

veterinary
EMBRYOLOGY

fertilization

LUCIA M. RIGOS, DVSM, PhD

Associate Professor

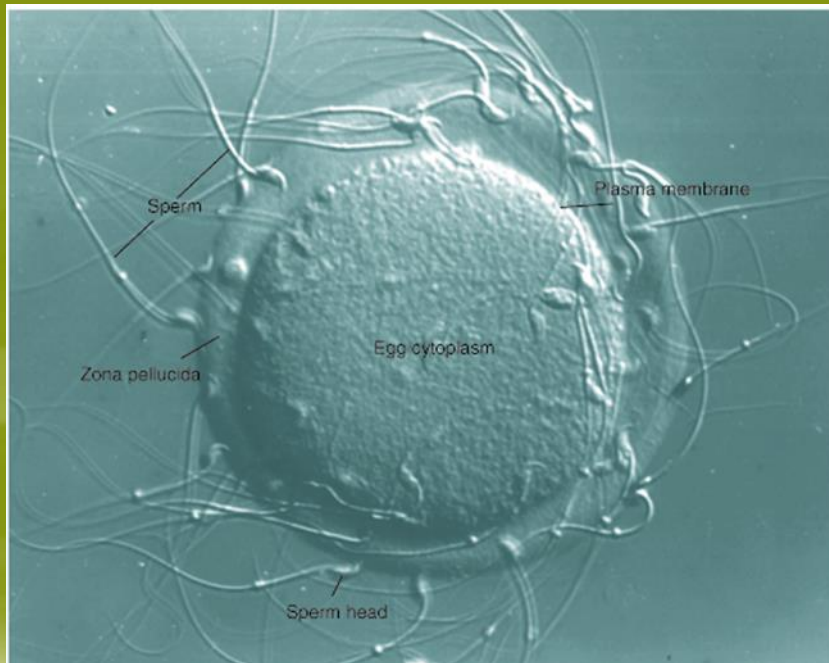
Department of Morphophysiology and Pharmacology

College of Veterinary Science and Medicine

Central Luzon State University

Fertilization

Union of male female gametes/sex cells



But before fertilization, mating or copulation must occur

- Mating can be natural, artificial or in vitro.
- **mating**—sexual intercourse of a male and female with the deposition of semen in the female reproductive tract



Artificial Insemination (AI)

- can be the classic AI and observing the occurrence of estrus or Fixed Time AI (FTAI) via hormonal intervention. Monitoring/observation of estrus is no longer done/problem because AI is at a fixed time



In In-Vitro fertilization

- Ovum are picked up (OPU) via guided Ultrasonography
- Ovum aspirated from the collected ovaries from abattoir /slaughterhouse



