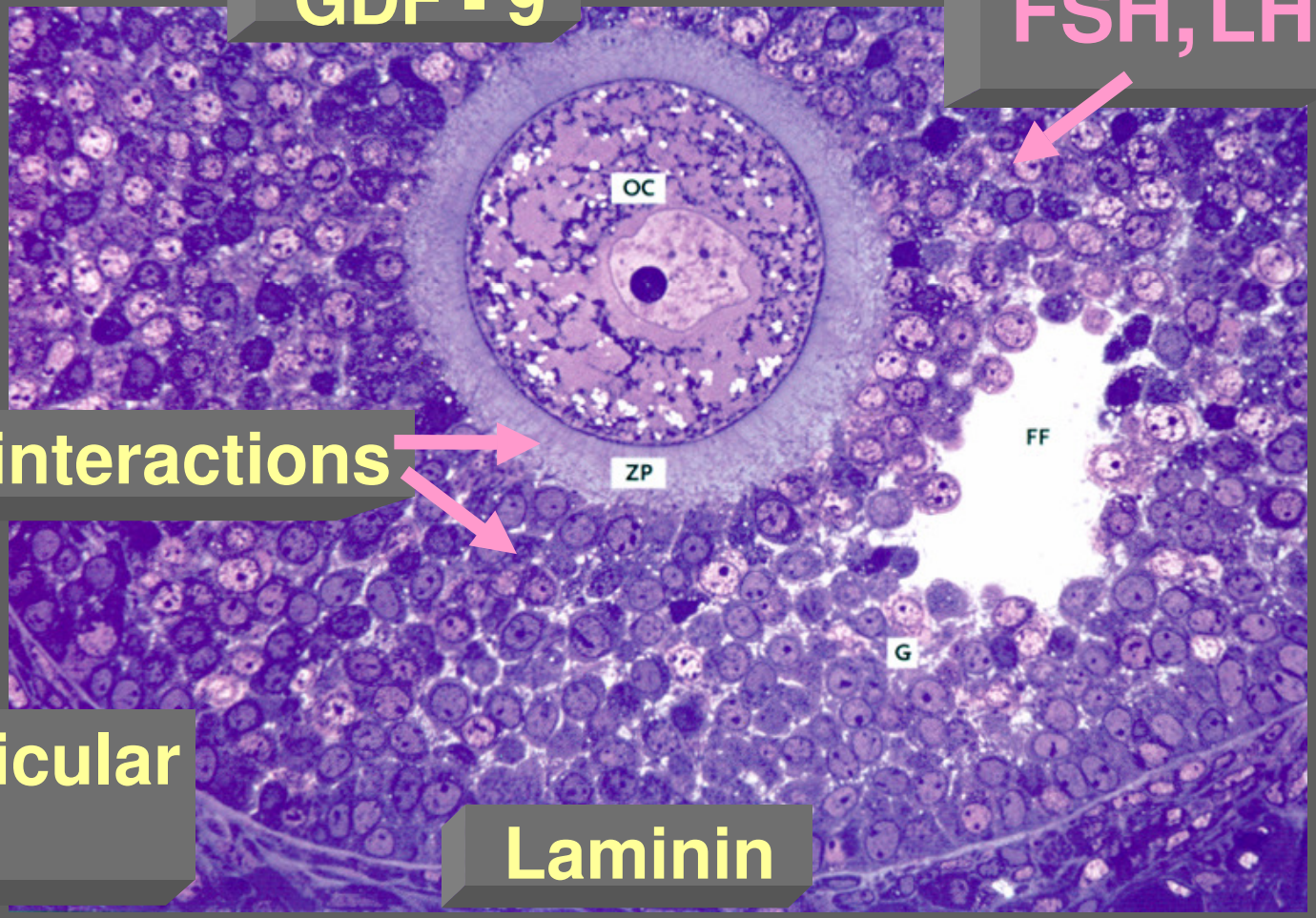
Oogenesis

Spermatogenesis

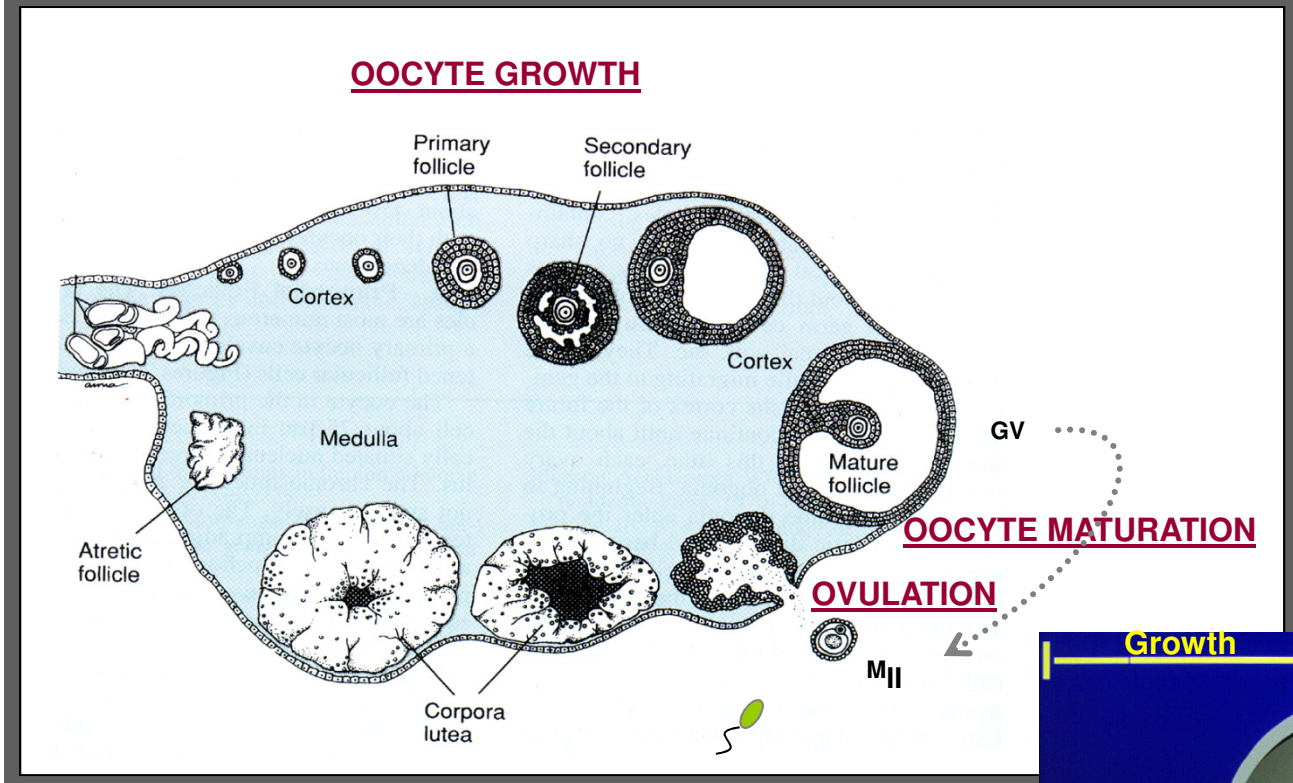
J Bowles and **P Koopman** (**2007**) Retinoic acid, meiosis and germ cell fate in mammals. Development 134:3401-3411.

Oogenesis takes place inside the **Ovarian Follicle**

Factors involved in oogenesis and folliculogenesis



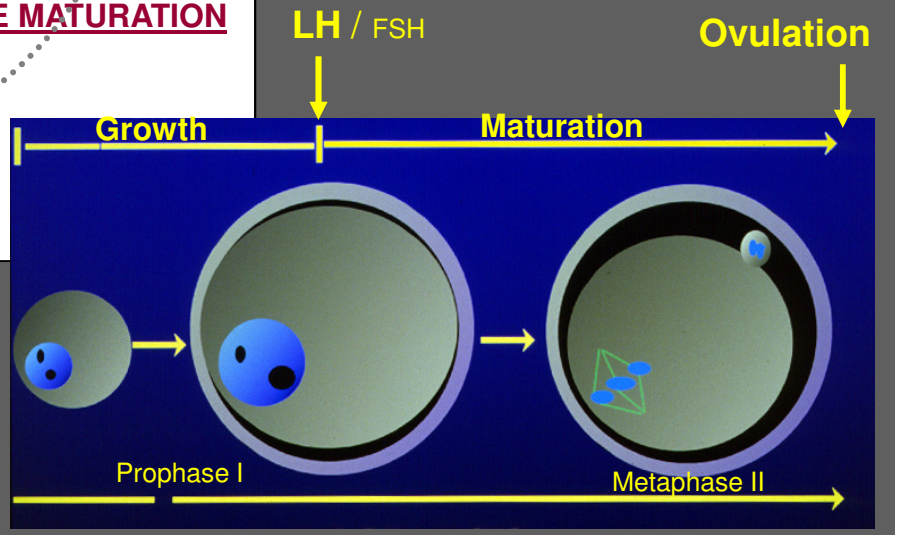
Oogenesis involves both growth and maturation phases



FERTILIZATION

Resumption of meiosis → PN formation
Embryo development

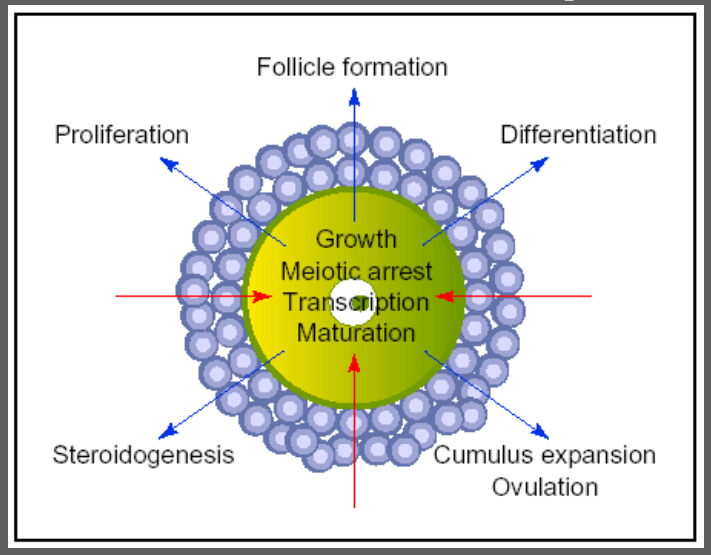
- **Growth Phase:**
  - Oocyte diameter increases
  - High transcriptional and translational activity
  - Accumulation of RNA / proteins
  - Building of new structures (zona pellucida, cortical granules)
- **Oocyte Maturation:**
  - Organelle redistribution
  - Nuclear / cytoplasmic events with resumption of meiosis and arrest at M_{II} shortly before ovulation



