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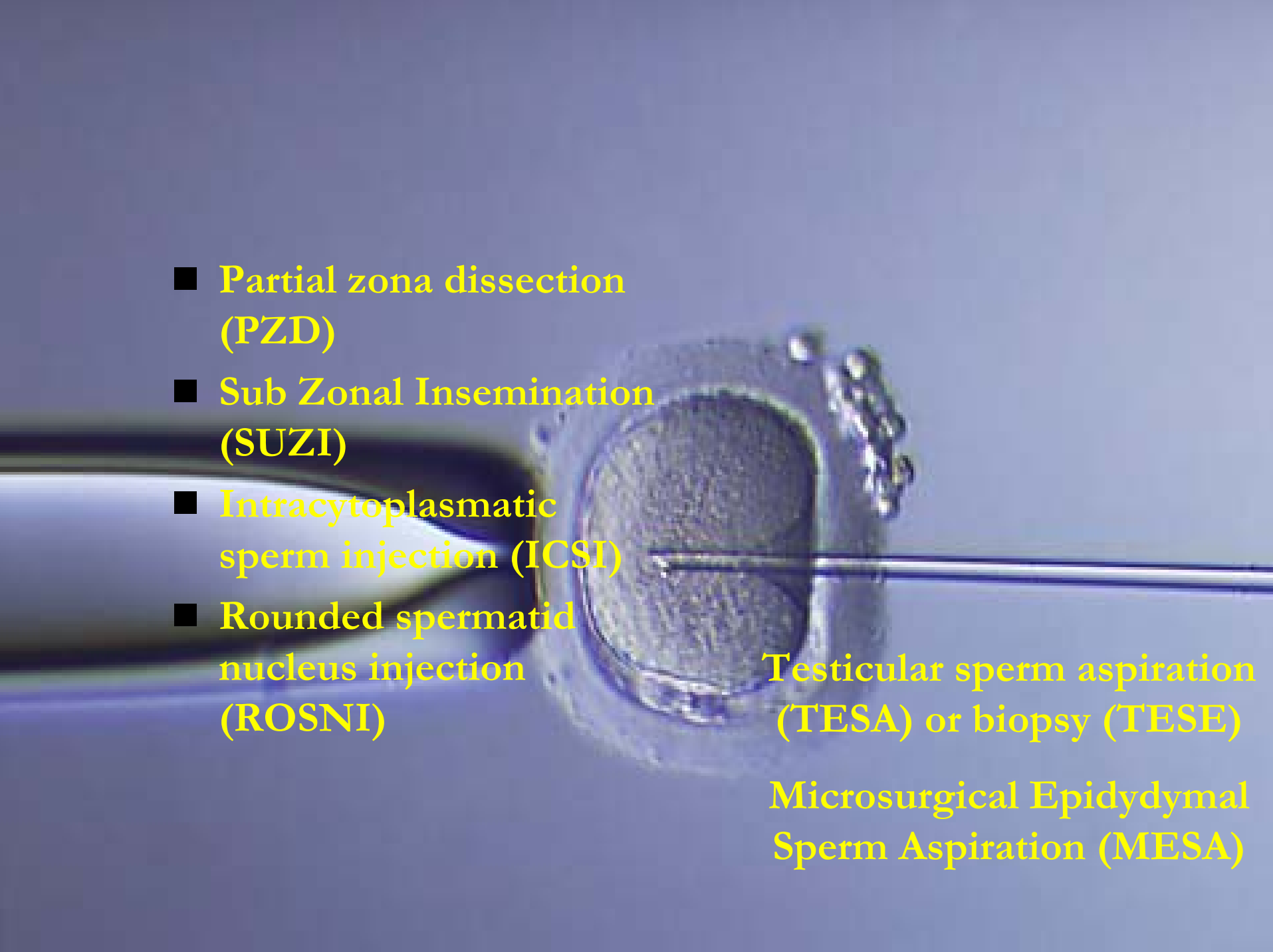
Fertilisation and implantation

