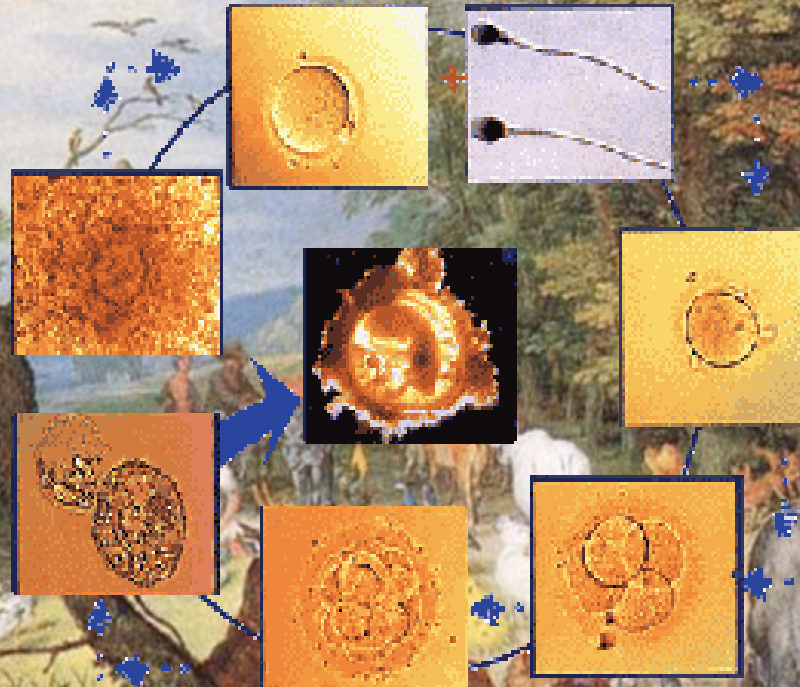


# IN-VITRO CULTURE OF ANIMAL CELLS (IVM, IVEF, IVG)

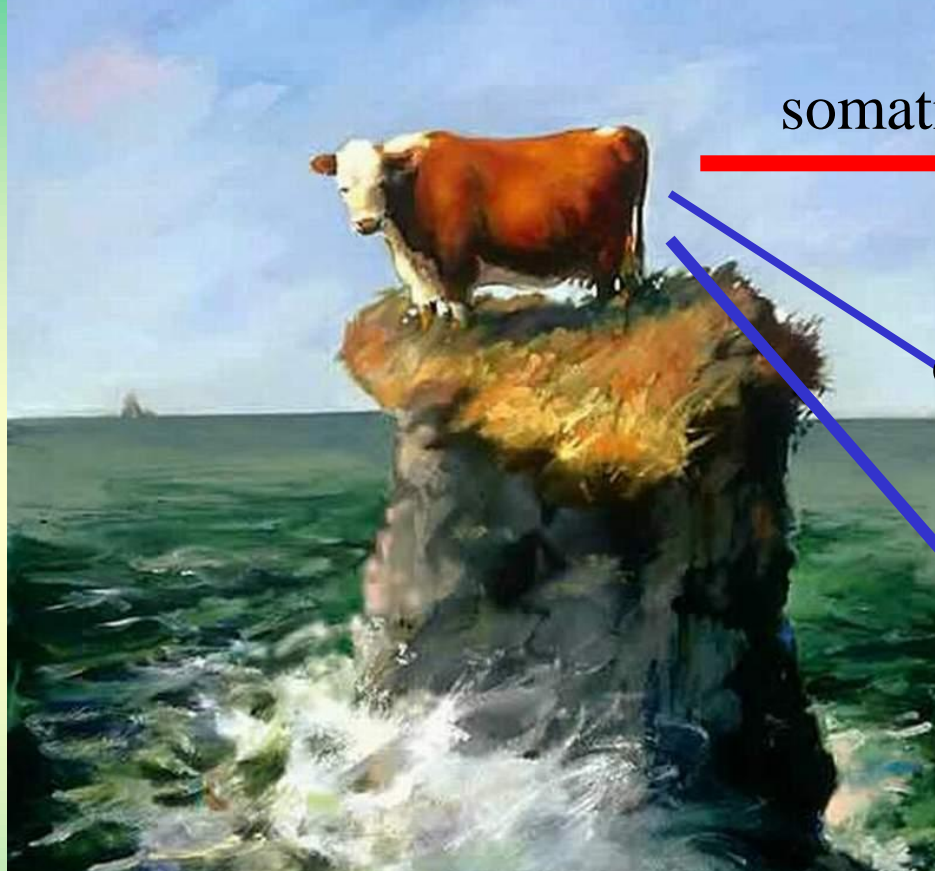


Gatot Ciptadi

Brawijaya University Malang

[www.bankselgamet.com](http://www.bankselgamet.com)

# PENDAHULUAN



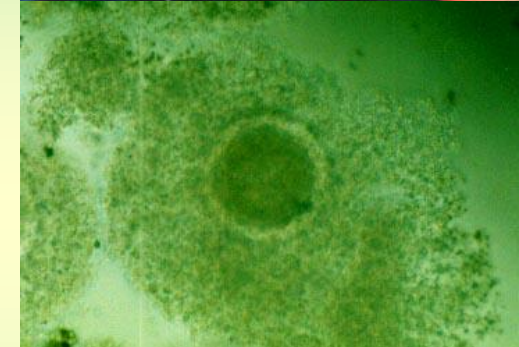
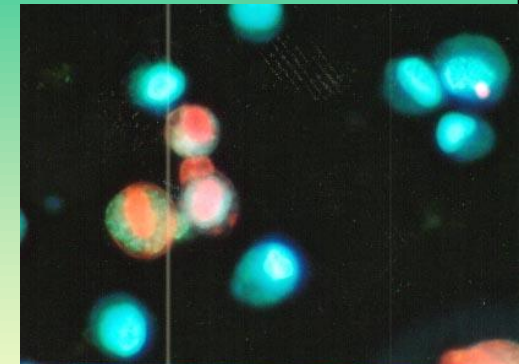
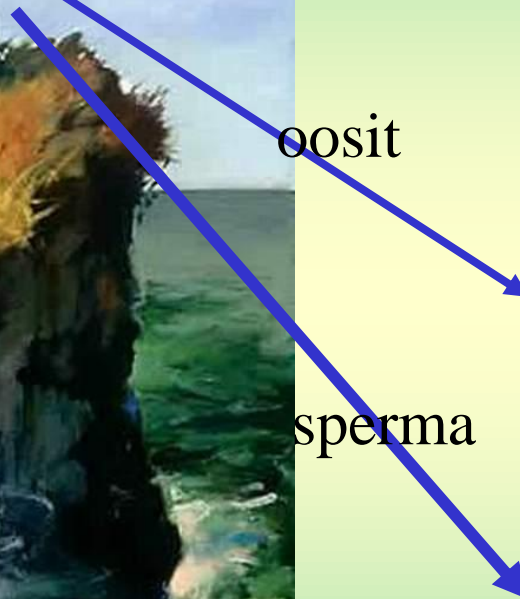
somatis