The Oocyte secretes dominant paracrine effectors

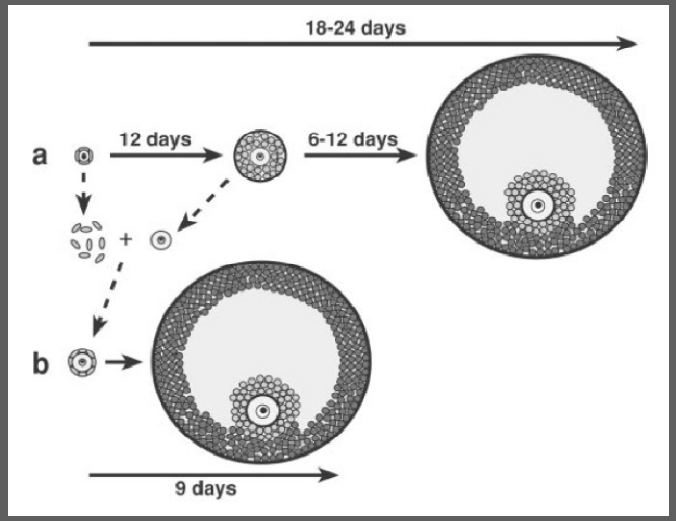
(GDF-9, BMP-15)

and



Dong et al. (**1996**) **Nature** 383: 531.
Galloway et al. (**2000**) **Nature Genetics** 25: 279.
Juengel et al. (**2002**) **Biol Reprod** 67: 1777.

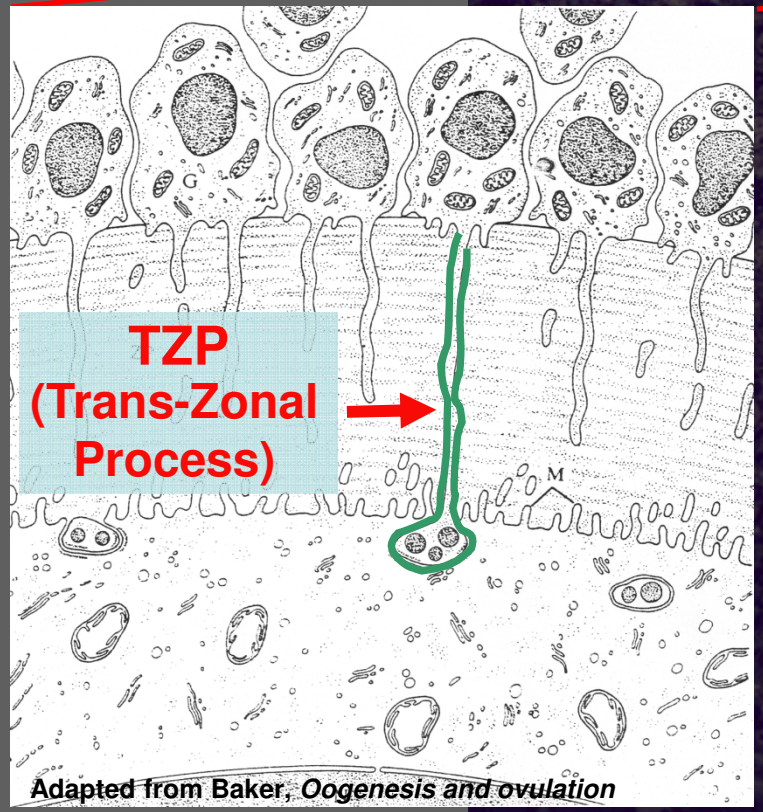
dictates the rate of follicle development



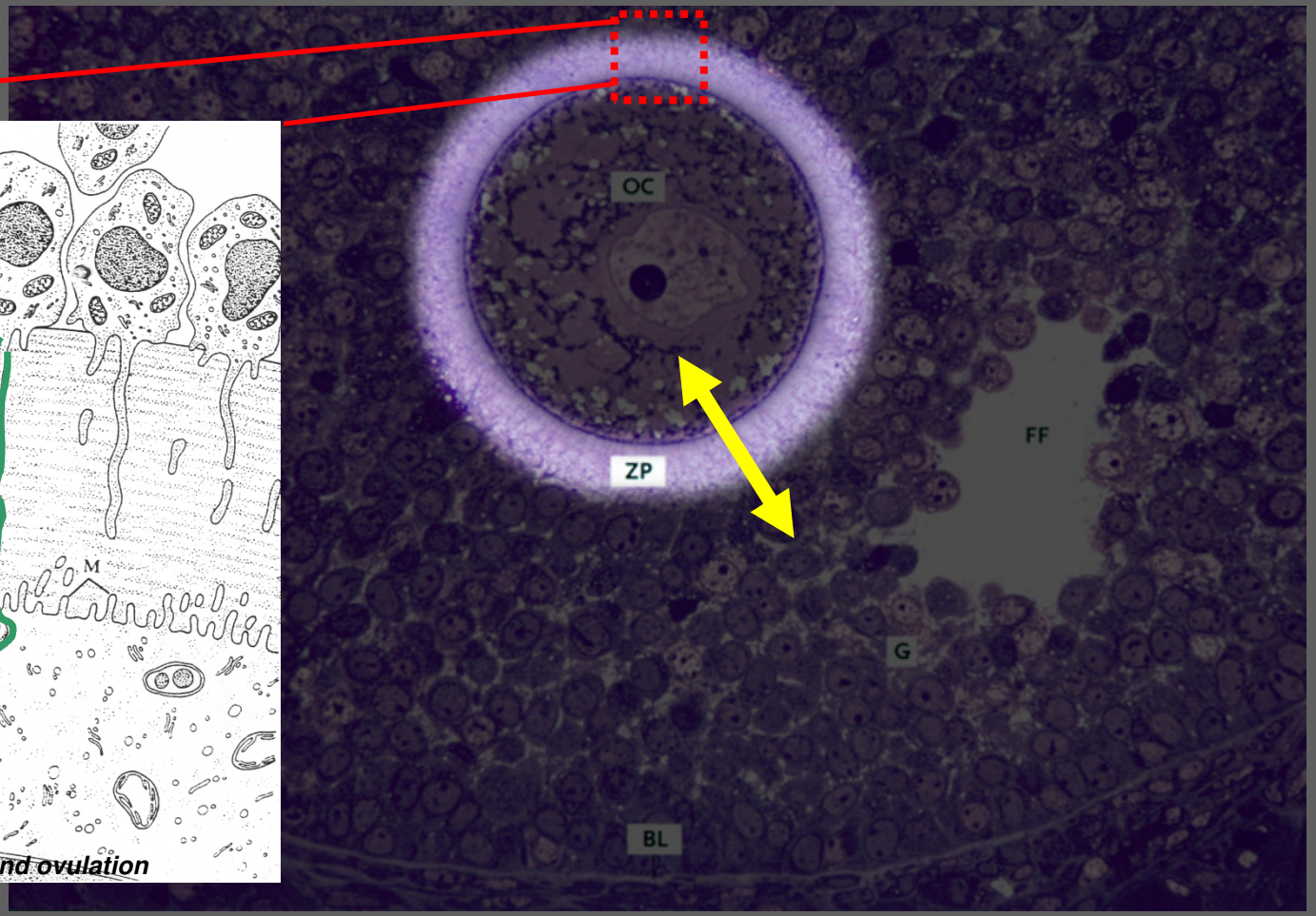
JJ Eppig (**2001**) **Reproduction** 122: 829.

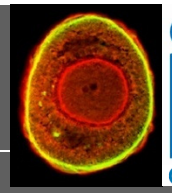
JJ Eppig, K Wigglesworth, FL Pendola (**2002**) The mammalian oocyte orchestrates the rate of ovarian follicular development. **PNAS** 99:2890–2894.

All stages of oogenesis require communication through **specialized somatic-germinal cellular interactions**



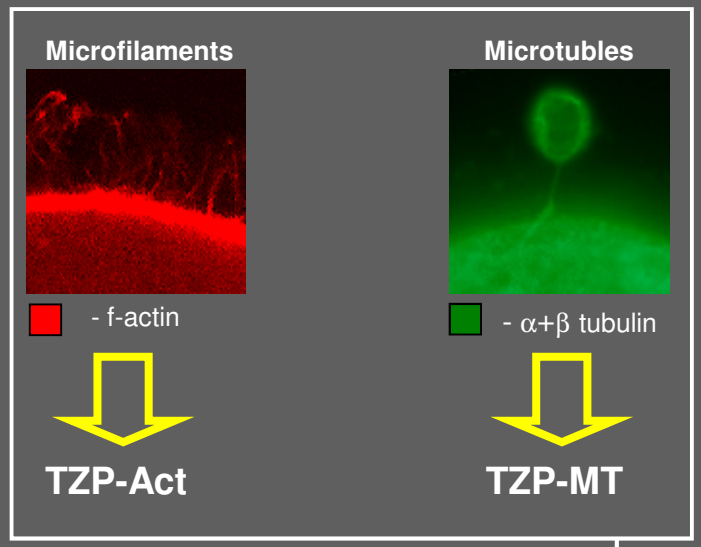
Adapted from Baker, *Oogenesis and ovulation*



