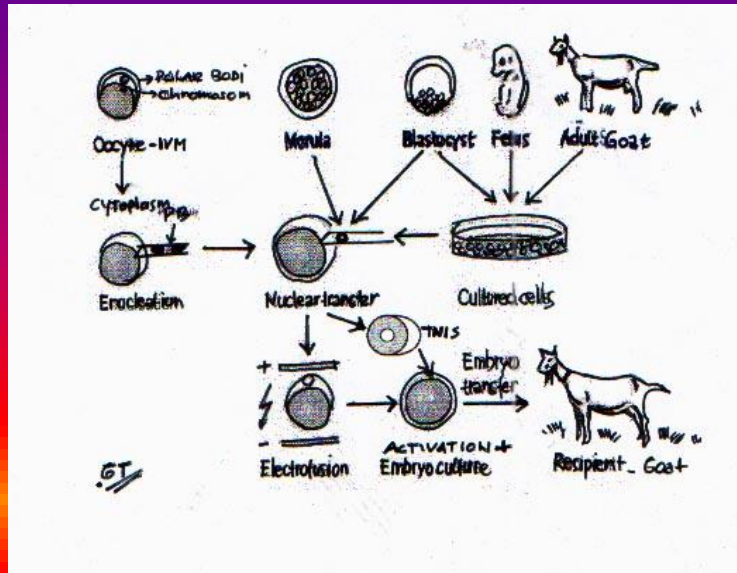


# IN-VITRO MATURATION AND CULTURE ANIMAL CELLS (OOCYTES)

**Gatot Ciptadi**  
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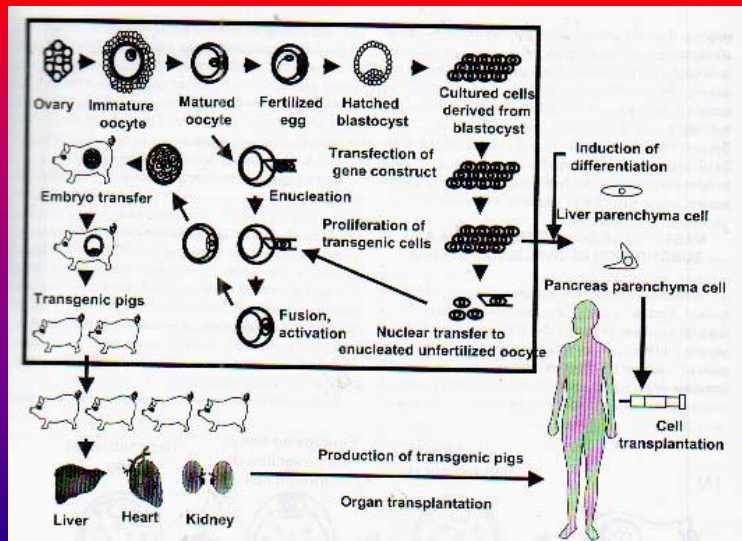
# Perkembangan Teknologi Reproduksi