Training in Research in Reproductive Health
Geneva 2005

Human fertilisation

- 1878 First attempts at in vitro fertilisation in mammalian eggs
- 1880 First successful embryo culture
- 1930 First successful ivf of mammalian eggs resulting in a live birth
- 1935 First successfully fertilised human eggs in vitro
- 1978 Birth of Louise Brown
- 1990 Preimplantation diagnosis
- 1992 Intracytoplasmatic sperm injection developed in humans



- Partial zona dissection (PZD)
- Sub Zonal Insemination (SUZI)
- Intracytoplasmatic sperm injection (ICSI)
- Rounded spermatid nucleus injection (ROSNI)

Testicular sperm aspiration (TESA) or biopsy (TESE)

Microsurgical Epidydymal Sperm Aspiration (MESA)

Fertilisation/implantation in the human species

A very non efficient process

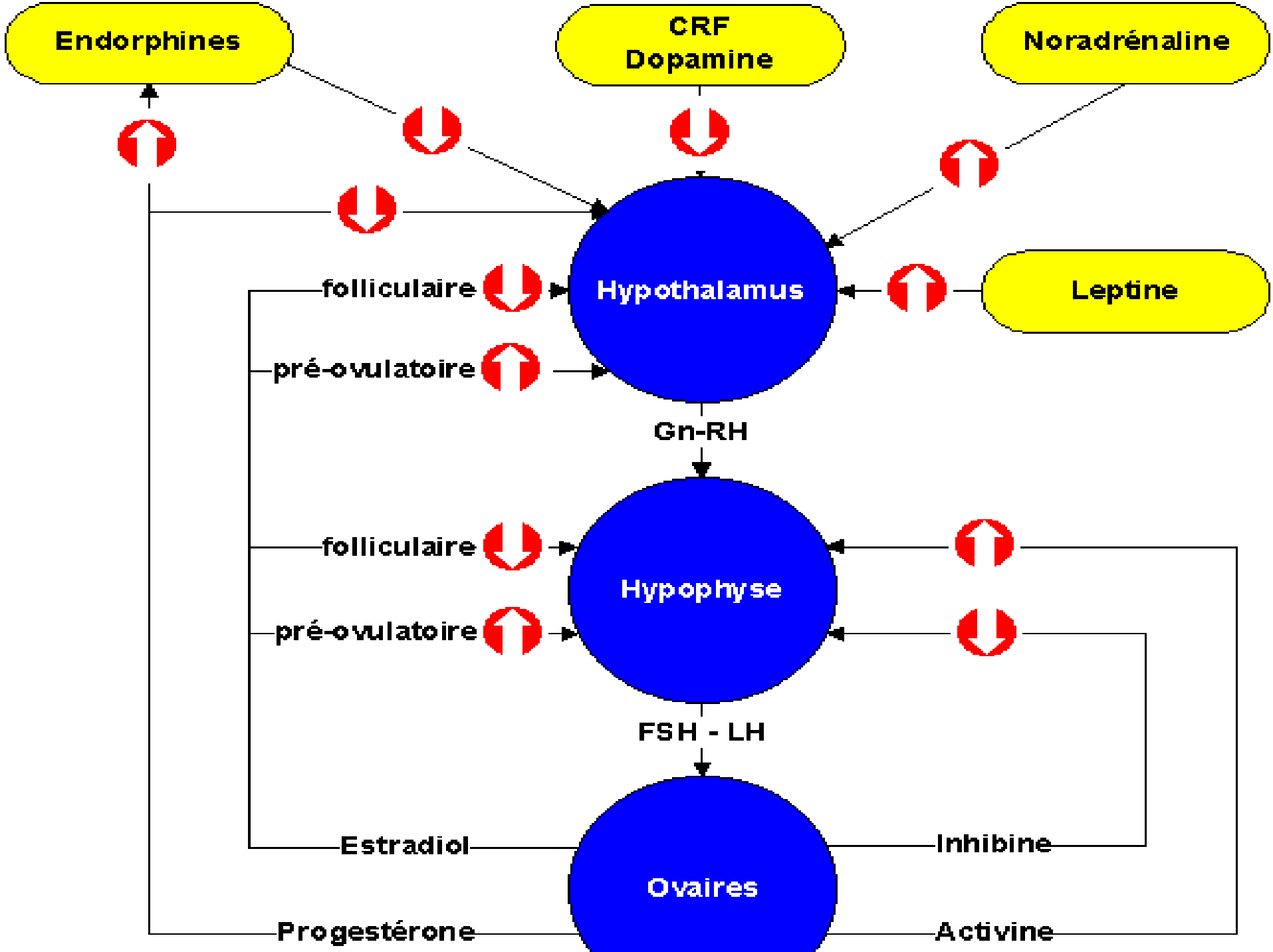
Over 50% of fertilised ova are aborted

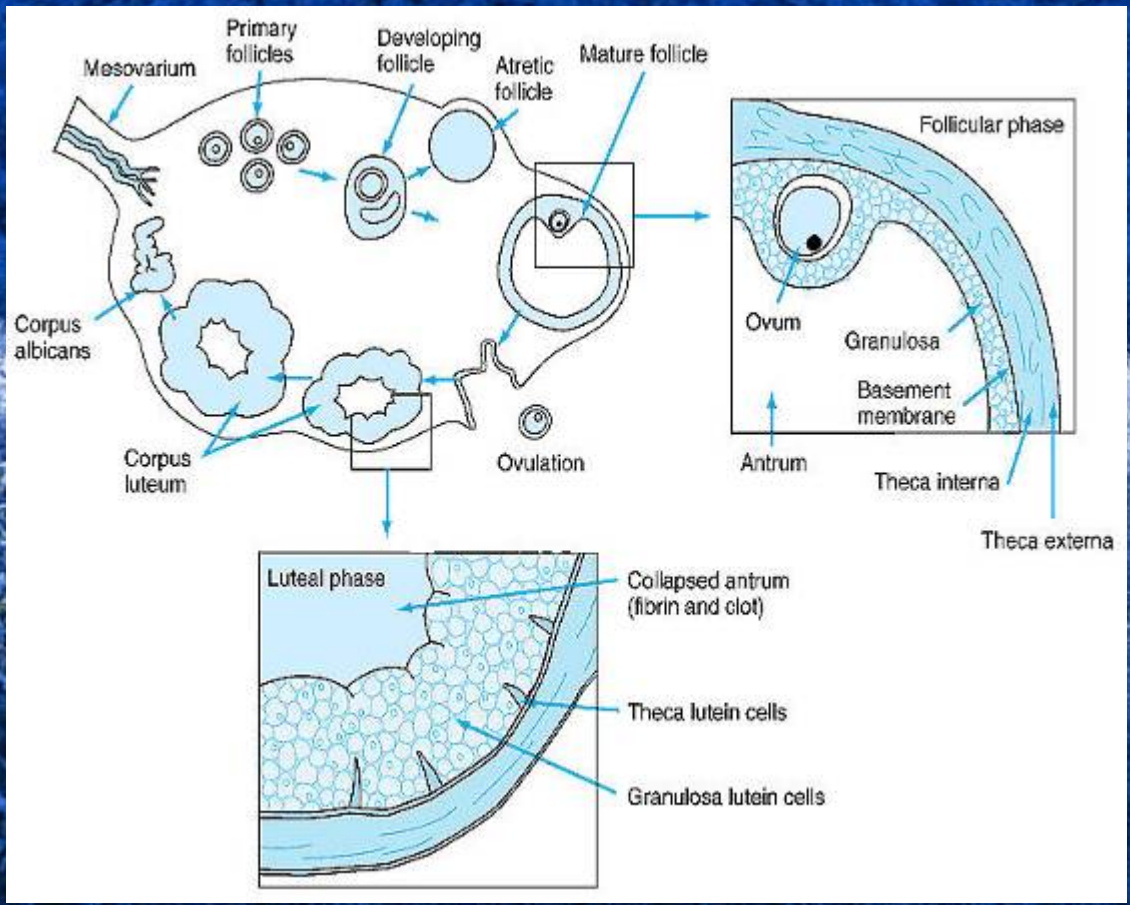
The embryo

- Cell division
- Cell migration
- Cell differentiation

Follicular/oocyte development

- In the human oocytes enter meiosis early in fetal life (~ 12 weeks).
- Oocytes progress to the diplotene stage of meiosis I (GV stage).
- Oocytes stay in this state until just before they resume ovulation
- This event is gonadotropin-dependent and triggered off by the mid-cycle LH surge