oosit



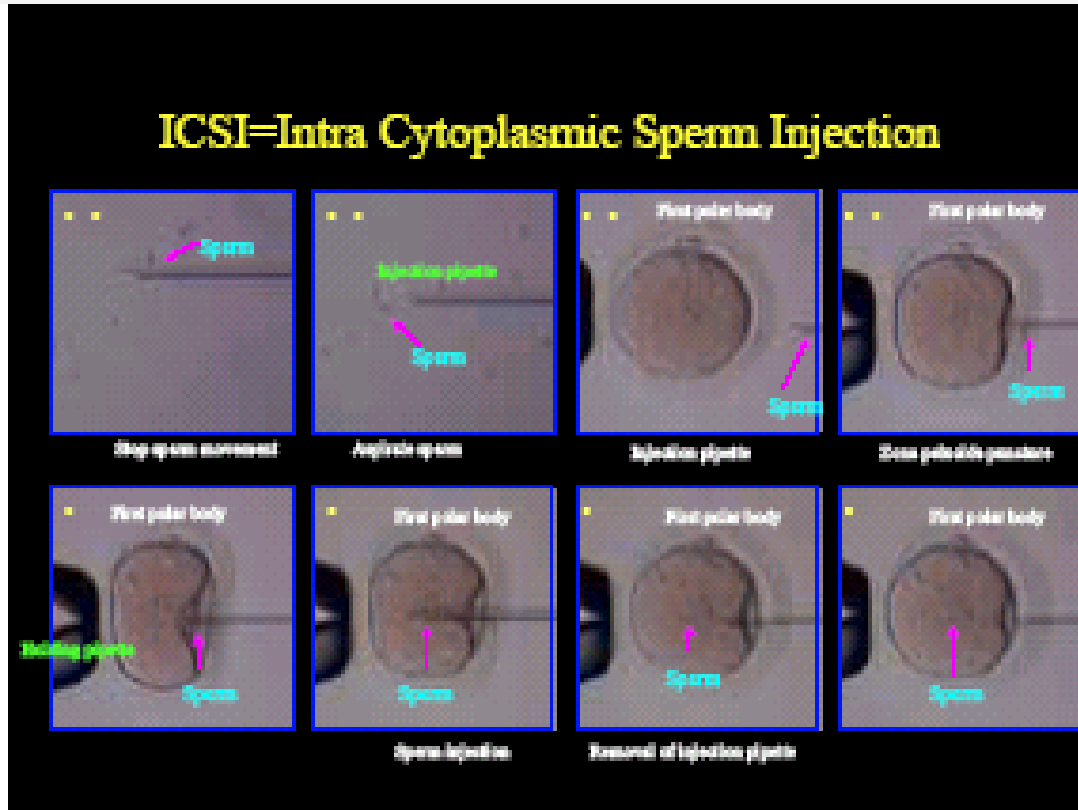
sperma



**BIOTEKNOLOGI REPRODUKSI**  
MAMPU MENYELAMATKAN  
**SUMBER GENETIK HEWAN LANGKA**

[www.bankselgamet.com](http://www.bankselgamet.com)