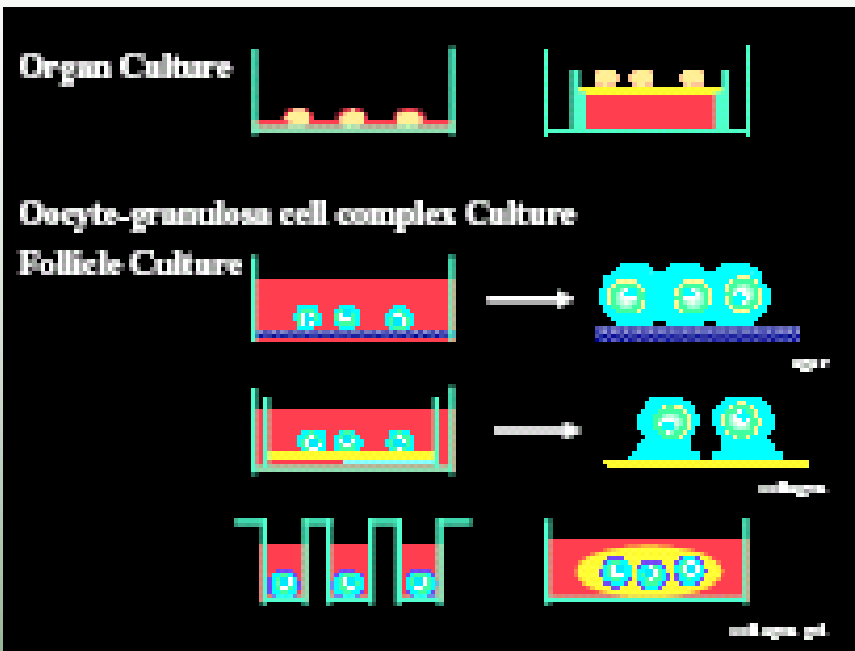
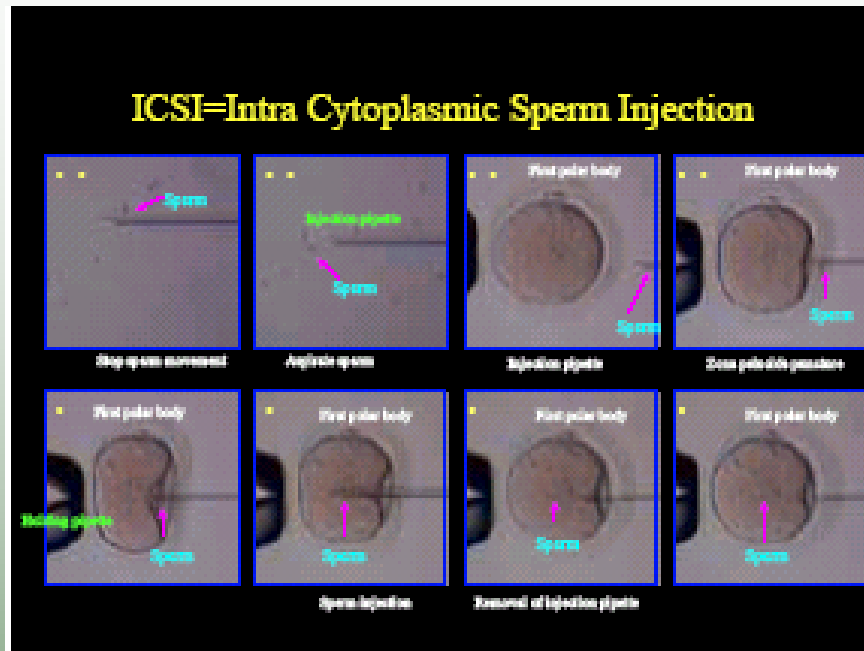
## Teknologi kloning (TRANSFER NUKLEUS)

### MANFAAT :

- Peningkatan produksi
- Bio farmasi-kedokteran
- **Transplantasi Organ Tubuh**
- Konservasi Plasma nutfah
- Pengembangan Sciences



# Contoh:Perkembangan Bioteknologi Reproduksi: Hanya perlu 1 spermatozoa untuk 1 oosit



In vitro Growth (IVG) Culture System :  
Pengembangan IVM,  
dilakukan untuk optimalisasi sumber oosit M-II terbatas  
(Miyano, 2016)