Fertilized in the laboratory



Transferred into a recipient animal

Sperm deposition



Species	Site of spermatozoa deposition in the female reproductive tract
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Cat	Vagina
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Dog	Uterus
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Cattle	Vagina
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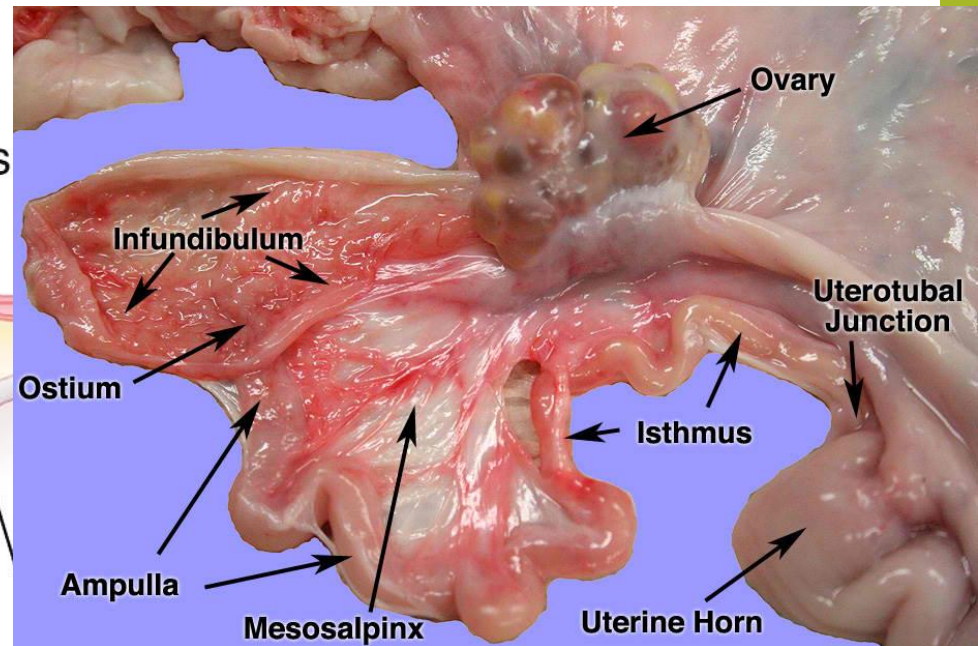
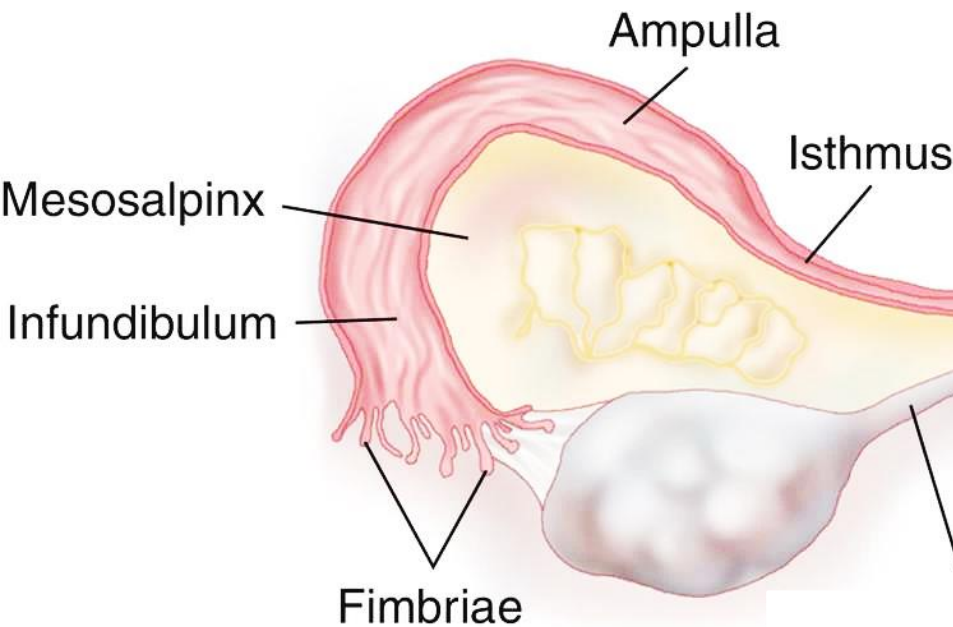
Horse	Uterus
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Sheep	Vagina
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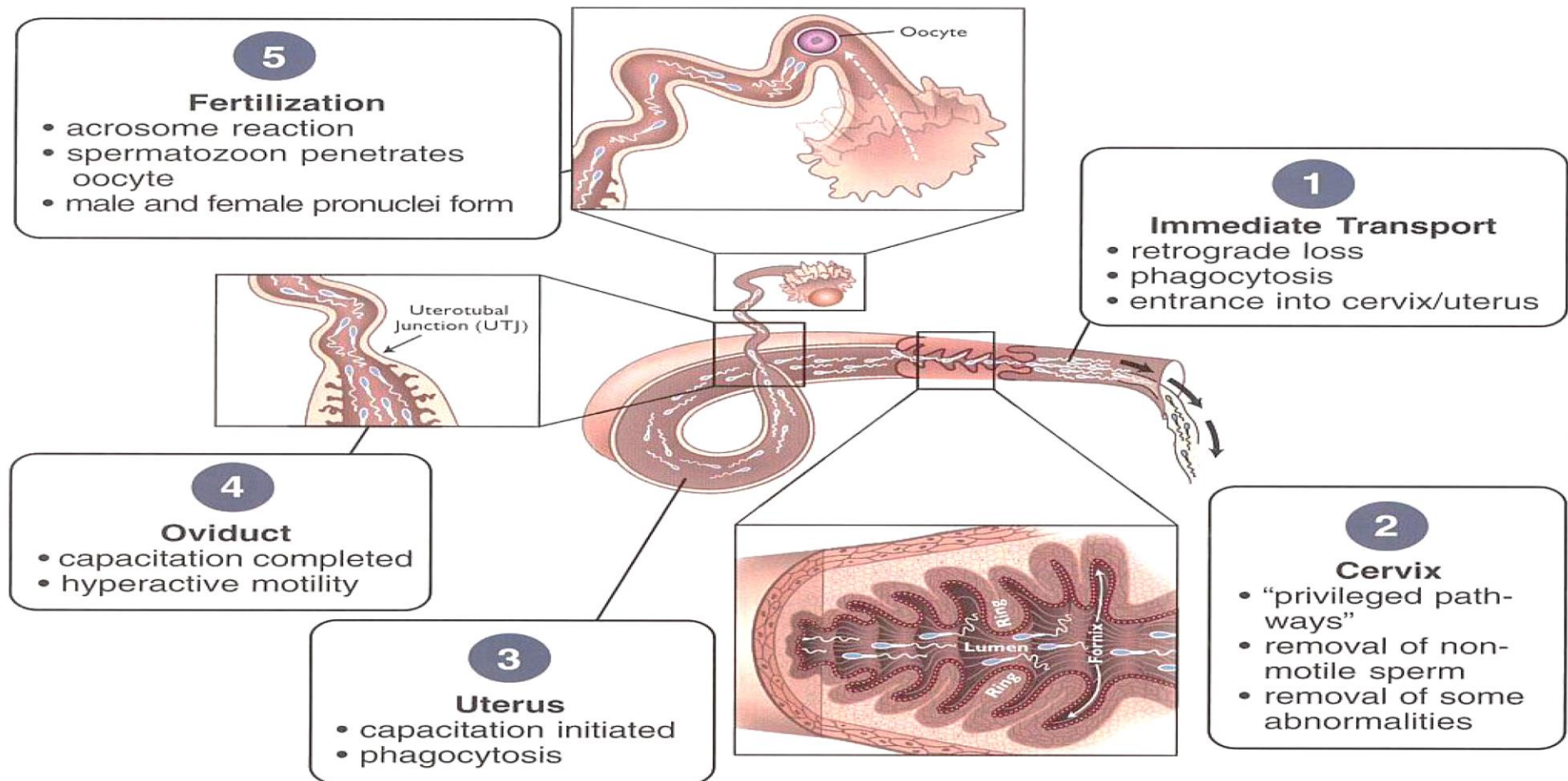
Pig	Cervix/Uterus
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Fertilization