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



KA.: 1 milyar spz

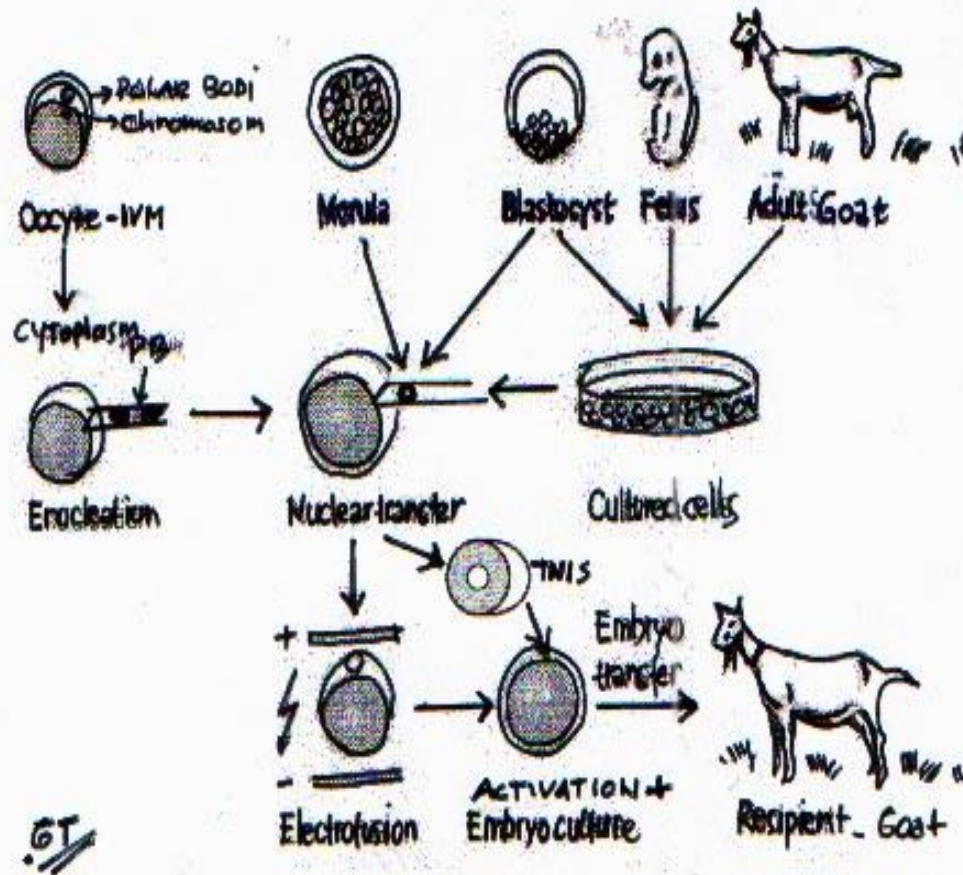
IB: 20-40 juta spz

IVF: 0,5– 1,0 juta spz

ICSI: 1 spz

Cloning- NT: 0 spz

# Perkembangan Terakhir Teknologi Cell In vitro : IVM, IVF, IVC, IVG , Kloning



## Teknologi kloning (TRANSFER NUKLEUS)

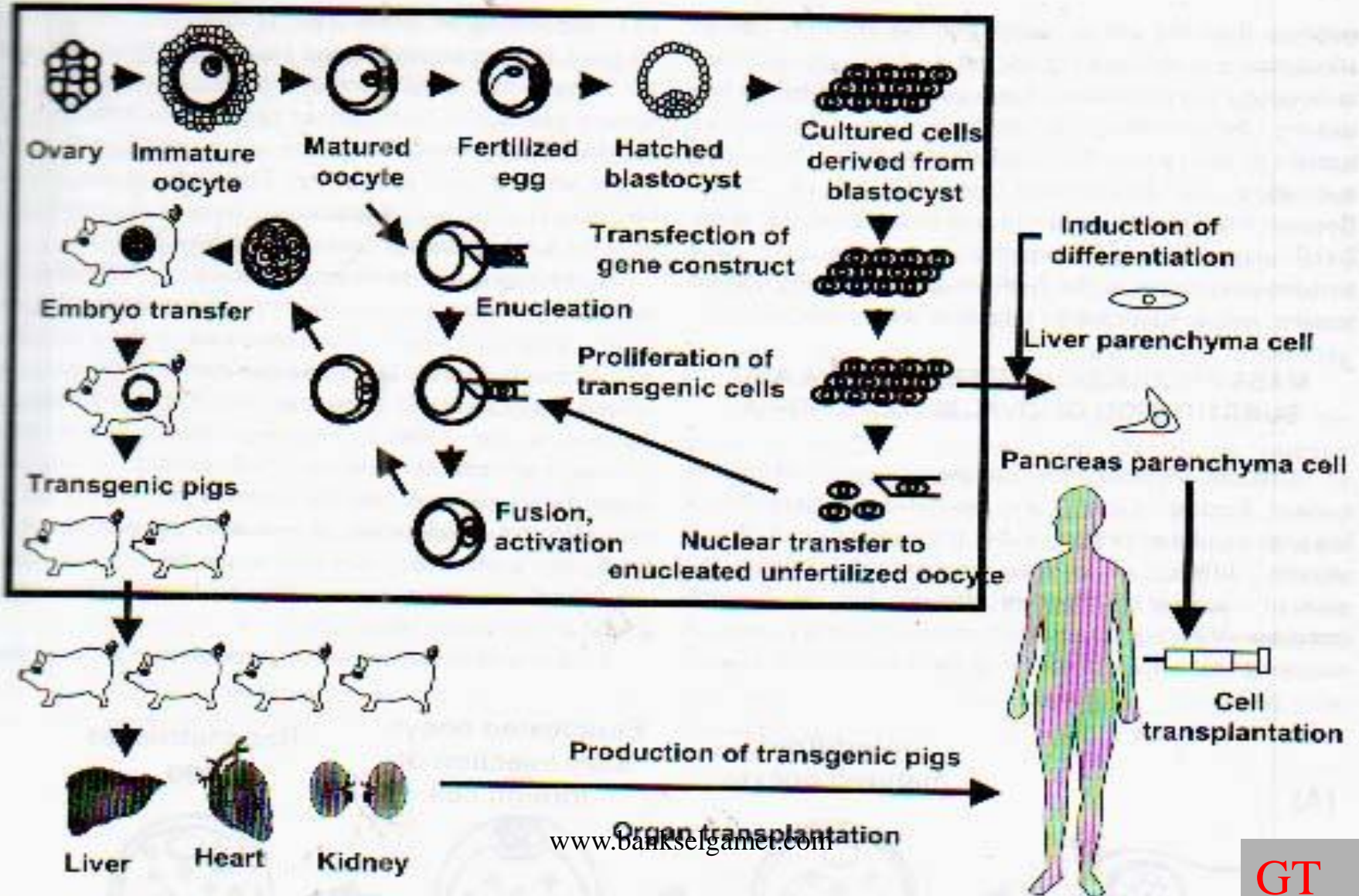
### MANFAAT :