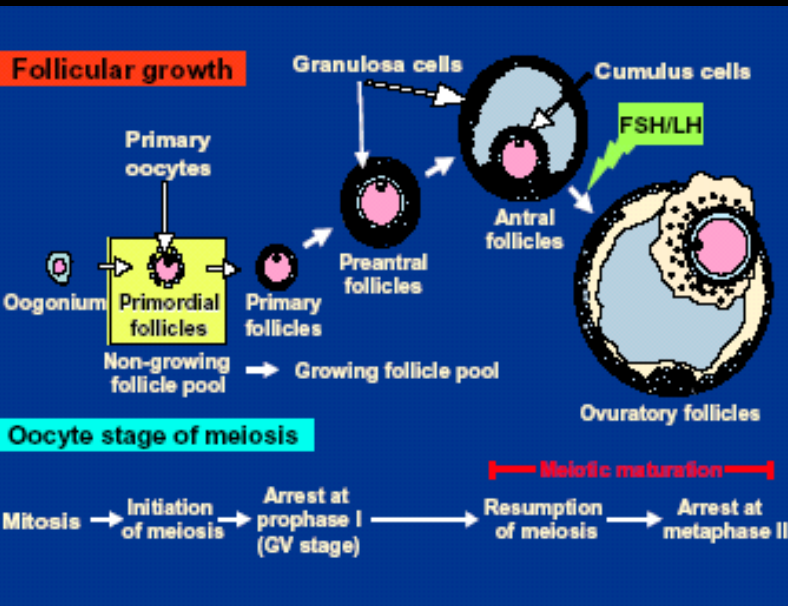
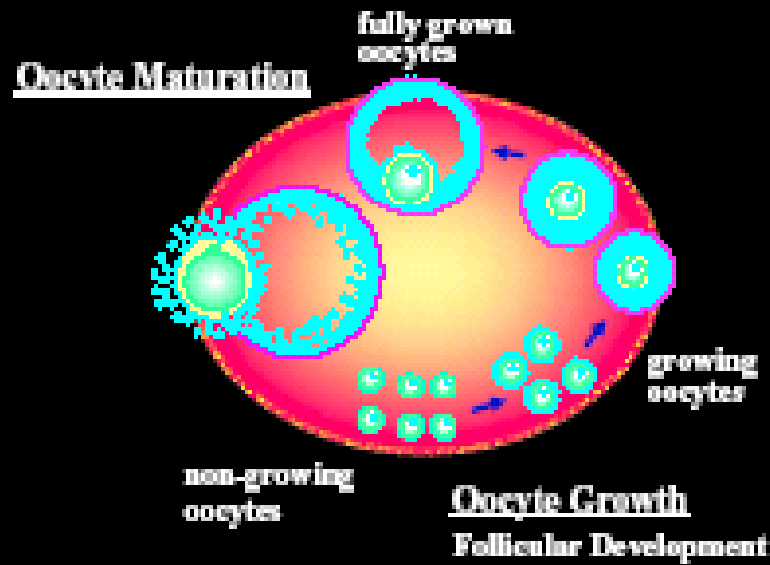
# Potential of In vitro culture System:

Sato, E. 2006.

## Numbers of ovarian follicles in mammals

Species	Primordial follicles	Developing follicles
Mouse	4,270	676
Sheep	105,450	475
Cow	120,000	300
Pig	420,000	84,000
Human	302,000	12,090

Mean number per pair of ovaries (Cooper and Tallar, 1987; Erickson, 1988)



## In vitro Maturation of Oocytes:

1. Oocyte at the GV stage mature to M-II (maturation)
2. Maturation can be induced in vitro (IVM)
3. Oocyte maturation : cumulus expansion, emission 1 st PB