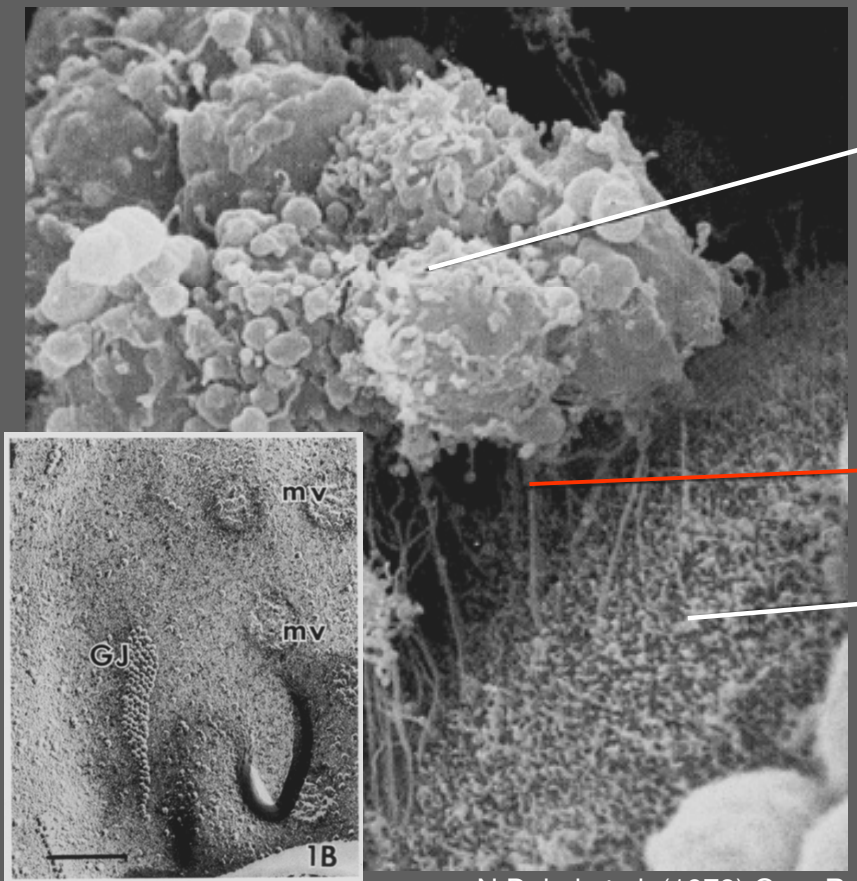


Trans-Zonal Processes

Granulosa cell extensions that transverse the zona pellucida and terminate at the oocyte cell surface
(Hertig and Adams, 1967; Anderson and Albertini, 1976)



TZPs have an heterogeneous cytoskeletal content



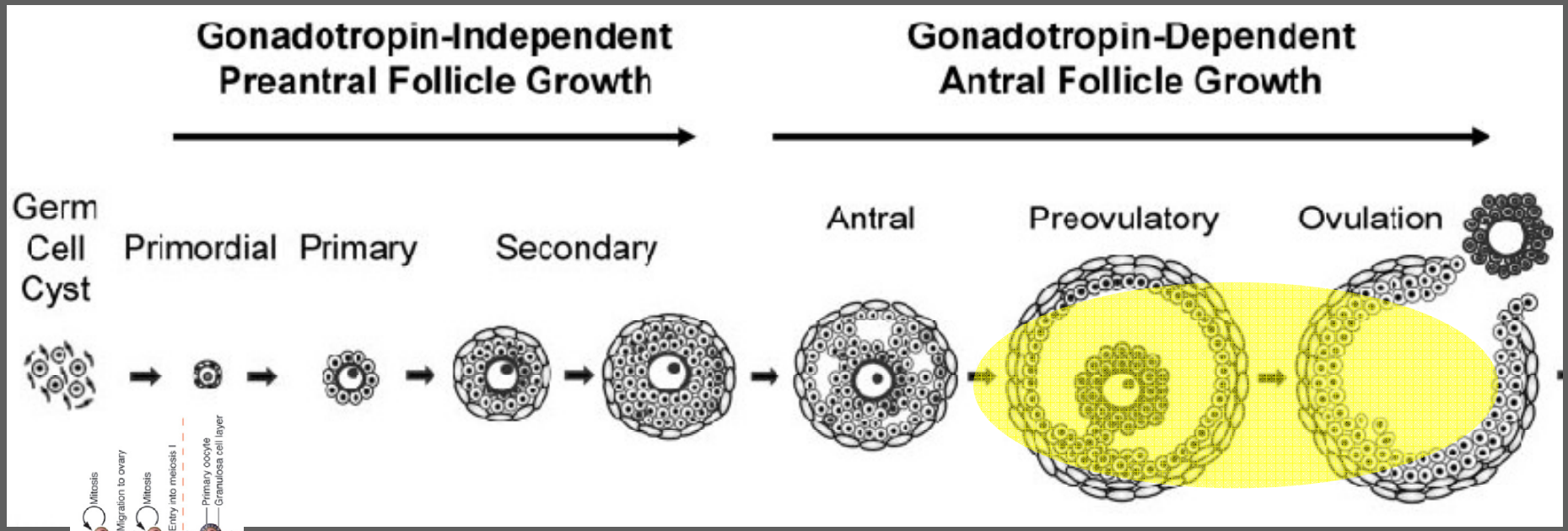
Gap junctions

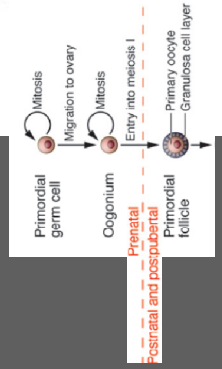
N Dekel et al. (1978) Gam Res 1:47.

Ovarian follicle development in humans

Primordial → ~120-180 days → Preantral → ~90 days → ovulation

Poorly understood (except Gonadotropin-dependent last stages)





Period of current ART intervention

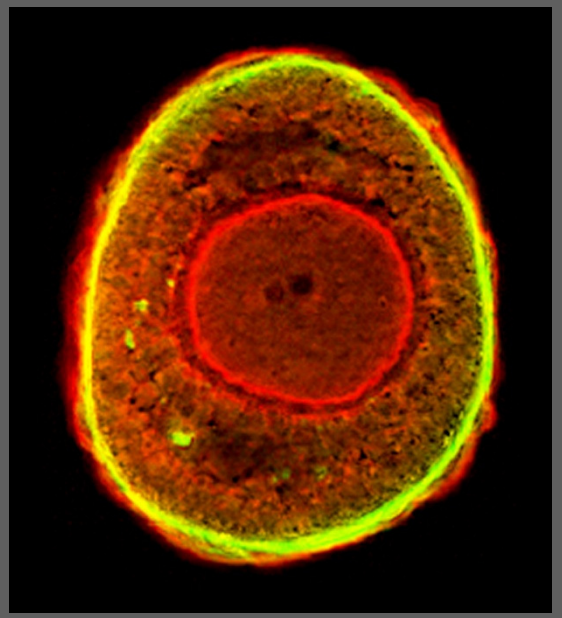
MA Edson et al.. (2009) The Mammalian Ovary from Genesis to Revelation. Endocrine Reviews, 30:624-712.

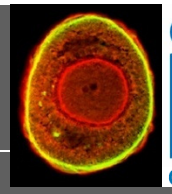
Oogenesis as acquisition of functional competencies

1. Meiotic
(capacity to reach the metaphase II arrest)

2. Activation
(capacity to finish meiosis, block polyspermy, and form pronuclei at fertilisation)

3. Developmental
(capacity to trigger and support embryonic development at activation)





Meiotic competence

Oocyte ability to leave the first arrest of the meiotic cell cycle in prophase I and to reach a new arrest at metaphase II

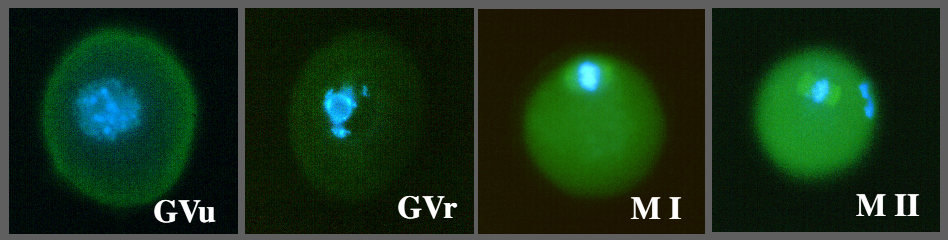
Mouse

⇒ During the oocyte growth phase (to about 60-65µm: 80% of full size)

⇒ Sequential acquisition, first of the capacity to re-initiate meiosis, than to reach metaphase I and finally to reach metaphase II

⇒ Associates with chromatin and microtubule configuration modifications and with centrosome phosphorylation during the prophase I arrest

⇒ Associates with differential accumulation / localization of several cell cycle related molecules ($p34^{cdc2}$, cyclin B1, cdc25C, wee1)



Sorensen and Wassarman (1976) Dev Biol 50:531-536.

Mattson and Albertini (1990) Mol Reprod Dev 25: 374-383.

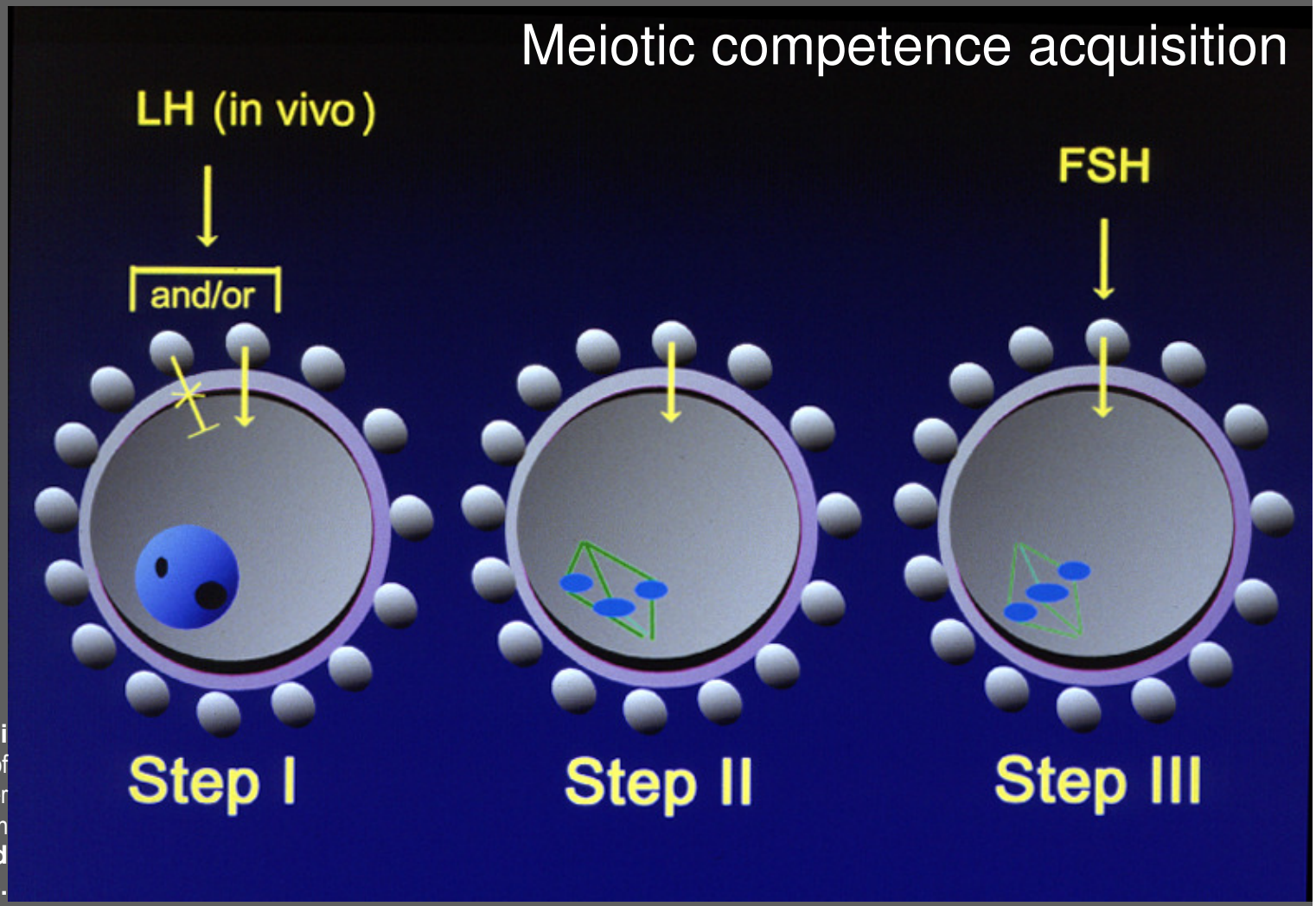
Wickramasinghe and Albertini (1992) Dev Biol 152: 62-74.