Endorphines
CRF
Dopamine
Noradrénaline
folliculaire
Hypothalamus
Leptine
pré-ovulatoire
Gn-RH
folliculaire
Hypophyse
pré-ovulatoire
FSH - LH
Estradiol
Inhibine
Ovaires
Progestérone
Activine


Mesovarium
Primary follicles
Developing follicle
Atretic follicle
Mature follicle
Corpus albicans
Corpus luteum
Ovulation
Follicular phase
Ovum
Granulosa
Basement membrane
Antrum
Theca interna
Theca externa
Luteal phase
Collapsed antrum (fibrin and clot)
Theca lutein cells
Granulosa lutein cells

Sperm characteristics

Human sperm quality is usually defined to by standard WHO semen analysis parameters: number, motility morphology

WHO laboratory manual for the examination of human semen and sperm –cervical mucus interaction. 1999 Cambridge University Press

Morphology I

Normal forms

Pyriform

Macrocephalic

Microcephalic

Round

Amorphous

Morphology II

Mid piece anomalies

Tail anomalies

Effects of maternal age on the oocyte developmental competence

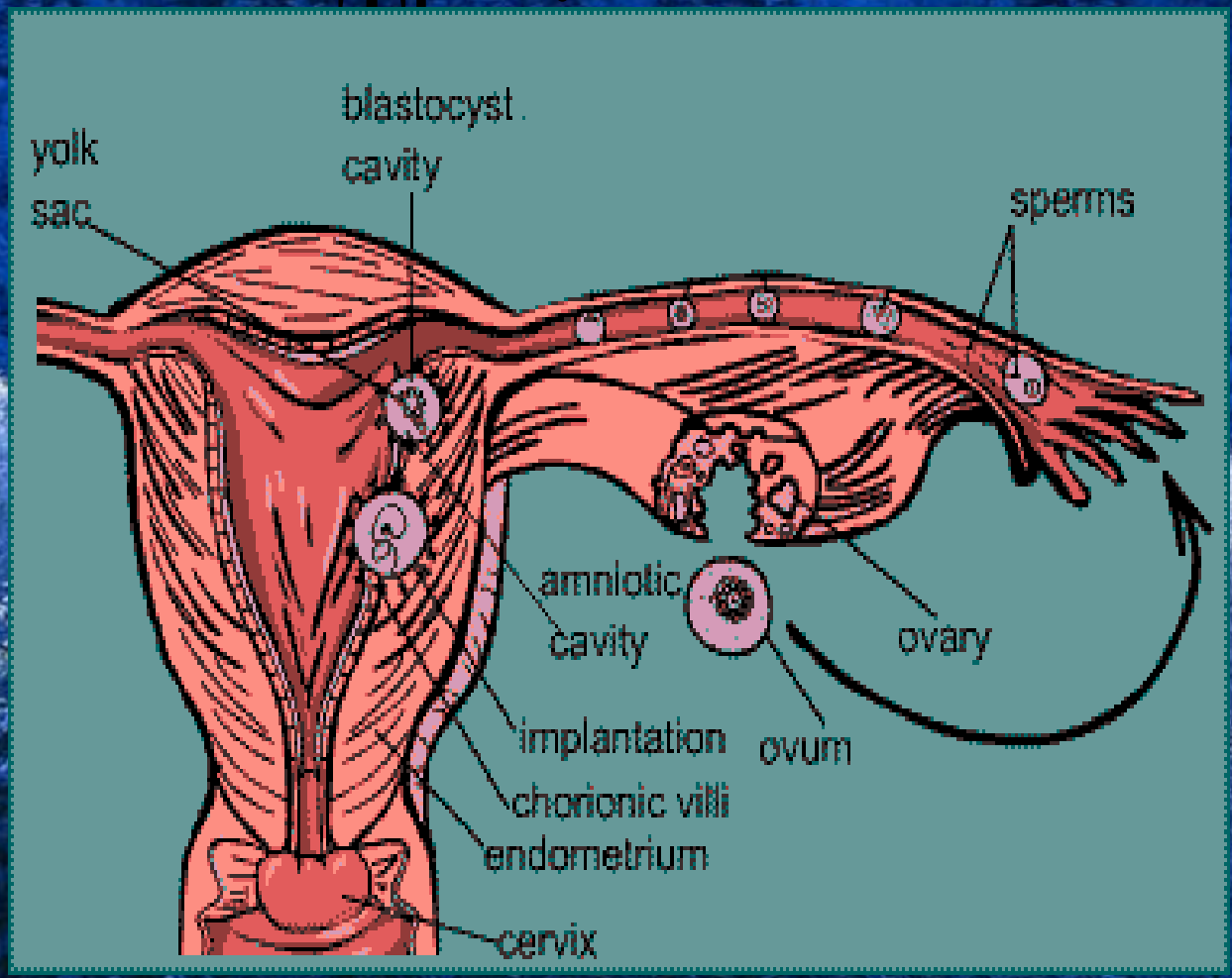
- Meiotic incompetence (effects on fertilisation)
- Errors in meiosis (genetic abnormalities)
- Cytoplasmatic deficiencies (anomalies at different stages of development (before or after fertilisation)
    - ***Armstrong DT Theriogenology 55, 1303-22, 2001***