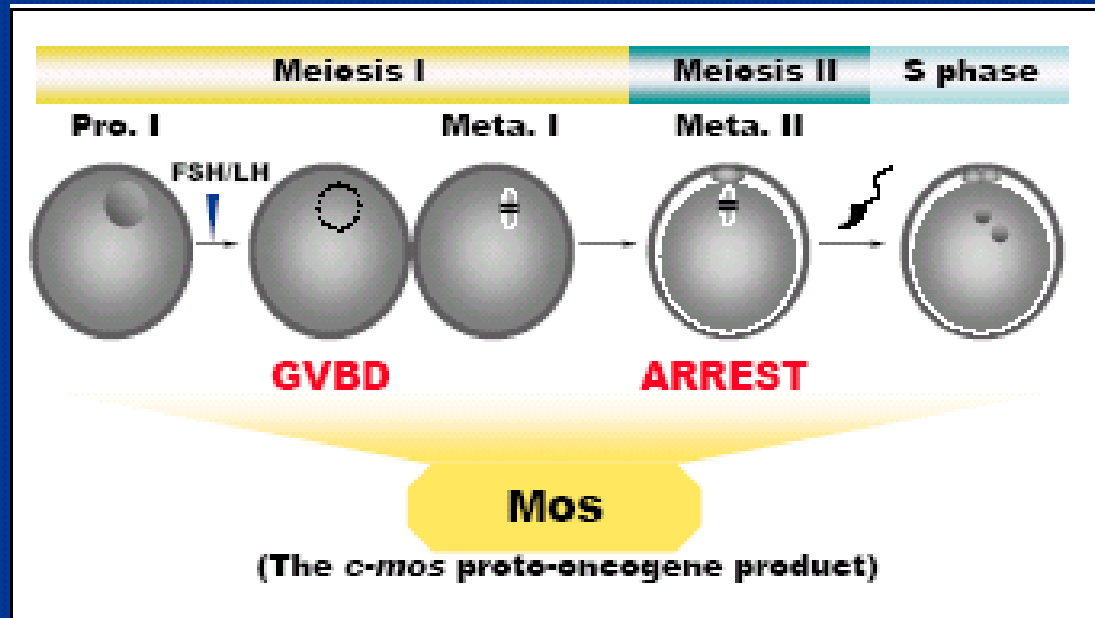
# OOCYTE MATURATION

3 Aspect important (maturation of):

- Nuclear (PB-I)
- Membrane (fixation and Penetreting sperms)
- Cytoplasmic (protein synthetis)