- Takes place in the ampullary region of the oviduct



- Sperm cells travel the female reproductive tract to meet the egg cell. However, only one lucky sperm wins the ovum.....*
- Sperm cells as they travel the segments of female tract decrease in number due to environmental conditions in each specific segments of the tract.*



Sperm transport

- Can be divided into three phases:

RAPID TRANSPORT PHASE

Delivers spermatozoa to the fertilization site very shortly after copulation (15-30 mins)

Slow/SUSTAIN ED TRANSPORT PHASE

Delivers spermatozoa to the ampulla in a more uniform manner over a sustained period of time (6-12 hrs)

Intercornual transport

Movement of sperm cells from one horn to another (usually during AI)

During the sperm transport

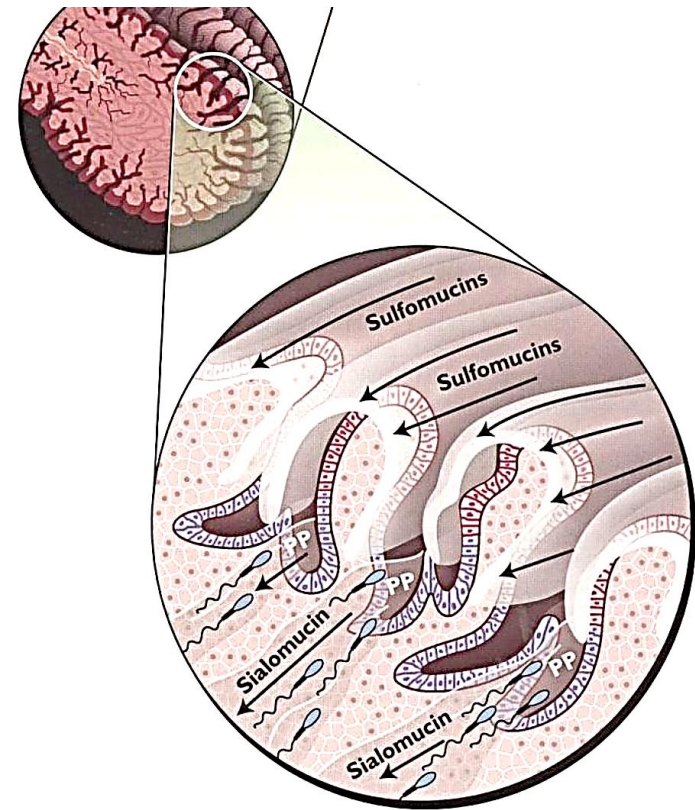
- Cervix produces mucus rich in:

Sialomucin

- **Low** in viscosity
- Produced by cells in the **basal** areas of the cervical crypts

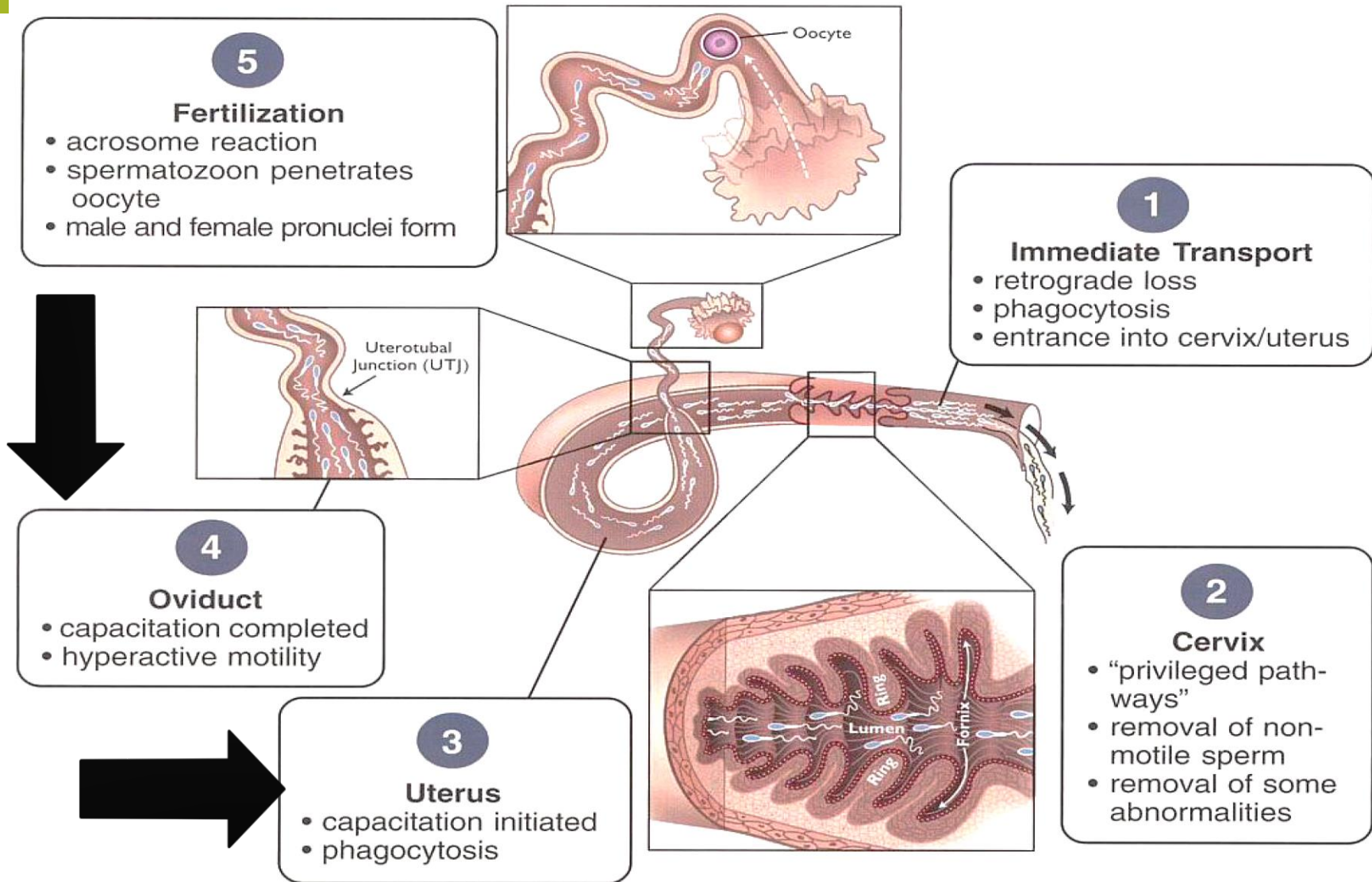
Sulfomucin

- **Viscous**
- Produced in the **apical** portions of the cervical epithelium



- Less viscous mucus favors sperm transport as they can easily swim