- Peningkatan produksi
- Bio farmasi-kedokteran

### Transplantasi Organ Tubuh

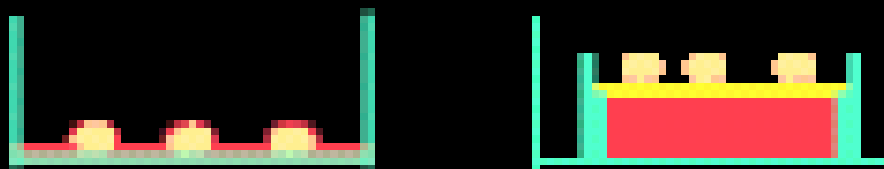
- Konservasi Plasma nutfah

# Bioteknologi cell culture: Sumbangan pada Ilmu Pengetahuan



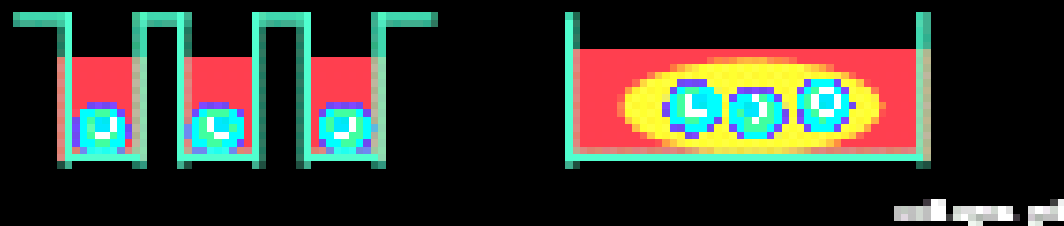
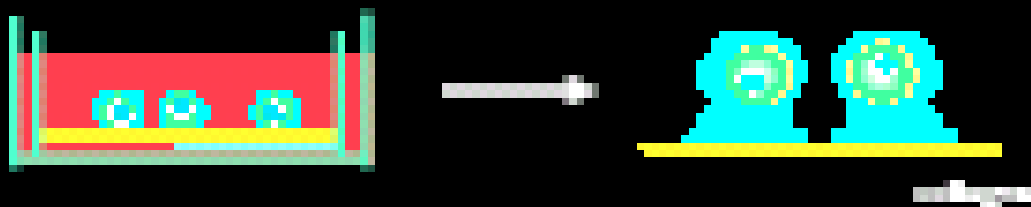
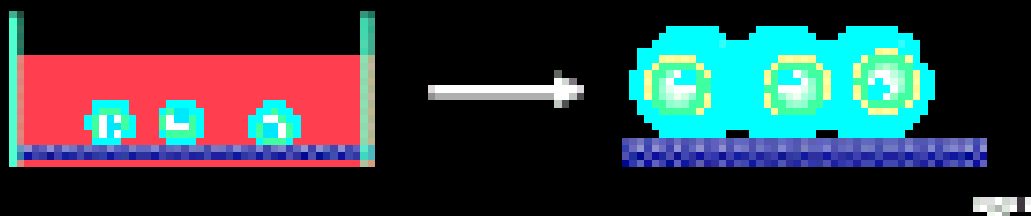
# In vitro Growth (IVG) Culture System

Organ Culture



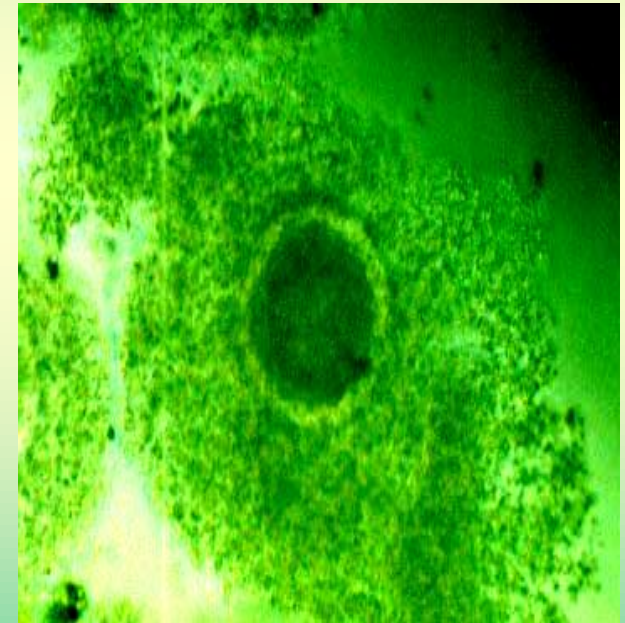
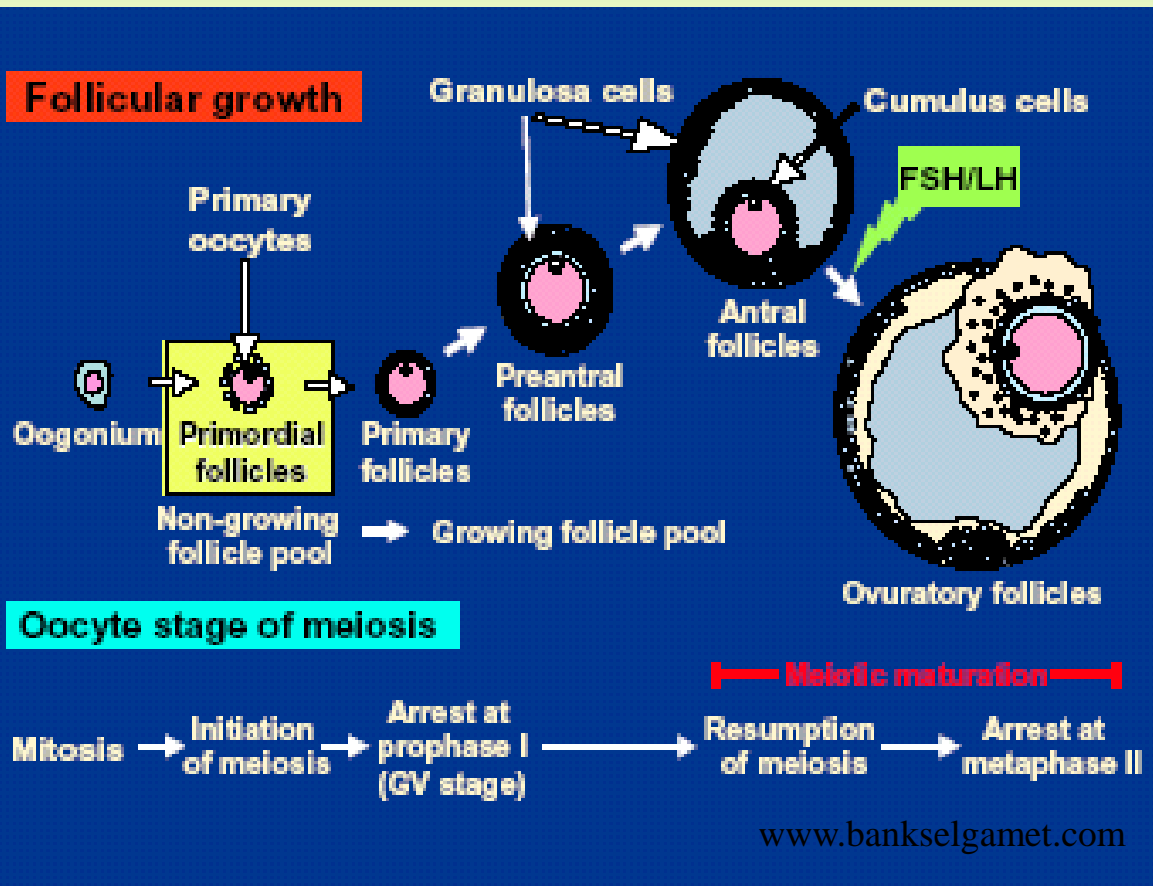
Oocyte-granulosa cell complex Culture