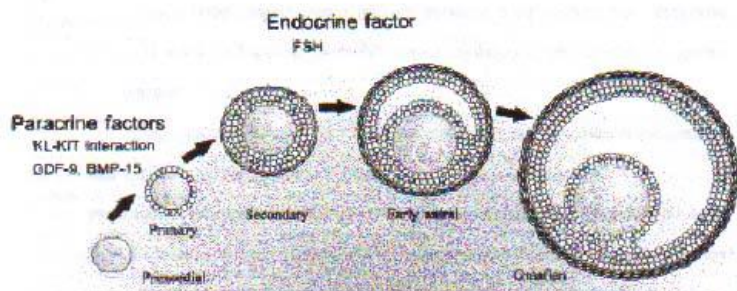
## OVERVIEW

### Background



- Overview of oocyte maturation -

Miyano, 2006,

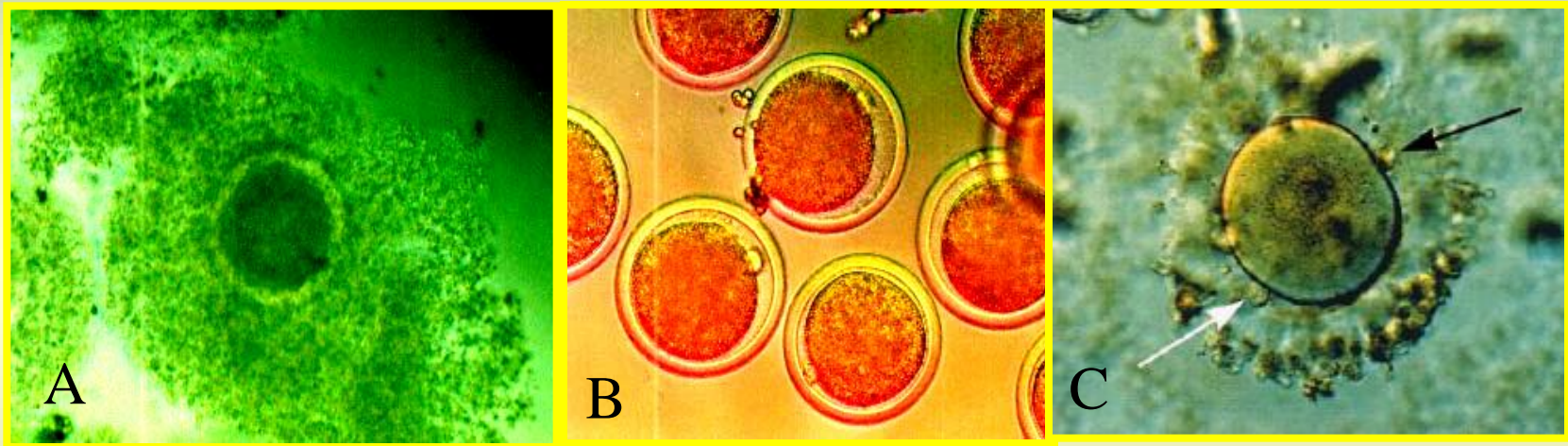


## METHOD of in vitro maturation of Oocyte (Goat)

- Material : Oocyte.-Cumulus-Granulosa Cell Complexes  
from large antral follicles (4 – 6 mm)
- Medium : TCM199, (10 % FCS, 0.1 ng/ml Na- pyruvate,  
Antibiotics.
- Hormon : FSH and LH
- Temperatur : 38.5 – 39 0C
- Gas % : 5 % CO<sub>2</sub> in air
- Dish : Falcon 35 mm
- Drop : 100 ul ( 10 ul/oocyte), covered with meniral  
oil/ parafin oil

# RESULT AND DISCUSSION:

## Oocyte Evaluation (IVM Result)



- A. Goat oocyte with cumulus cells expansion (Ciptadi, 2005): 77.8 %