Eppig et al. (1994) Dev Biol 164: 1-9.

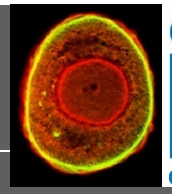
Kanatsu-Shinohara et al. (2000) Biol Reprod 63: 1610-1616.

MA Edson et al.. (2009) Endocrine Reviews, 30:624-712.

Lessons from the hamster model



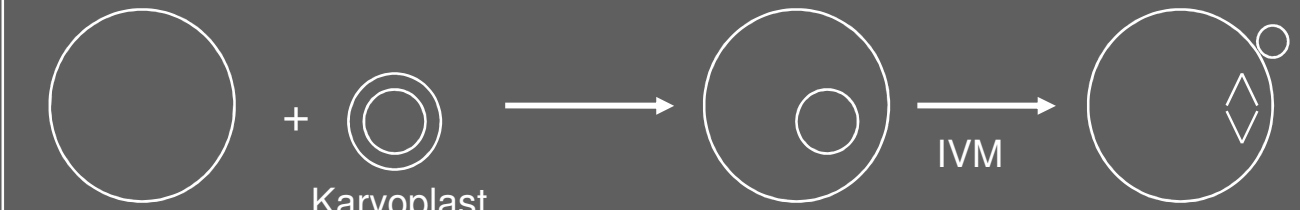
CE Plancha, DF Albertini (1994) Hormonal regulation of meiotic maturation in the hamster oocyte involves a cytoskeleton mediated process. **Biol Reprod 51:852-864.**



Meiotic competence acquisition

Mouse

⇒ Although the visible expression of this competence is the nuclear compartment, **it is the cytoplasm that confers meiotic competence**



Full grown ooc. (enucleated GV) (**competent**)

Karyoplast (non-growing ooc.) (**incompetent**)

Kono et al. (1996) Nat Genet 13: 91-94.

Ovine, Bovine, Human

⇒ After the oocyte growth phase

⇒ Human primary oocytes recovered from antral follicles ›3mm already acquired meiotic competence and can reach metaphase II *in vitro*

⇒ **Studies on acquisition of meiotic competence in human oocytes are needed**

Trounson et al. (1998) Hum Reprod 13 (Suppl. 3): 52-62.
MA Edson et al.. (2009) Endocrine Reviews, 30:624-712.

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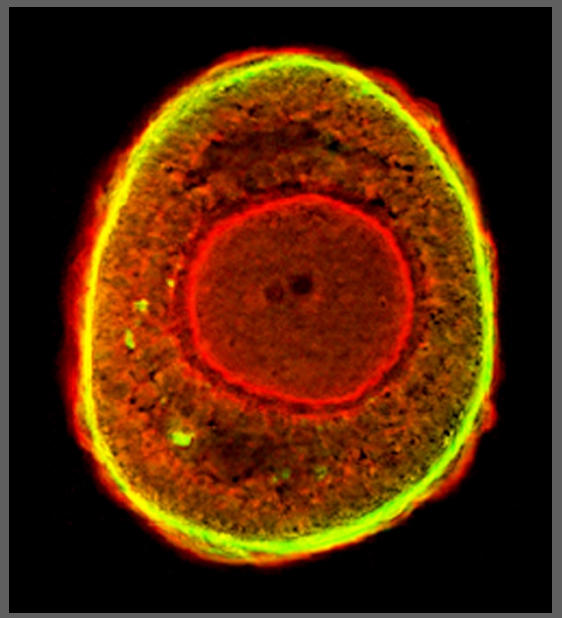
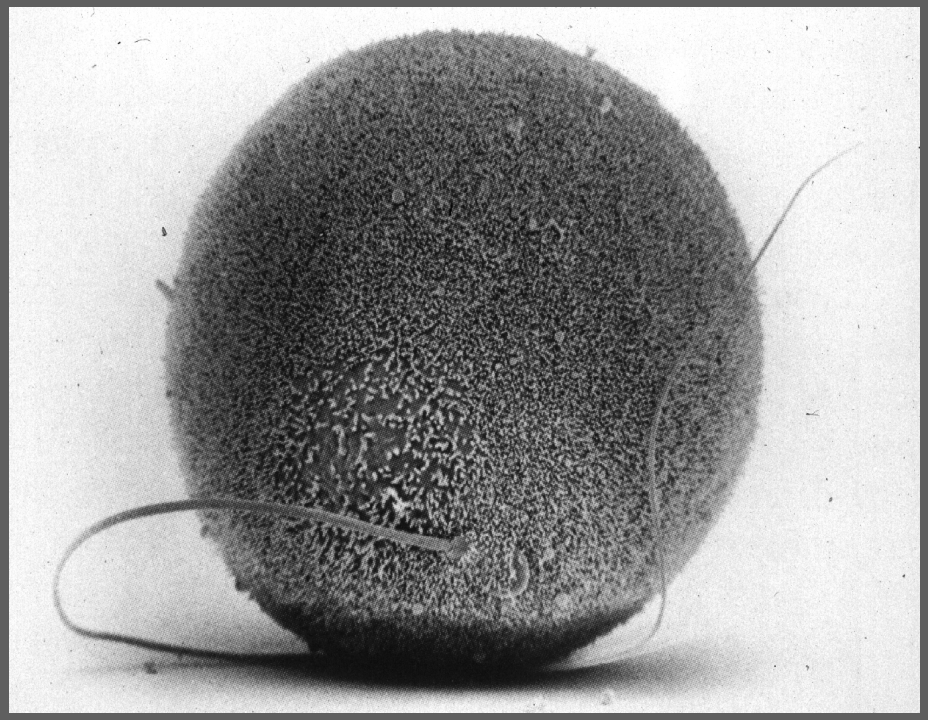


Activation competence

Oocyte ability to be successfully activated at fertilisation

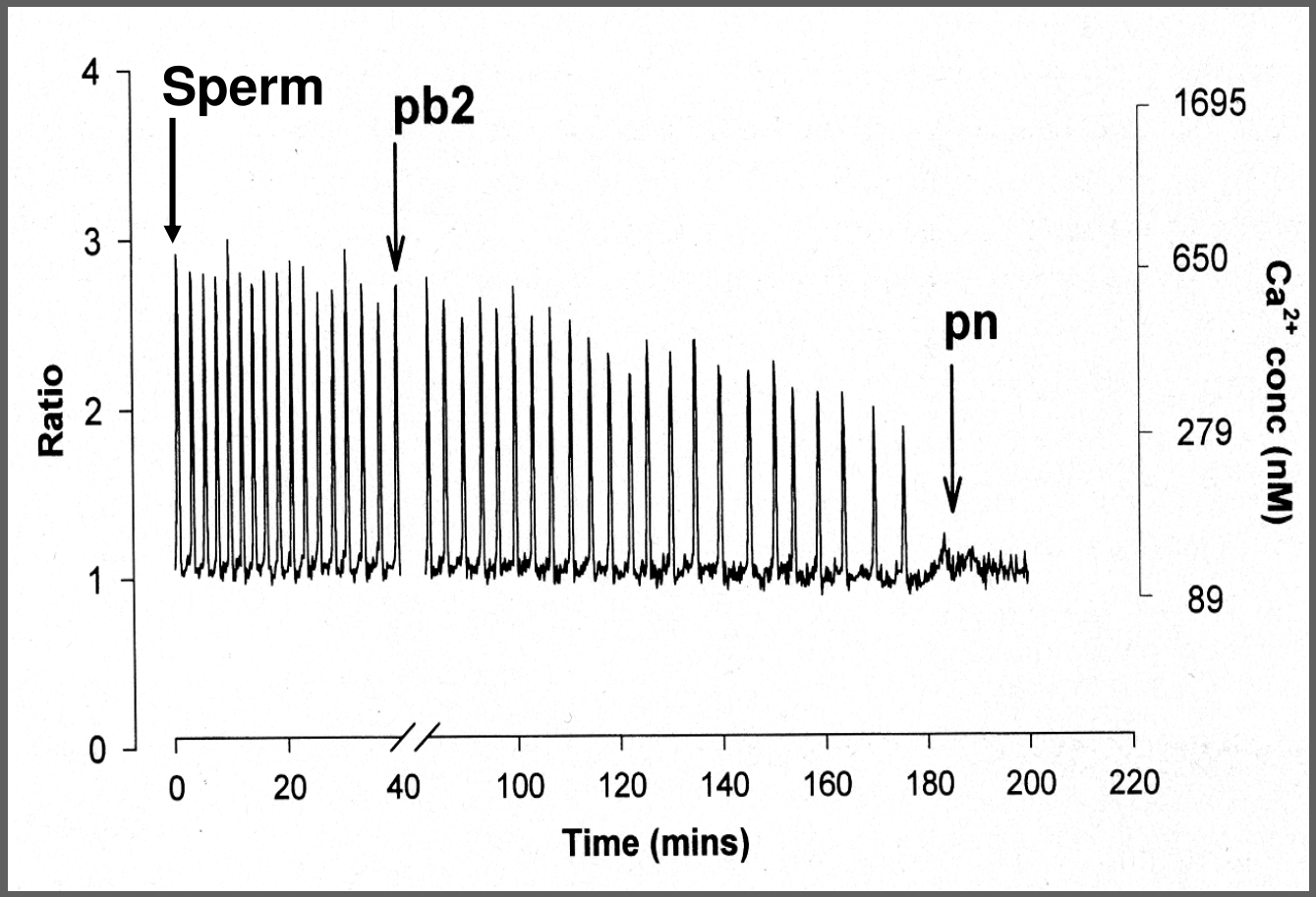


Activation involves:

- ➔Induction of oocyte intracelular [Ca^{2+}] oscilations
- ➔Cortical reaction and block to polyspermy
- ➔Conclusion of meiosis
- ➔Decondensation of sperm chromatin
- ➔Pronuclei formation

Activation competence

Mouse

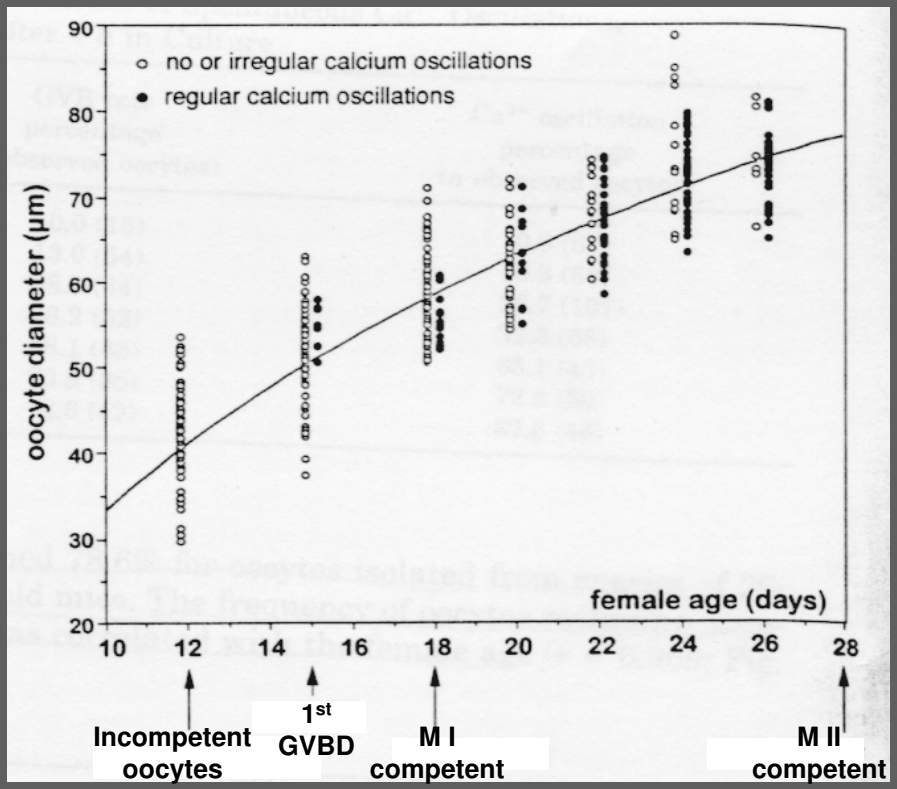


Intracelular [Ca^{2+}] oscilations at fertilisation

Jones et al. (**1995**) **Development** 121:3259-3266.

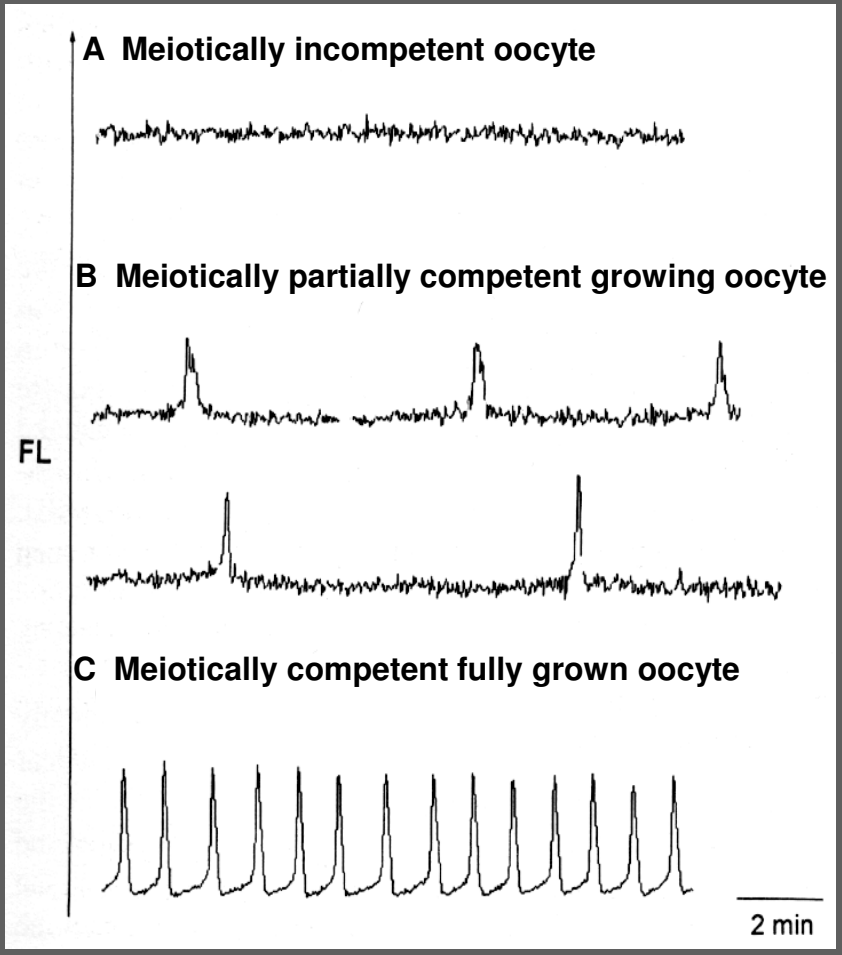
Activation competence acquisition

The mechanisms of Ca^{2+} signalling become functional with female age and with oocyte growth



Lefèvre et al. (**1997**) **Exp Cell Res** 236:193-200.

Mouse

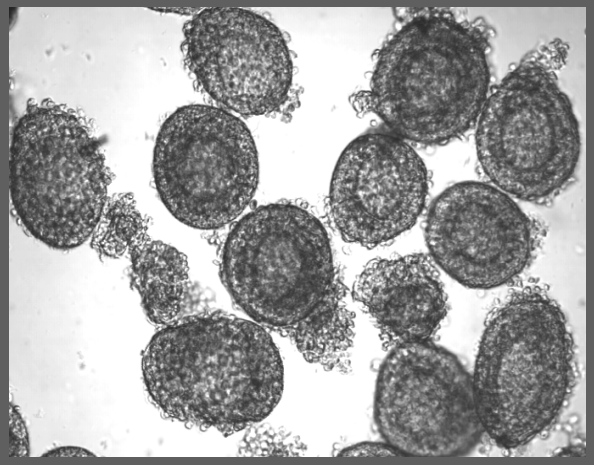


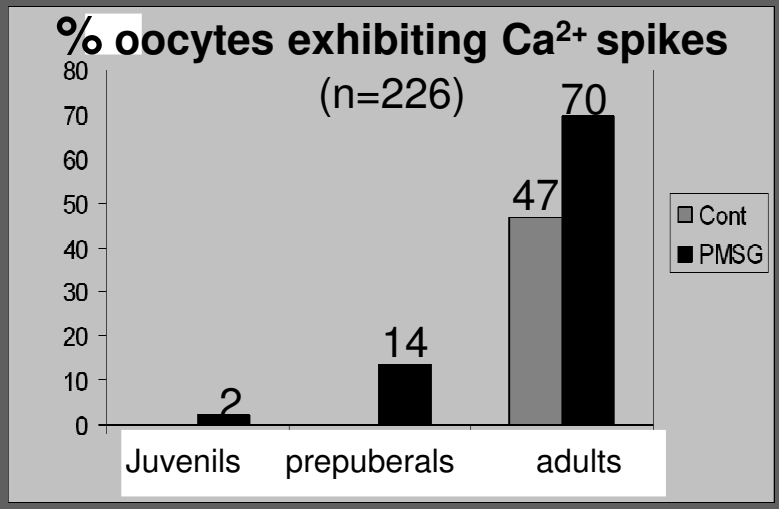
Carroll et al. (**1994**) **Development** 120:3507-3517.

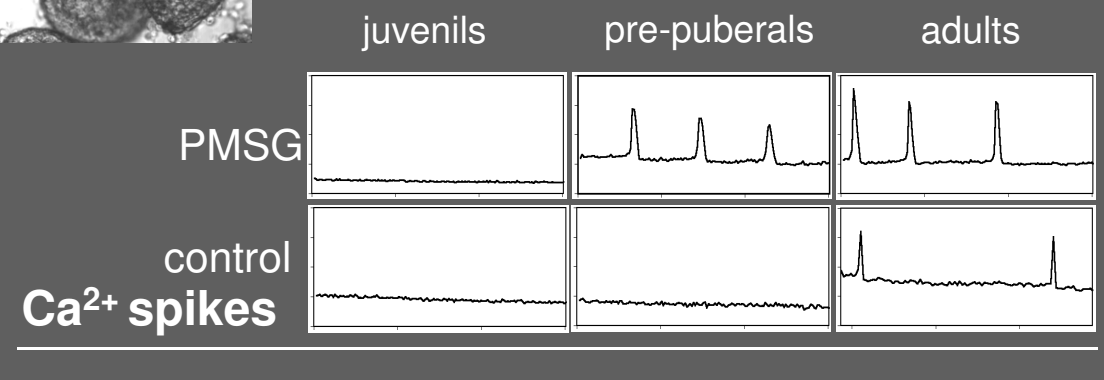
Activation competence acquisition

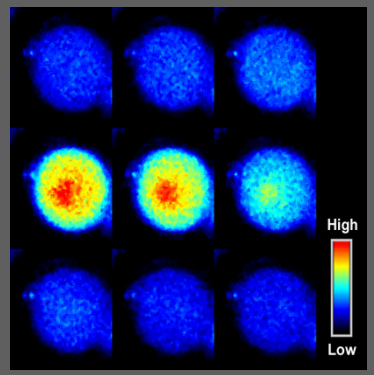
In a defined preantral follicle population, age and PMSG stimulation significantly increase the proportion of oocytes presenting Ca^{2+} spikes

Mouse

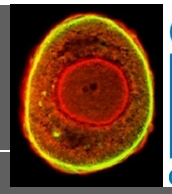








Gomes et al (**1999**) Int J Dev Biol 43: 839-842.