Follicle Culture



# In vitro Maturation of Oocytes:

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

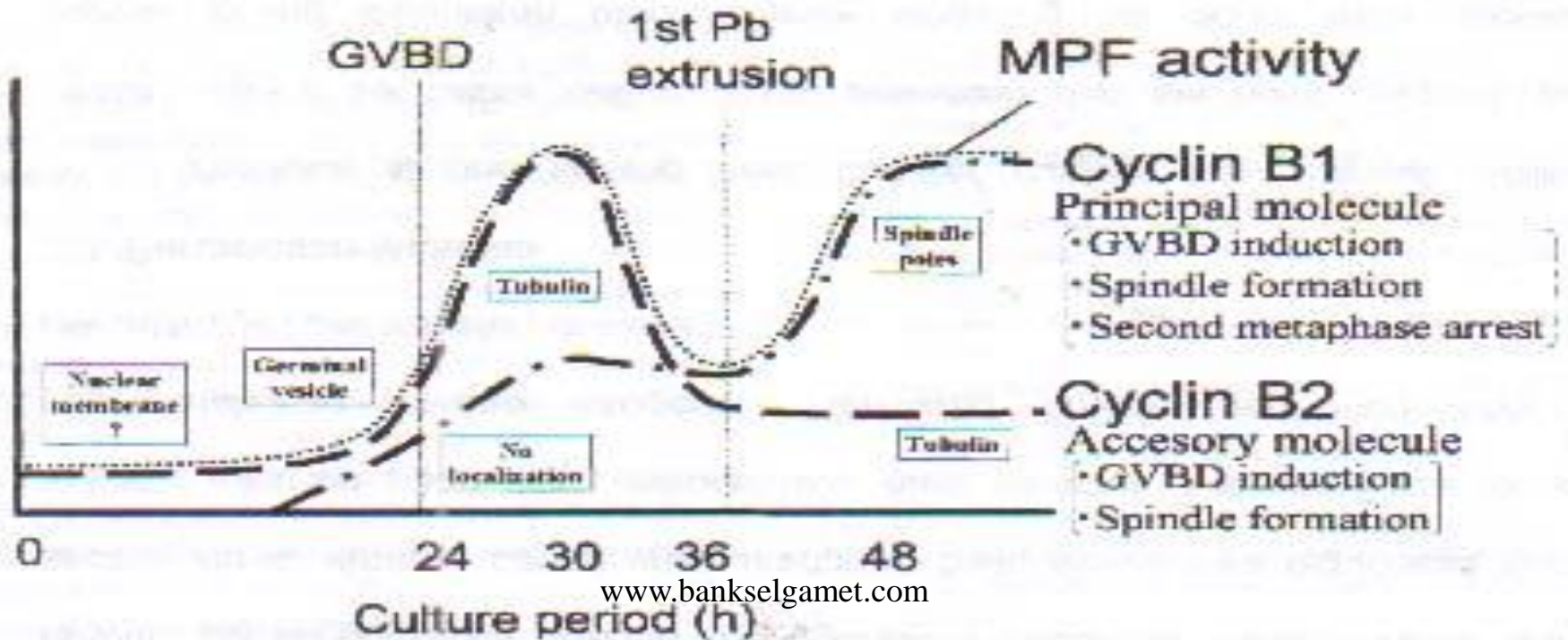
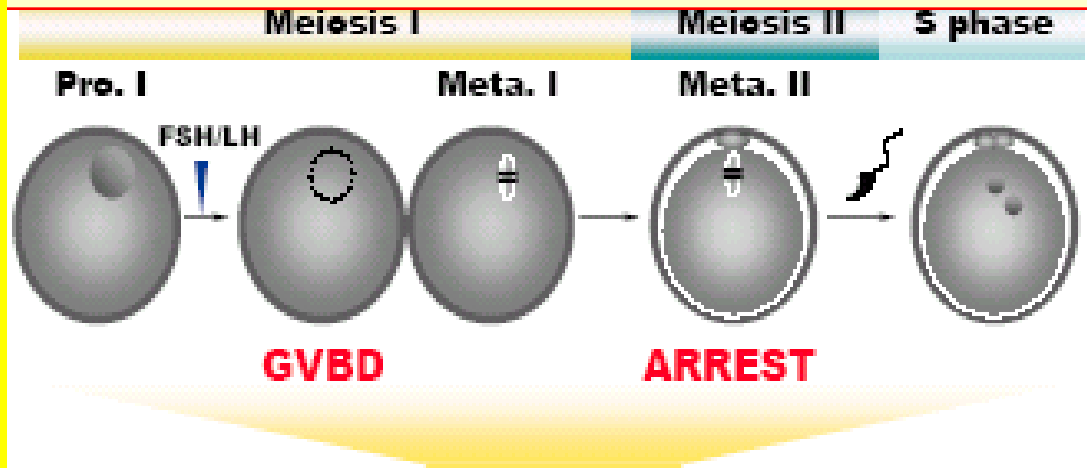