- B. Polar body 1 st. Extruction after cumulus removed (Ciptadi, 2005): 95.32%
- C. Human oocyte maturation (Trounson *et al.* 1998)

## R.E.C.E.N.T.. R.E.S.U.L.T.S:

IVM and IVF rate (insemination) : 62.6 – 37.7 %  
(ICSI) : 53.9 – 69.3 %

IVM (28 – 36 h) : similar rate maturation, fertilization and cleavage.

**Coefisien correlation** (r) : KW1: NM = 0.58 – 0.89.

Factor affecting IVM: culture condition.  
size of the follicles.

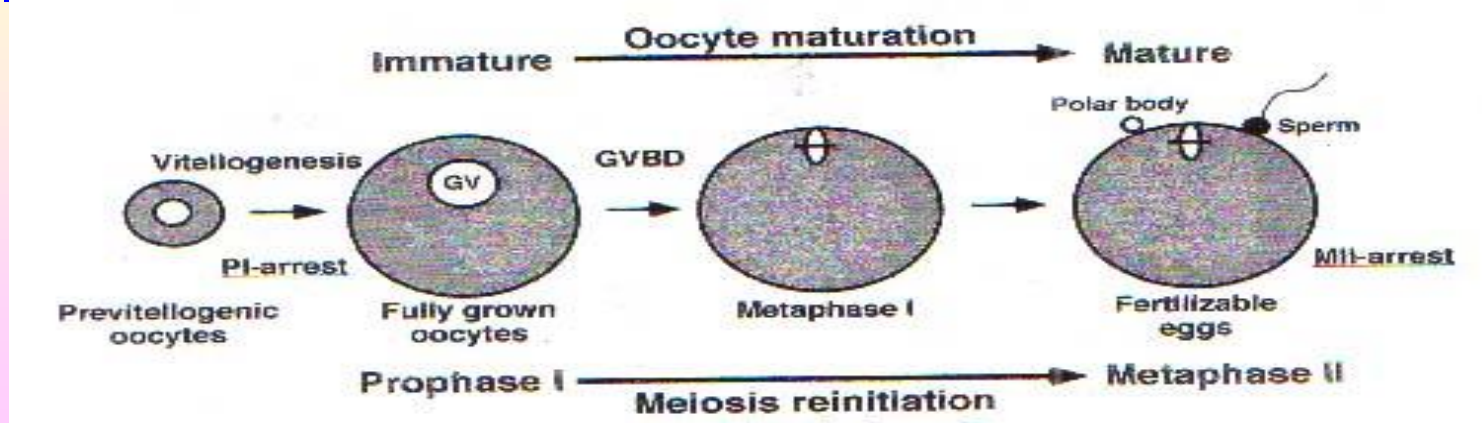
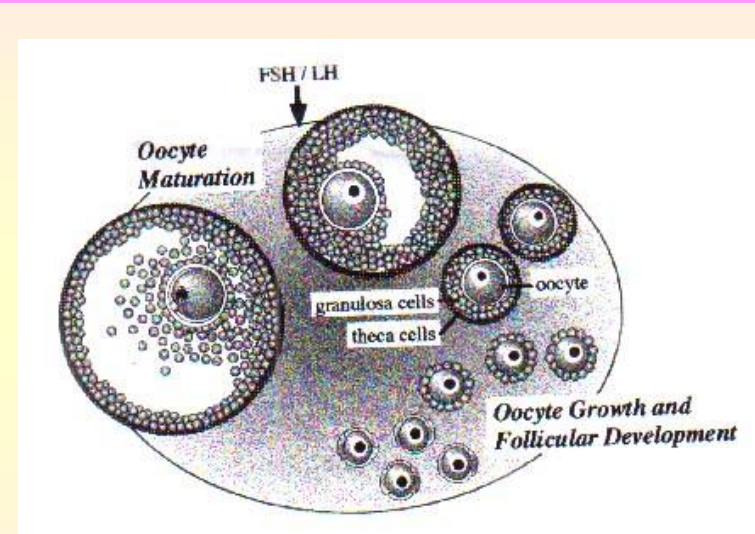
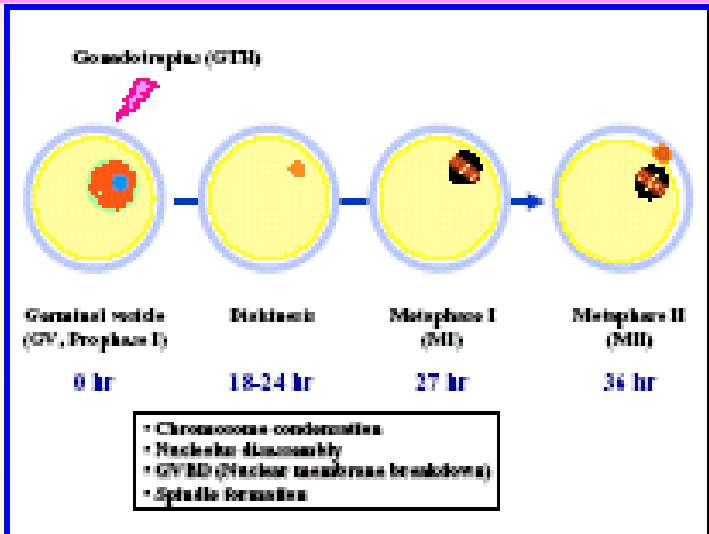
### **Pada manusia\***