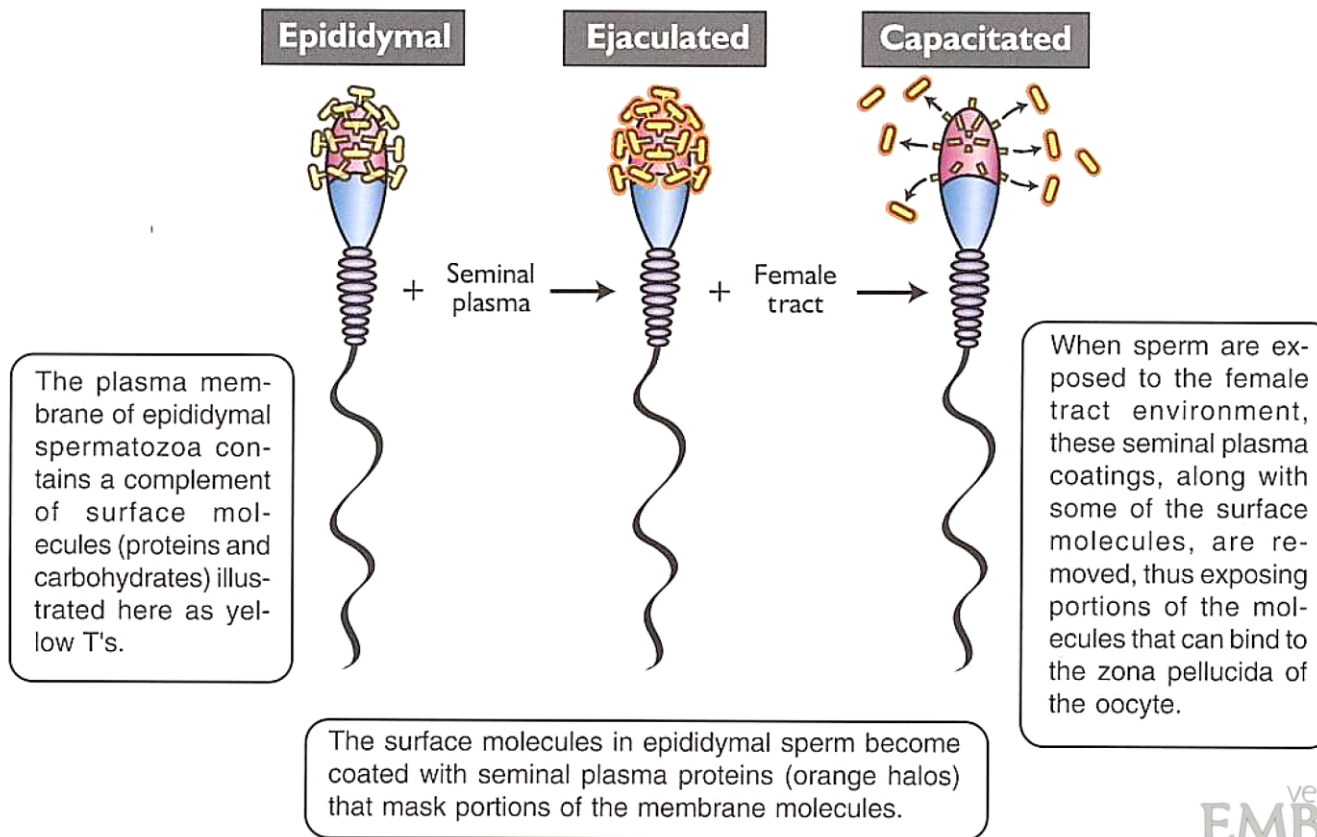
fertilization



- Sperm cells swim up the ovum while the ovum moves down and meet the sperm cells in the ampulla of the oviduct

But the sperm cells have to undergo **CAPACITATION**

- a biochemical process that occurs in the female tract whereby spermatozoa acquire the capacity to fertilize the egg.