Activation competence acquisition

Mouse

The cytoplasmic reorganization during oocyte maturation confers definitive competence for activation

- Involves the calcium stores (SER) and the cortical granules
- Corresponds to part of the classic cytoplasmatic component of oocyte maturation
- Mechanisms proposed:

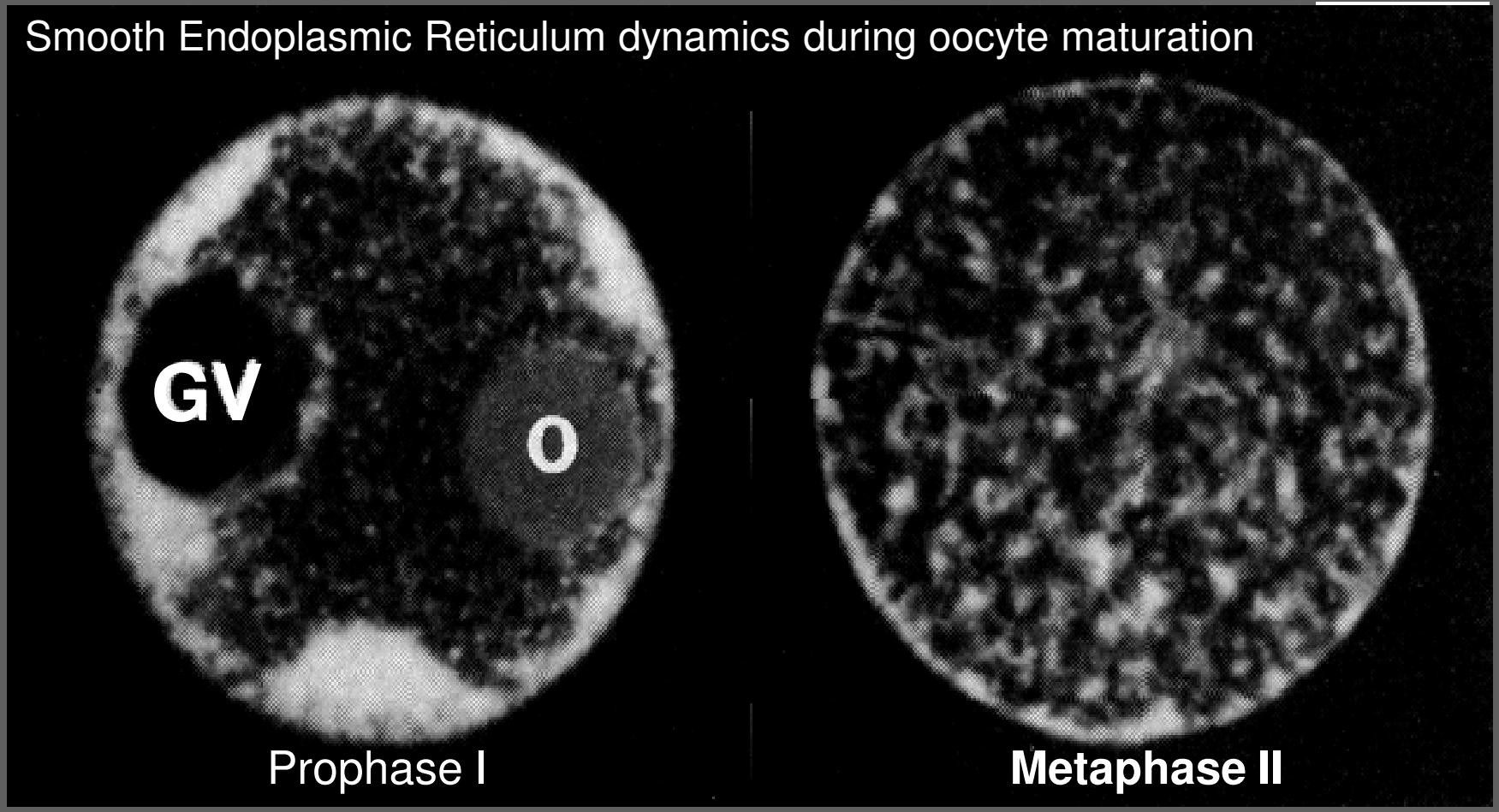
1. changes in the regulation and an increase in levels of InsP3 receptor
2. changes in the structure of the calcium stores (SER)
3. changes in the size of the calcium store itself

Ducibella (**1996**) The cortical reaction and developmental of activation competence in mammalian oocytes. **Hum Reprod Update** 2:29-42.

Cheung et al (2000) Hum Reprod 15: 1389-1395.

Activation competence acquisition

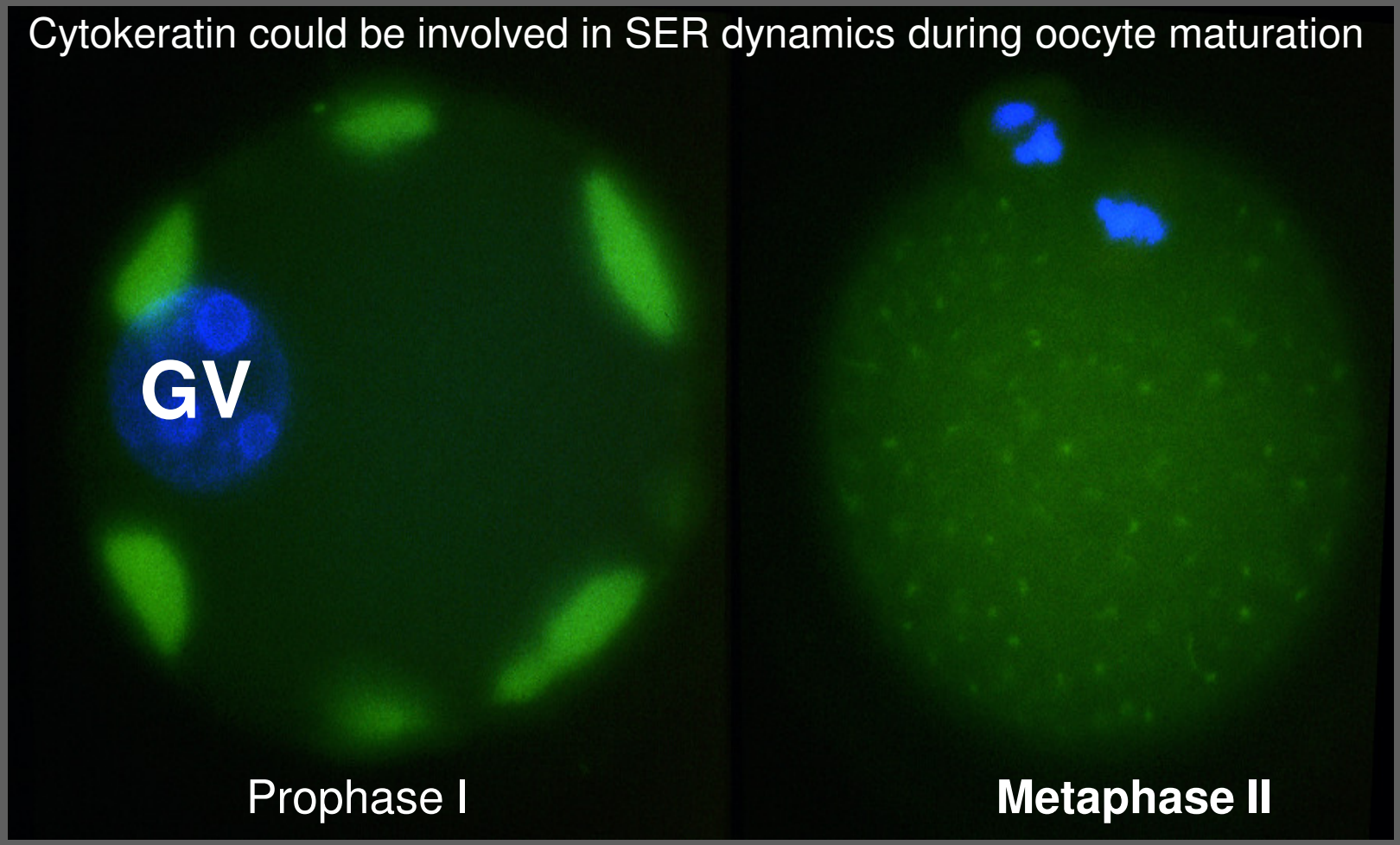
Hamster



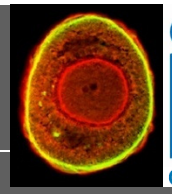
Shiraishi et al. (**1995**) Developmental changes in the distribution of the endoplasmic reticulum and inositol 1,4,5-triphosphate receptors and the spatial pattern of Ca^{2+} release during maturation of hamster oocytes. **Dev Biol** 170:594-606.

Activation competence acquisition

Hamster



Plancha (**1996**) Cytokeratin dynamics during oocyte maturation in the hamster requires reaching of metaphase I. **Differentiation** 60:87-98.



Activation competence acquisition

Human

As with other mammals, the maximal sensibility to the mechanisms of calcium release seems to occur at the end of oocyte maturation, just before ovulation.

That sensibility increase can also be due to the redistribution of the intracellular calcium deposits in the oocyte.

Studies on acquisition of activation competence in human oocytes are needed.

Ducibella (**1996**) The cortical reaction and developmental of activation competence in mammalian oocytes. **Hum Reprod Update** 2:29-42.