Male fertility declines with age?

- French group found a negative correlation between the fertilisation rate and the age of the husband on a oocyte donor program
- Fertilisation rate for men less than 39 years old was 60,2 % and only 51,3% for men over 39.

Risk of miscarriage and age

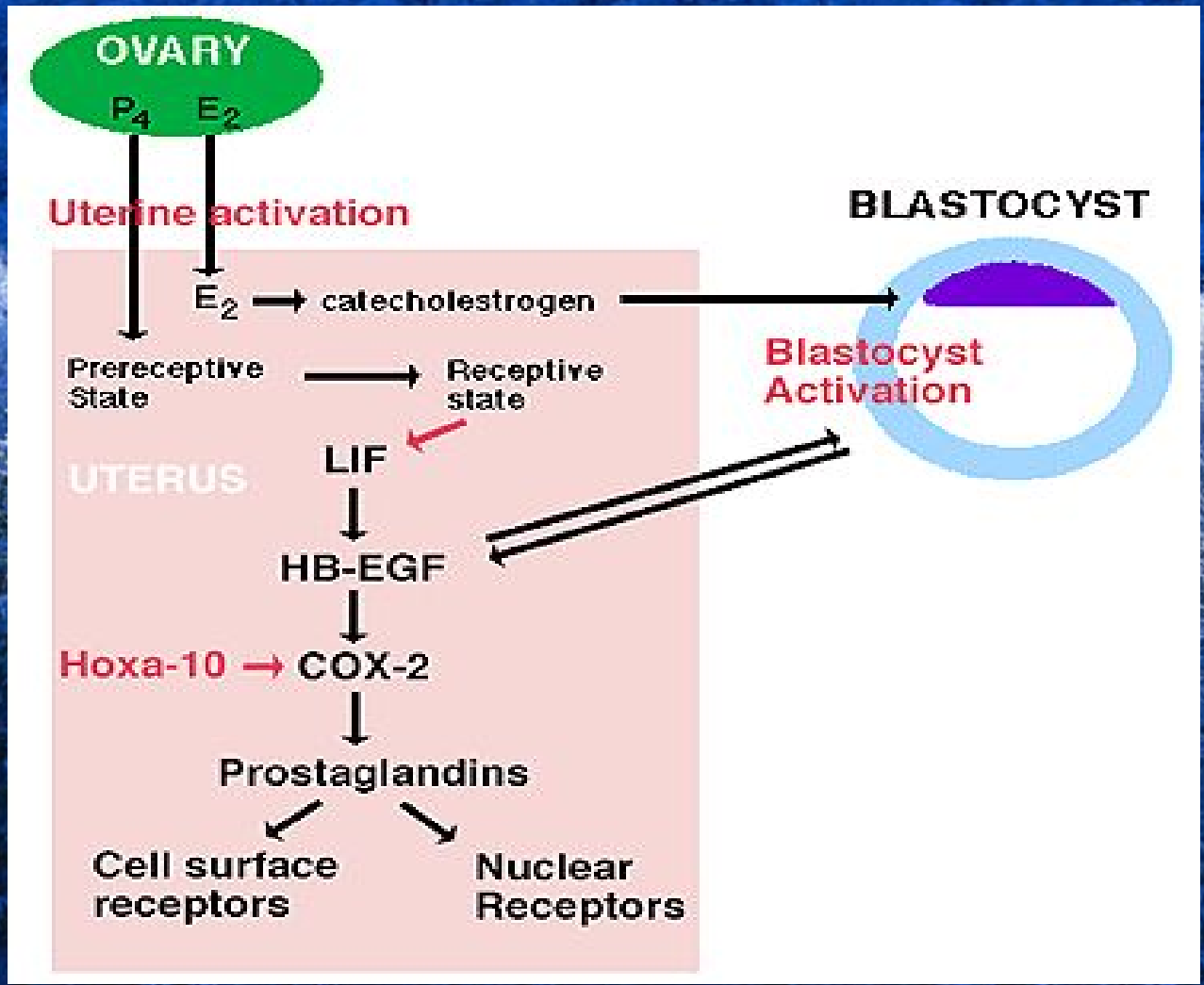
- **Fetal loss is the possible destiny of 13 % of clinical pregnancies.**
- **At 42 more than half of pregnancies result in fetal loss.**
- **The risk of spontaneous abortion is 8.9% in the age group 20-24 and 74% in those aged 45 or more**
  - ***Andersen et al. BMJ 320,1780-1712, 2000***