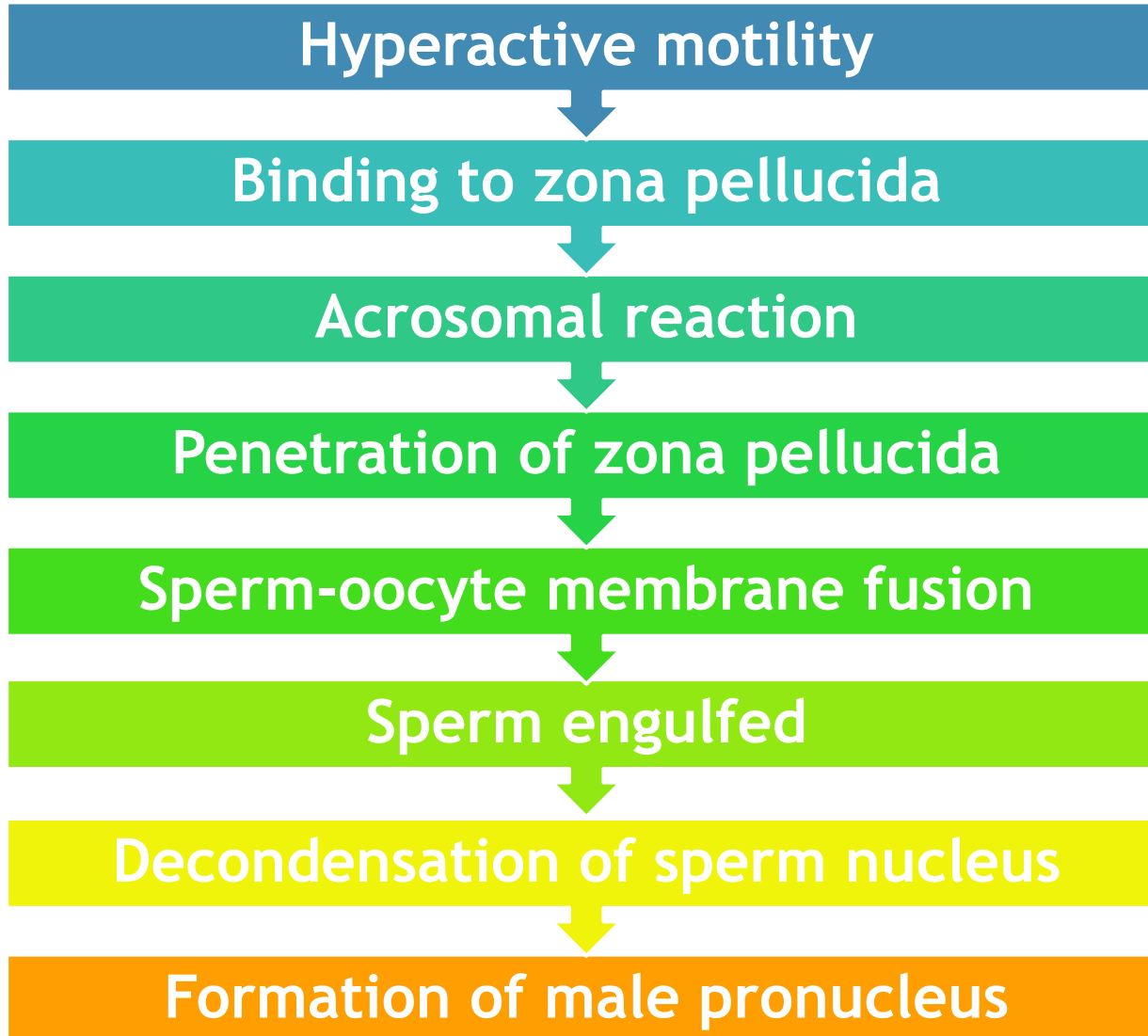


capacitation

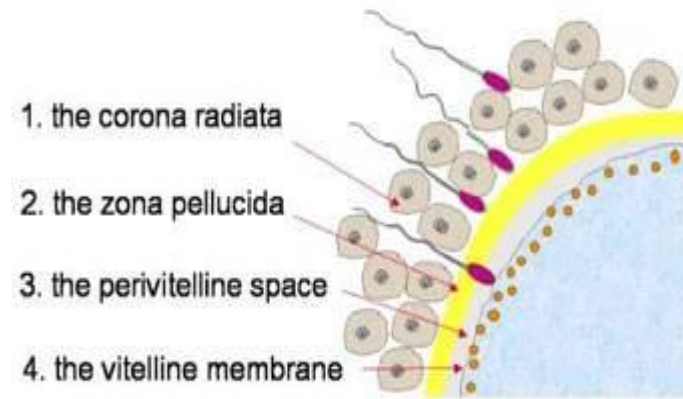
- If spermatozoa are deposited in the vagina, cervix or uterus (semen deposition varies among species)
- ❖ Capacitation may begin through the **cervix , uterus and isthmus respectively.**