Sato,E, 2006,  
Ciptadi,2005

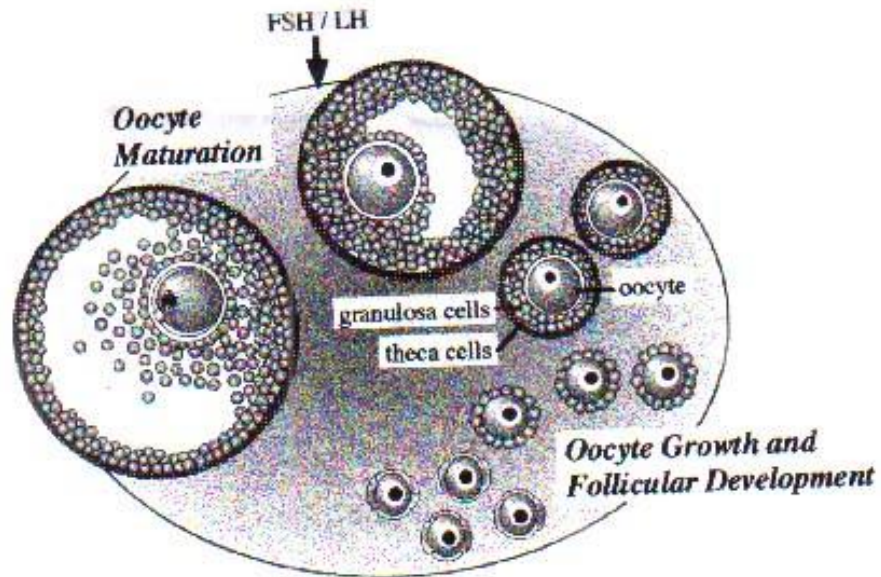
### 3 Aspect important (maturation of):

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

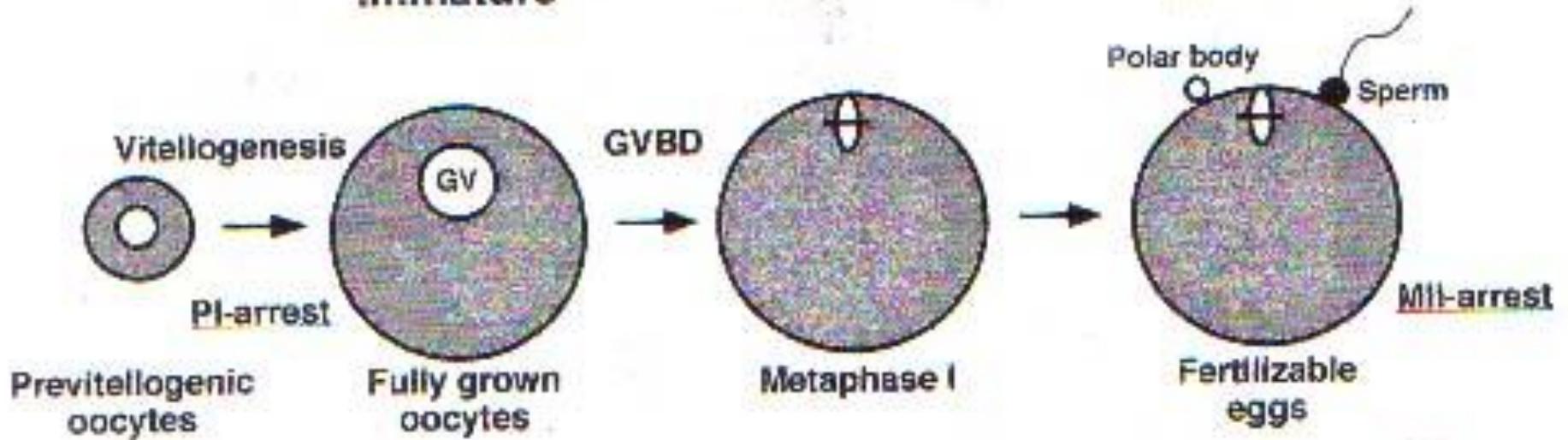
## OOCYTE MATURATION







Immature  $\xrightarrow{\text{Oocyte maturation}}$  Mature



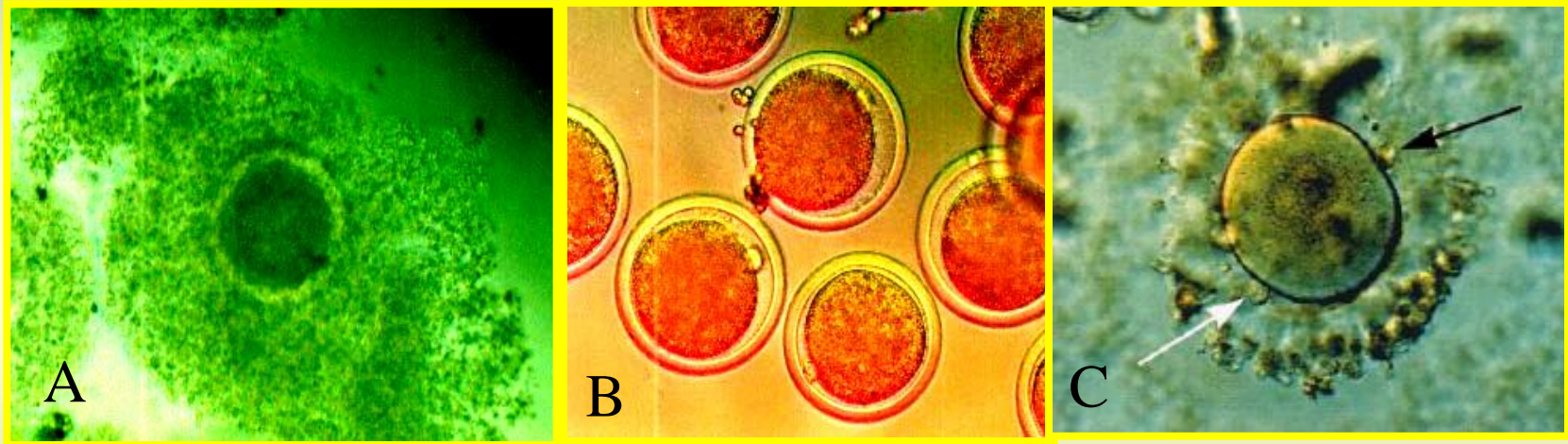
Prophase I  $\xrightarrow{\text{Meiosis reinitiation}}$  Metaphase II