blastocyst
cavity
yolk
sac
sperms
amniotic
cavity
ovary
implantation
ovum
chorionic villi
endometrium
cervix

Implantation and age

- Still matter of some controversy even if the «oocyte factor» seems determinant.
- The lack of knowledge of all the physiological variables that determine a successful nidation makes the analysis of the uterine receptivity difficult.

A matter of communication



Trophoblast control of maternal endocrine functions

In primates and horses, chorionic gonadotropins produced by the trophoblast mantains the ovarian corpus luteum in an active state.

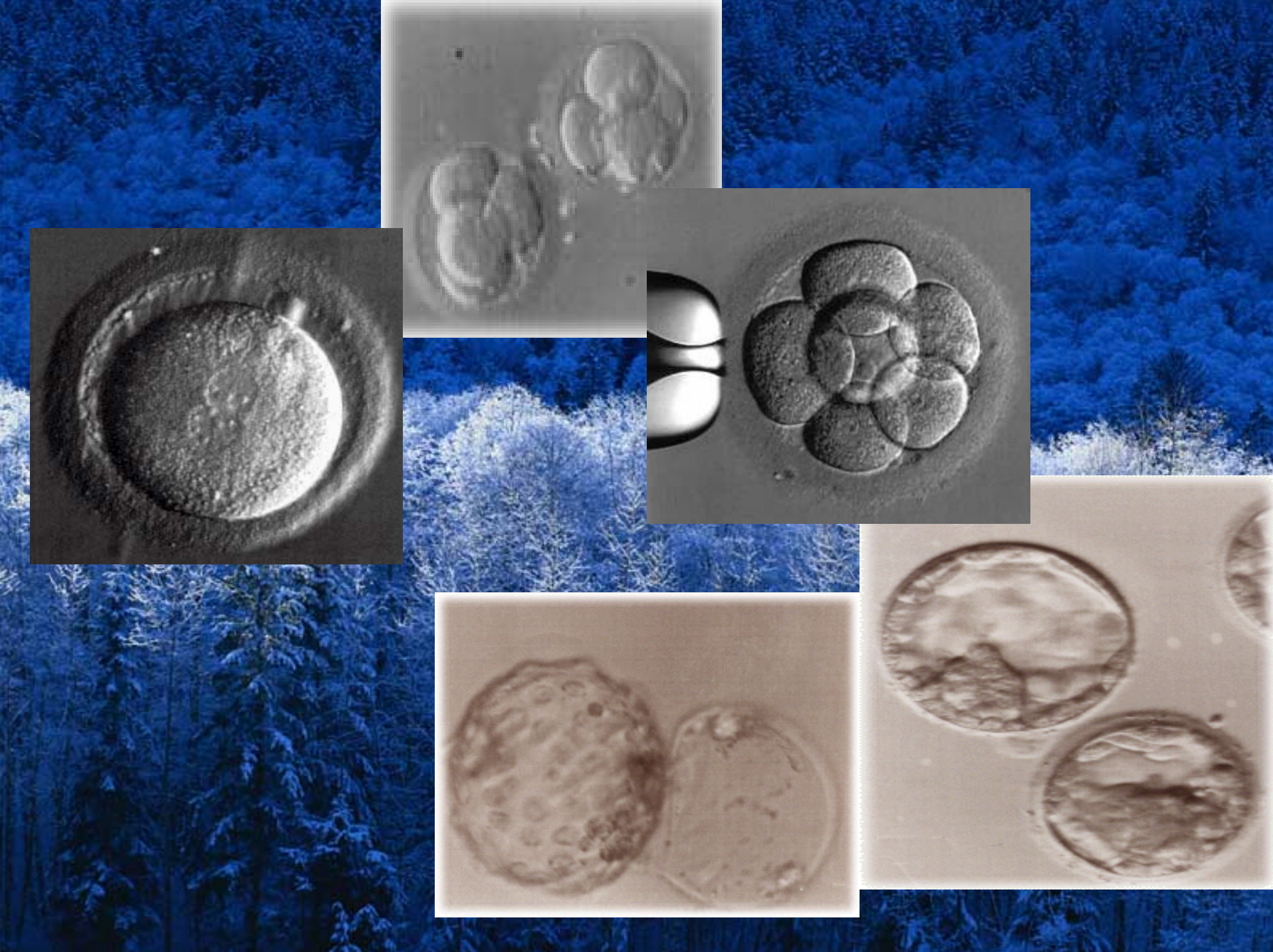
In other mammals the trophoblast also produces somatomammotrophin (placental lactogen)