IVM successfully used for infertile patients

Rate of clicical pregnancy(30 – 35 %) and implantation ( 10 – 15 %)

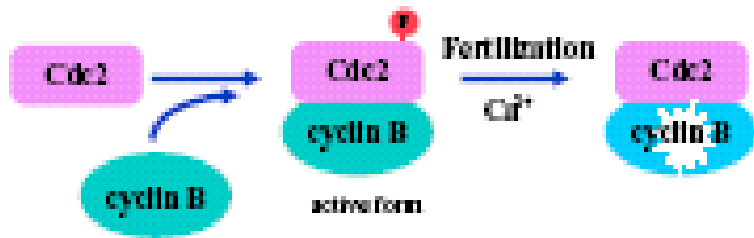


**Process of maturation** encompasses a complex series of molecular and structural events, culminating in the arrest of the oocyte chromosomes on the M-II plate in anticipation of sperm penetration and activation for fertilization



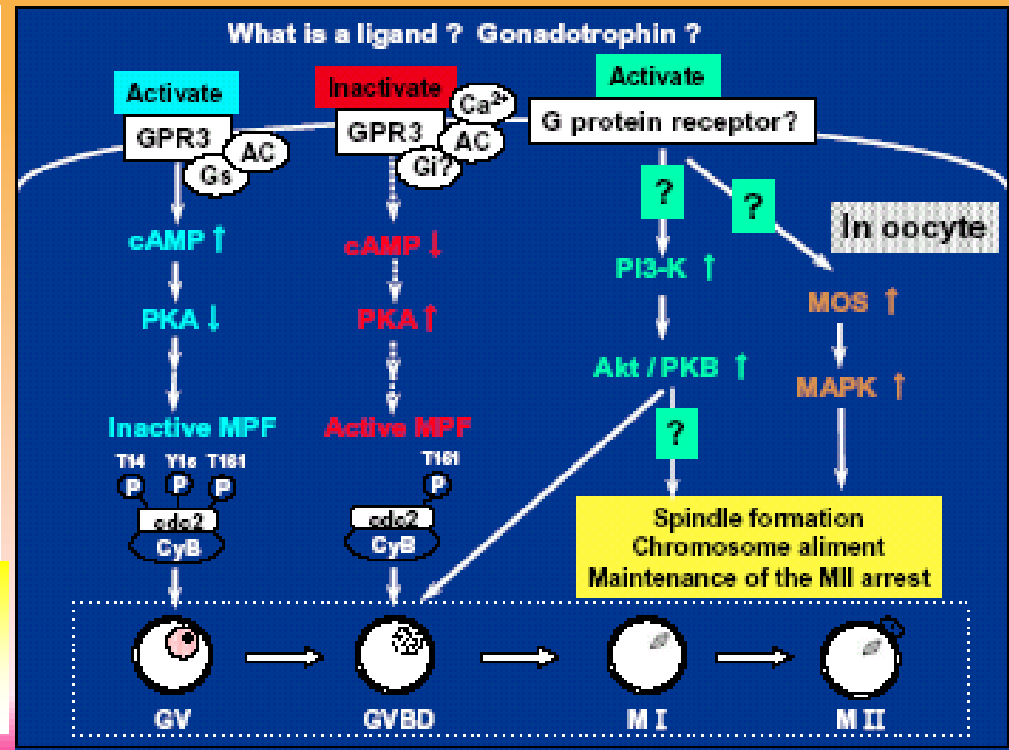
# Molecular Mechanism of activation MPF

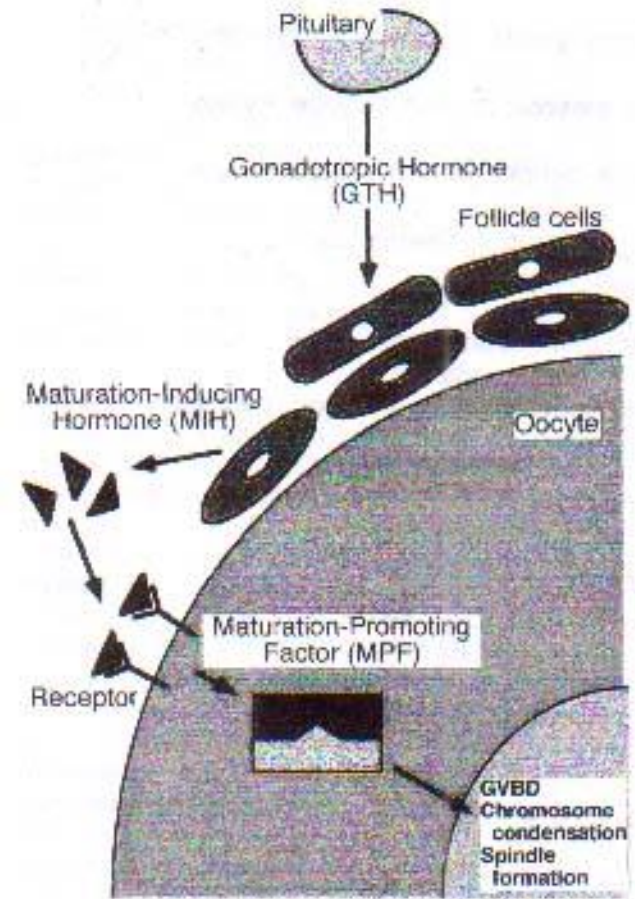
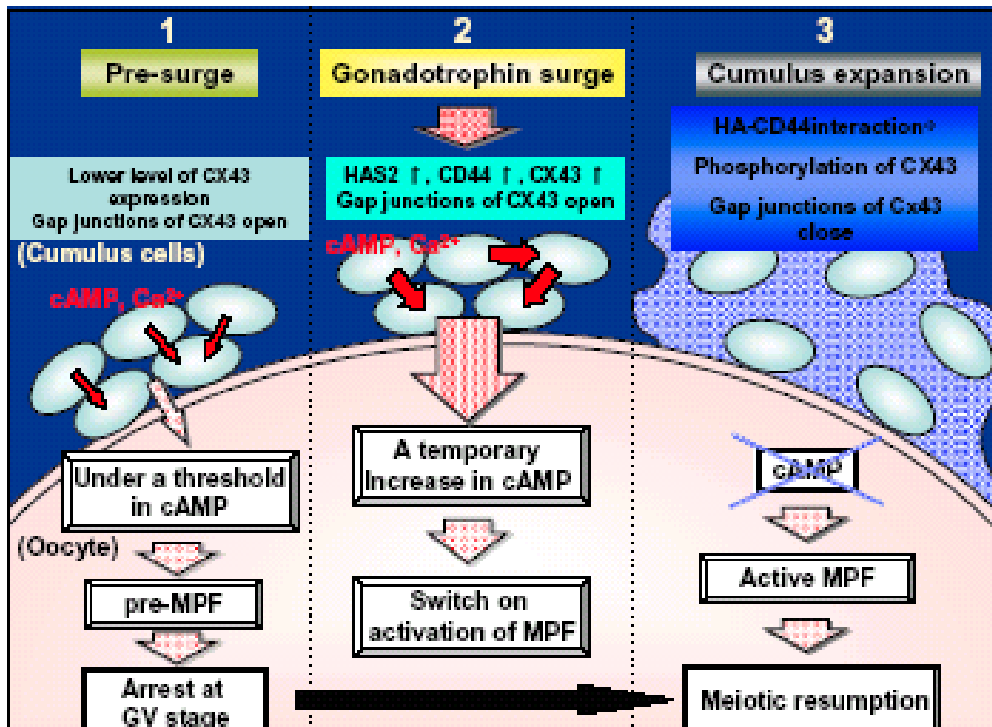
Cdc2 kinase (MPF) induces Oocyte Maturation