## 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 % CO2 in air
Dish	: Falcon 35 mm
Drop	: 100 ul ( 10 ul/oocyte), covered with meniral oil/ paraffin oil

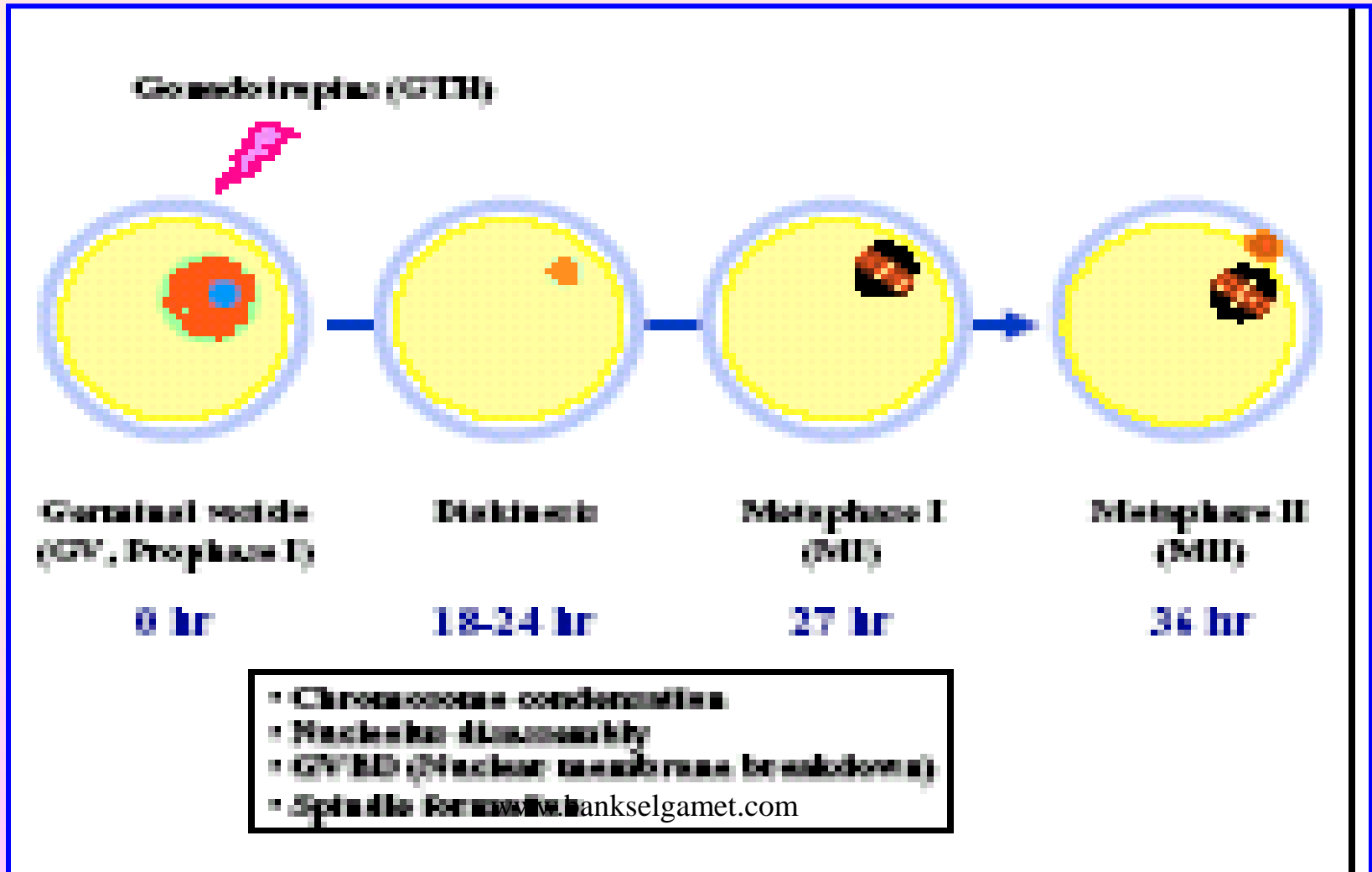
# RESULT AND DISCUSSION:

## Oocyte Evaluation (IVM Result)



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

**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



## 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.

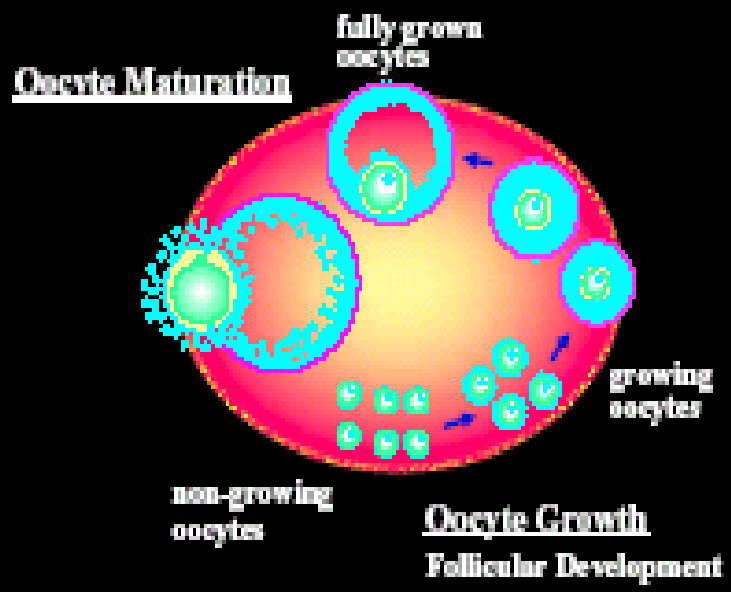
Factor affecting IVM: culture condition.  
size of the follicles.