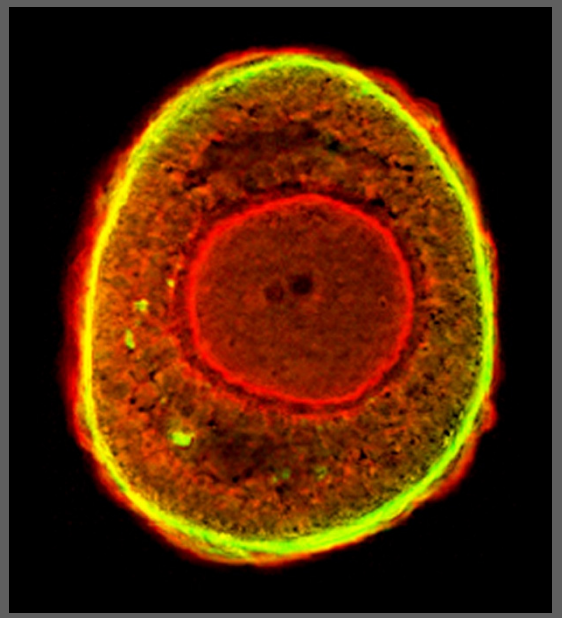
MA Edson et al.. (**2009**) The Mammalian Ovary from Genesis to Revelation. **Endocrine Reviews**, 30:624-712.

Oogenesis as acquisition of functional competencies

1. Meiotic
(capacity to reach the metaphase II arrest)

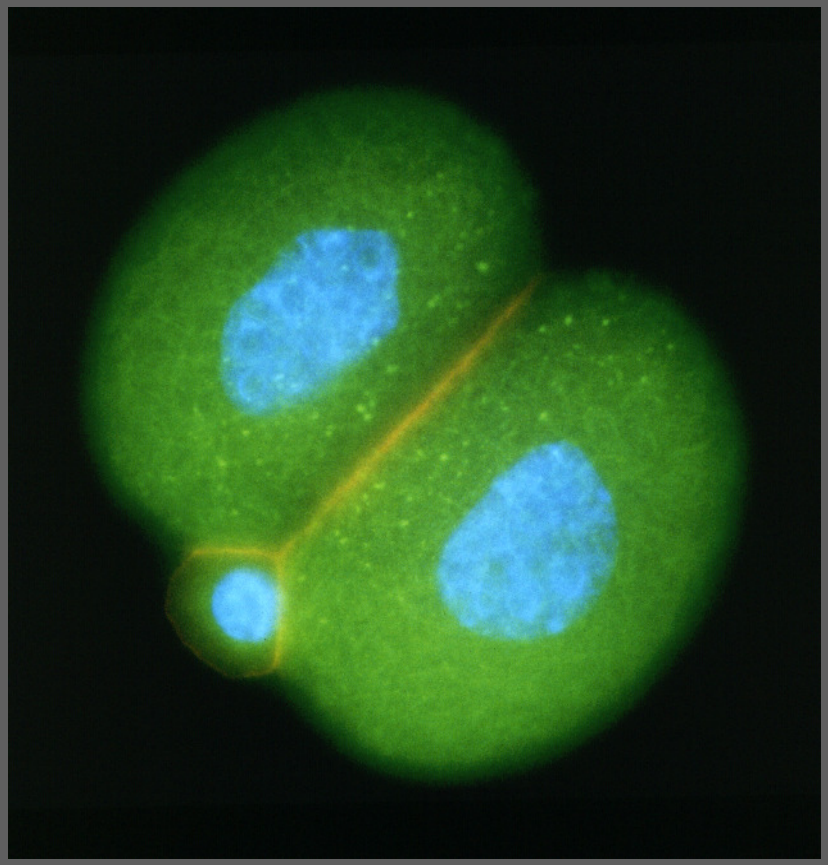
2. Activation
(capacity to finish meiosis, block polyspermy, and form pronuclei at fertilisation)

3. Developmental
(capacity to trigger and support embryonic development at activation)



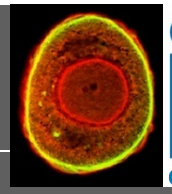
Developmental competence

Oocyte ability to trigger and support embryonic development at activation



From activation results:

- ➔Induction of oocyte intracelular [Ca^{2+}] oscilations
- ➔Beginning of embryonic development



Developmental competence acquisition

Mouse

⇒ During oocyte growth, (to full size ±80μm), before oocyte maturation

⇒ Sequential acquisition, first of the capacity to reach the 2-cell stage, and than to reach the blastocyst stage

⇒ Importance of animal age, follicular growth, gonadotropins and germ-somatic cell interactions

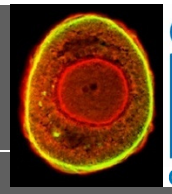
Eppig and Schroeder (**1989**) Biol Reprod 41: 268-276.

Eppig (**1993**) Serono Symposia, USA Series. (Barry Bavister, ed). Springer-Verlag New York, Inc., pp. 43-53.

⇒ Sequential acquisition, first for postimplantation development, and than for preimplantation development

Kono et al. (**1996**) Nat Genet 13: 91-94.

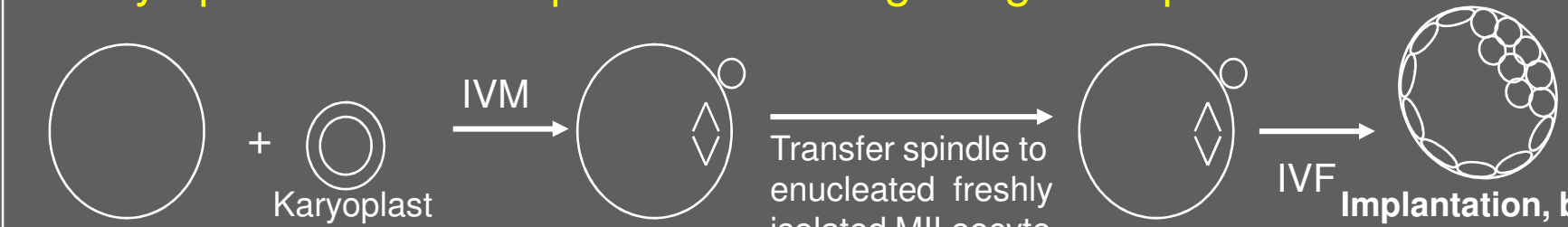
Bao et al. (**2000**) Biol Reprod 62: 616-621.



Developmental competence acquisition

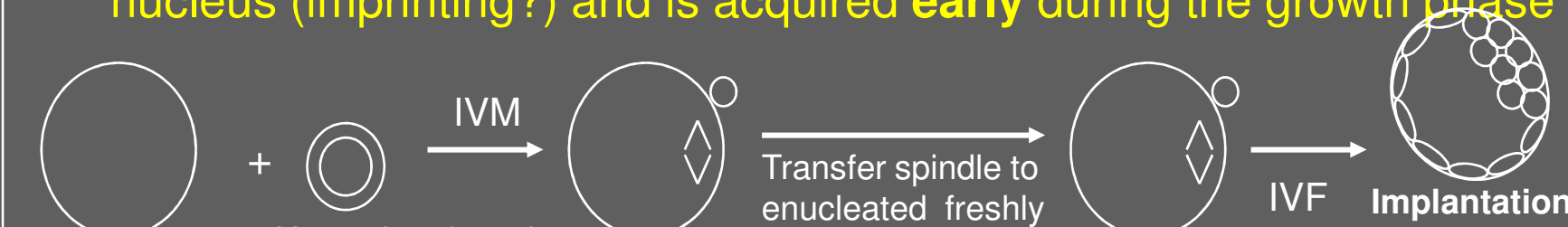
Mouse

⇒ Competence for preimplantation development resides in the cytoplasm and is acquired **late** during the growth phase

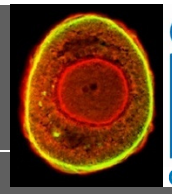


Kono et al. (**1996**) **Nat Genet** 13: 91-94.

⇒ Competence for postimplantation development resides in the nucleus (imprinting?) and is acquired **early** during the growth phase



Bao et al. (**2000**) **Biol Reprod** 62: 616-621.



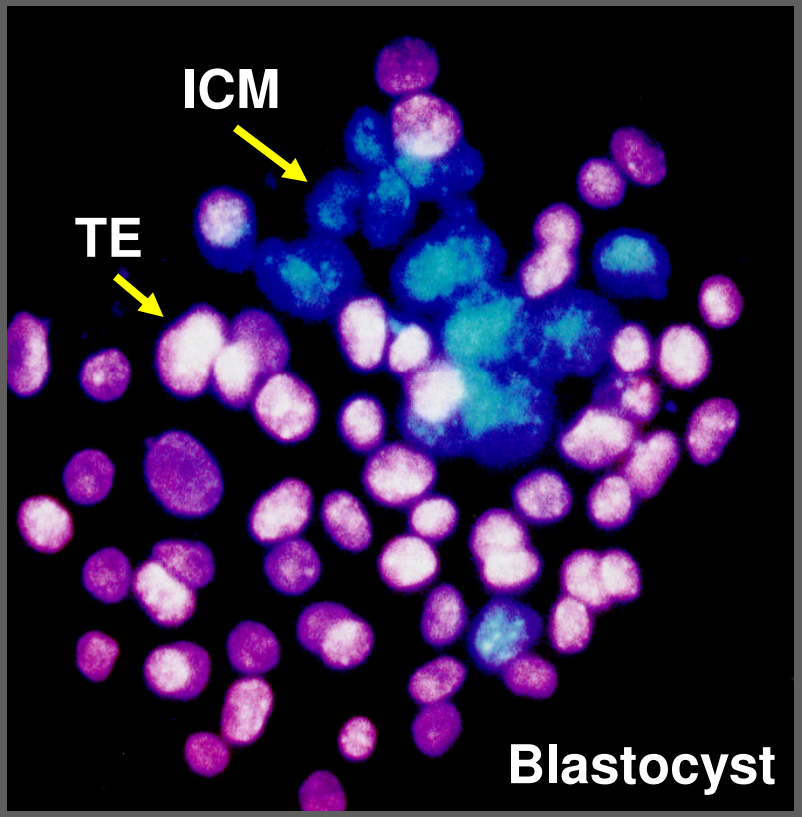
Developmental competence acquisition

Mouse

⇒Different patterns of the Ca^{2+} oscilations at fertilization and during the first cell cycle influence the ratio of ICM to TE cells in blastocysts