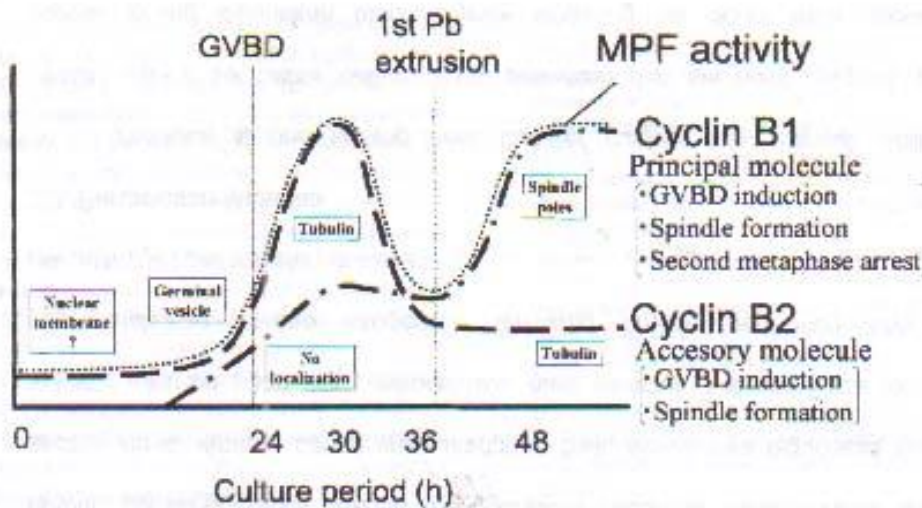
Sato, E 2006

Miyano and Hirao, 2003





Sato, E. 2006.



# The potential benefits of developing IVM:

Reduce both cost of of drug treatment and  
wastage of immature eggs collecting during standart IVF  
Could also lessen the risk of hyperstimulation syndrome  
May provide a valuable model for investigating  
the causes of meiotic aberattions and aneuploidies

**Might open to oocyte cryopreservation**

The potential clinical benefits of IVM  
Preserved ovarian tissue for future child-bearing

## **CONCLUSSION:**

Result from IVM oocytes are promising  
Further research: to improve culture condition  
and implantation rate