Transport

The blastocyst arrives in the uterus 132 to 144 hours after fertilisation

At this time the endometrium has undergone a series of changes leading to a period of receptivity called the « window of implantation »

The receptivity is present between the 20 and the 24 day of the cycle



Apposition

- The blastocyst is in contact with the endometrium
  - The embryo can be rinsed out of the uterus

Hatching

- The zona pellucida dissolves possibly because of the secretion of proteases by trophectodermal cells

Orientation

- The inner cell mass is orientated towards the endometrial epithelial lining
- Architectural changes concern ephitelial glands (increase of secretory aspect, vacuolisation and decrease of mitotic activity). Stroma becomes oedematous around day 21
- Electron microscopy studies have shown the presence of pinopods between day 19 and 24 of the cycle

Molecular changes at the endometrial level

- Expression of molecules on cell surface changes according to receptive or non receptive status
  - Mucins (glycocalyx)
  - MUC-1 in humans
  - Integrins, selectins, cadherins and immunoglobulins

Adhesion

- **Connections are established between the embryo and the endometrium**

Invasion

- Penetration of trophectoderm between epithelial cells

Digestion

- Integrins bind the trophoblast to the basal membrane this triggers the activity of proteases to digest the membrane.

Formation of the syncytium-trophoblast and villous formation

- Appearance of the syncytium and invasion of the extracellular matrix
- Migration of cytotrophoblast and of the fetal stroma inside the syncitium
  Appearance of villi
- Proliferation is tightly regulated

  (TGFb1 probably blocks the secretion of proteases, inhibitors are also present)

Placental control of the maternal immune system

The fœtus is an allograft

Selective inhibition of the maternal immune system

- T-cells are in a reversible tolerant state
- Expression of HLA G
- Specifically on cytotrophoblast

Non selective inhibition

- Progesteron
- aFP
- PL
- Activin
- Inhibin
- Interleukin 1b, 6 and 10

Metabolic changes

- The syncythiotrophoblast expresses the metabolic enzyme indoleamine 2,3 dioxygenese (IDO)
- This probably establishes a microenvironment that blocks T-cell proliferation or function

The end of implantation….. birth

- The onset of labor is controlled by high estrogen/progesteron ratios and secretion of oxitocin from the pituitary.

- Syntesis and secretion of prostaglandins and collagenases also contribute to the onset