### Pada manusia\*

IVM successfully used for infertile patients

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

# Potential of In vitro Growth (IVG) culture System:



## 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 (Gordon and Talbot, 1987; Erickson, 1985)

## 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

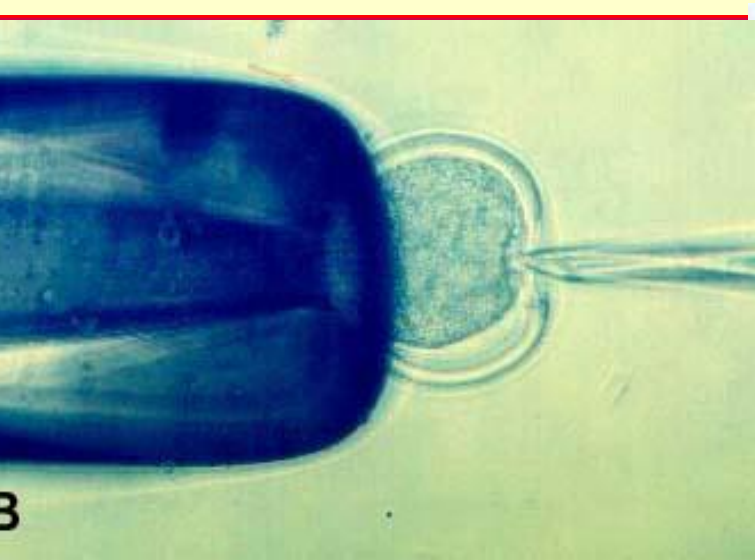
May provide a valuable model for investigating the causes of meiotic aberattions and aneuploidies

**Might open to oocyte cryopreservation**

The potential clinical benefits of IVM

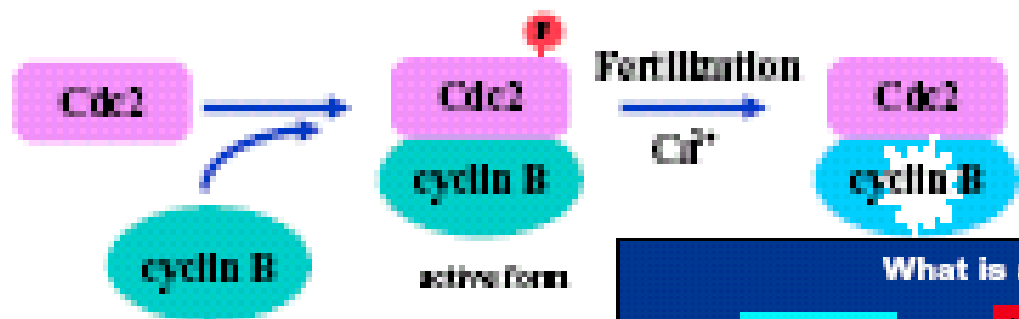
Preserved ovarian tissue for future child-bearing

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

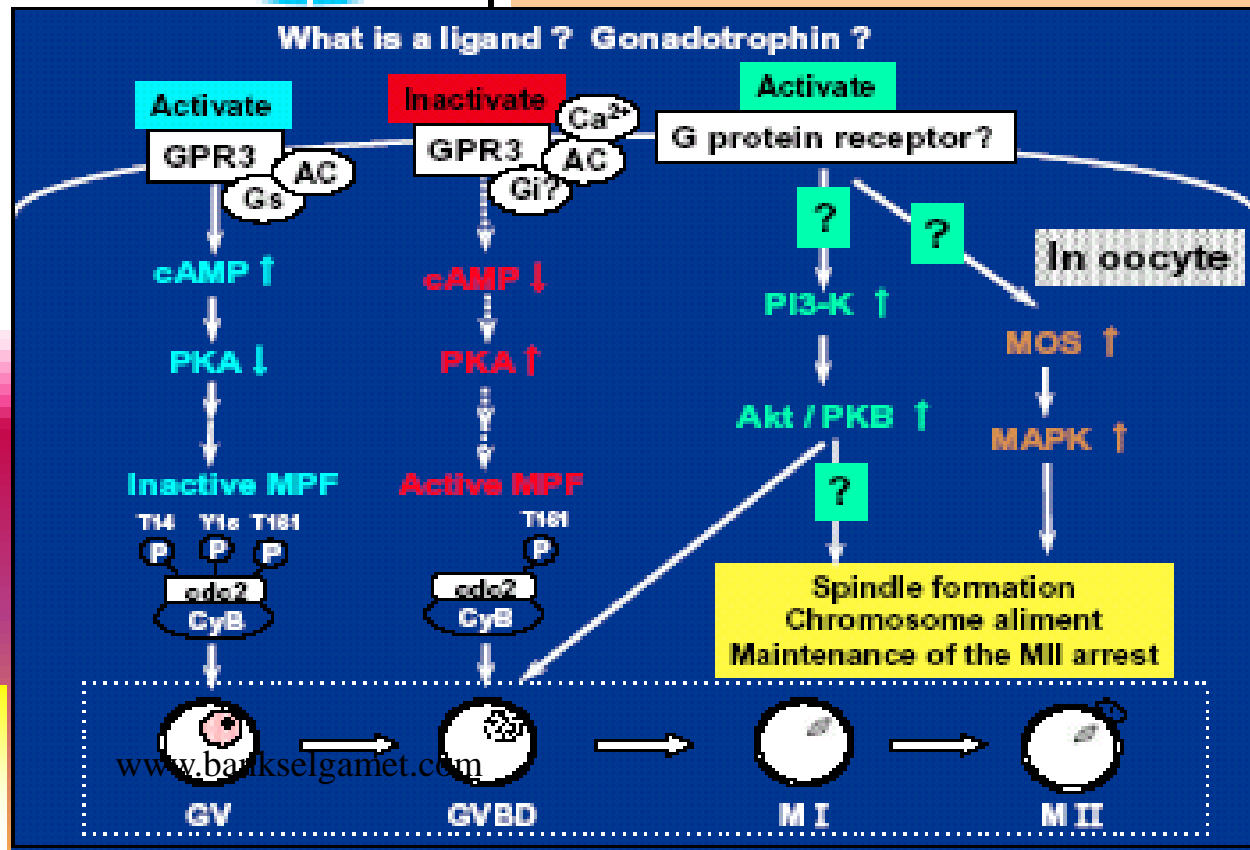




# Cdc2 kinase (MPF) induces Oocyte Maturation



# Molecular Mechanism of activation MPF



Sato, E 2006  
Miyano and Hirao, 2003