(influence cell diferentiation during pre-implantation development)

⇒The frequency of Ca^{2+} oscilations at activation influences the rate of implantation after embryo transfer



Stachecki and Armant (1996) Development 122:2485-2496

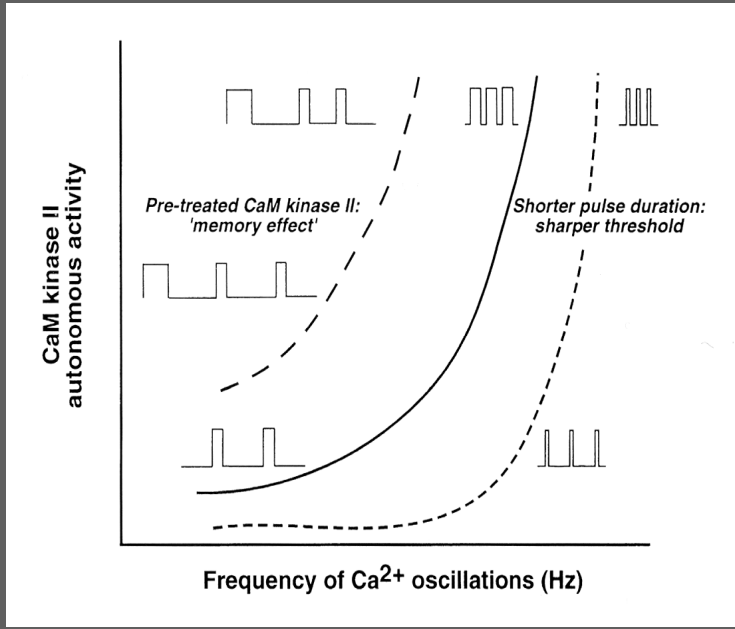
Bos-Mikich et al. (1997) Dev Biol 182:172-179.

Swann and Ozil (1994) J Physiol 483: 331-346.

Developmental competence acquisition

How could the frequency of Ca^{2+} oscilations be decoded by the oocyte?

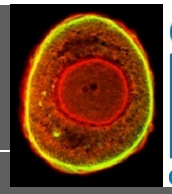
Ca^{2+} / Calmodulin kinase II could function as a frequency decoder of Ca^{2+} oscilations inside the oocyte



CaM kinase II activity ↑↑ exponentially with the frequency of Ca^{2+} spikes

Note: CaM kinase II is involved in MPF and CSF inactivation upon fertilization of *Xenopus* eggs

Dupont and Goldbeter (**1998**) **BioEssays** 20: 607-610.
De Koninck and Schulman (**1998**) **Science** 279: 227-230.



Developmental competence acquisition

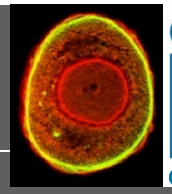
Bovine

⇒ Before oocyte maturation

Blondin et al. (1997) *In vitro* production of bovine embryos: developmental competence is acquired before maturation. Theriogenology 47:1061-1075.

⇒ Oocytes cultured inside antral follicles during 24 hours before IVM and IVF, acquire greater competence to embryonic development, relative to oocytes submitted to those processes imediatelly after collection.

Nashta et al. (1998) Maintenance of bovine oocytes in meiotic arrest and subsequent development *in vitro*: a comparative evaluation of antral follicle culture with other methods. Biol Reprod 59:255-262.



Developmental competence acquisition

Human

⇒ Probably, most antral follicles with a diameter inferior to 10mm contain developmentally incompetent oocytes to support development beyond the 4-cell stage. (Certain conditions (PCOS) may be exceptions.

⇒ After IVM (follicles 10-12mm) oocytes exhibit developmental competence, although at lower rates.

⇒ Trying to improve oocyte developmental competence by delaying oocyte IVM with drugs (6-DMAP, etc) ⇒ **variable effects !**

⇒ **It remains a research priority in Reproduction Medicine the identification of factors necessary to the acquisition of developmental competence by the oocyte both *in vivo* and *in vitro*.**

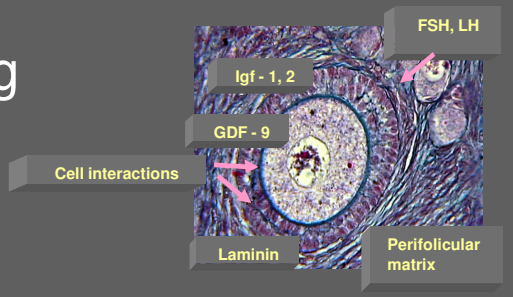
Trounson et al. (1998) Hum Reprod 13 (Suppl. 3): 52-62.

Anderiesz et al. (2000) Hum Reprod 15: 379-388.

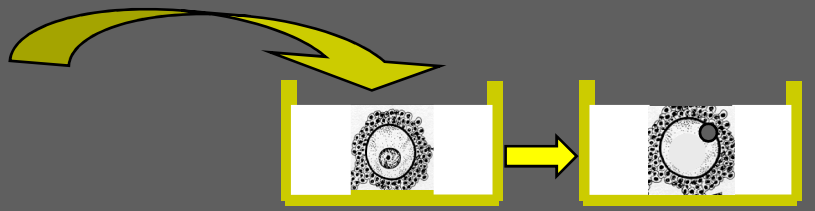
MA Edson et al.. (2009) Endocrine Reviews, 30:624-712.

Chalenges of research today

• To understand the biological mechanisms underlying oocyte competences acquisition during oogenesis



• To identify factors *(FSH, insulin, EGF, Midkine)* able to improve *in vitro* the acquisition of oocyte competences in IVG or IVM cultures (follicle / COC)



Eppig et al. (1998) Biol Reprod 59: 1445-1453.

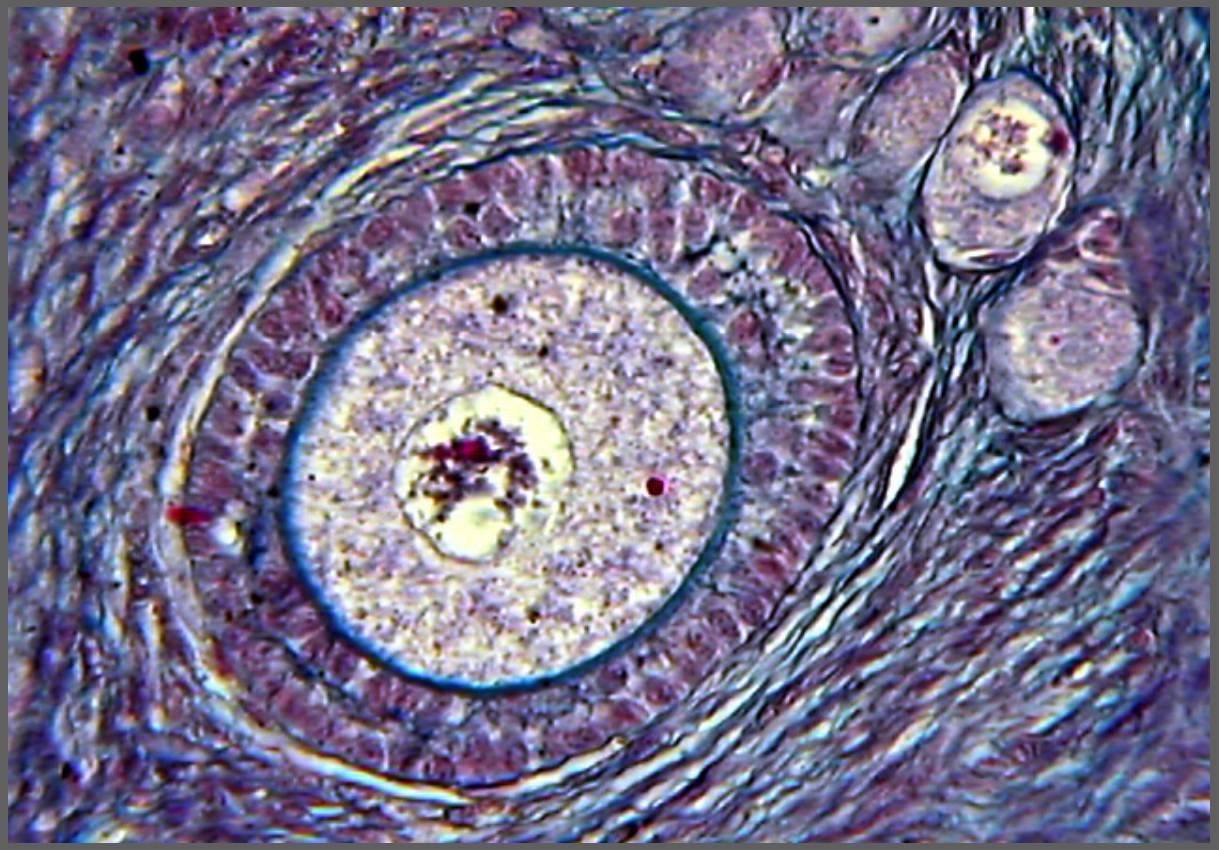
Rabindranath et al. (1999) Hum Reprod 14: 3060-3068.

Merriman et al. (1998) Hum Reprod 13: 690-695.

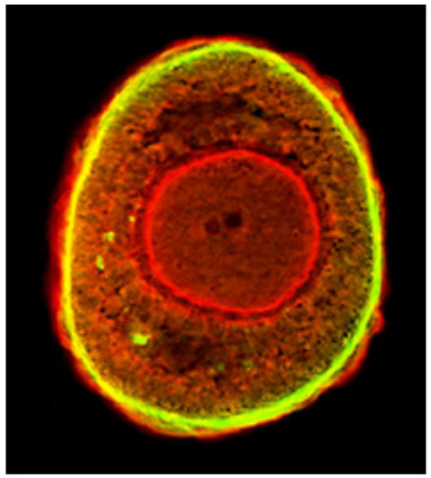
Ikeda et al. (2000) Biol Reprod 63: 1067-1074.

MA Edson et al.. (2009) Endocrine Reviews, 30:624-712.

"(…) embryogenesis begins during oogenesis"



Edmund B. Wilson (**1925**) The Cell in Inheritance and Development. 3rd edition. Macmillan, New York.



Oogenesis as acquisition of competences

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