POST-CAPACITATION

After capacitating, sperm cells undergo the foregoing events



Zona binding

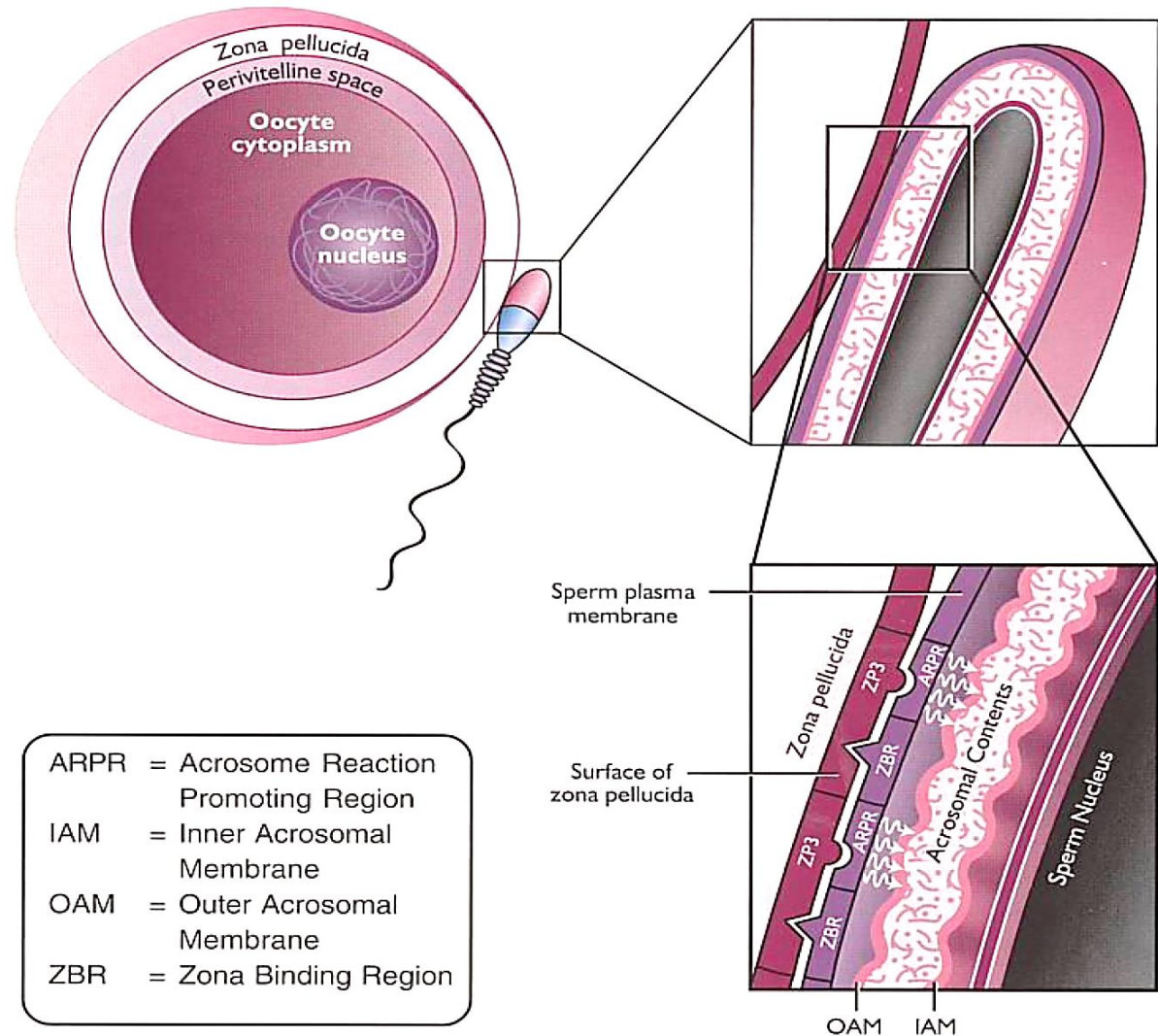


- **ZONA PELLUCIDA**
 - Consists of three glycoproteins:
 - **ZP₁** and **ZP₂**- structural proteins
 - **ZP₃**- receptor for sperm
- **Two zona binding sites**
 - Primary zona binding region
 - Acrosome reaction promoting ligand

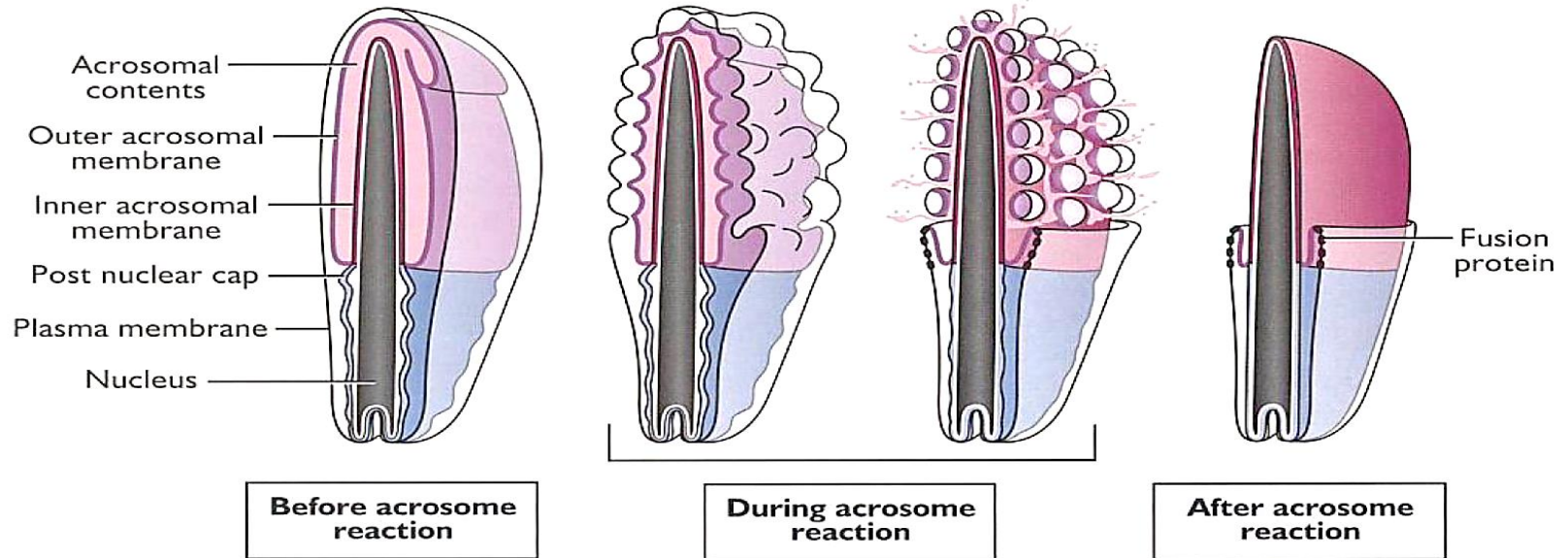
Zona binding

Proposed model for zona binding and the initiation of the acrosomal reaction in mammalian spermatozoa. The sperm plasma membrane overlying the acrosome contains two receptor-like regions. The first, called the zona binding region (ZBR), reacts with ZP3 to cause physical attachment of the sperm to the zona pellucida. A second membrane region, the acrosome reaction promoting region (ARPR), also binds to ZP3 and initiates the acrosome reaction by causing the sperm plasma membrane to fuse (arrows) to the outer acrosomal membrane.

ARPR = Acrosome Reaction Promoting Region
 IAM = Inner Acrosomal Membrane
 OAM = Outer Acrosomal Membrane
 ZBR = Zona Binding Region



Acrosomal Reaction



Before Acrosomal Reaction

Before the reaction begins, all membranes of the head are intact.

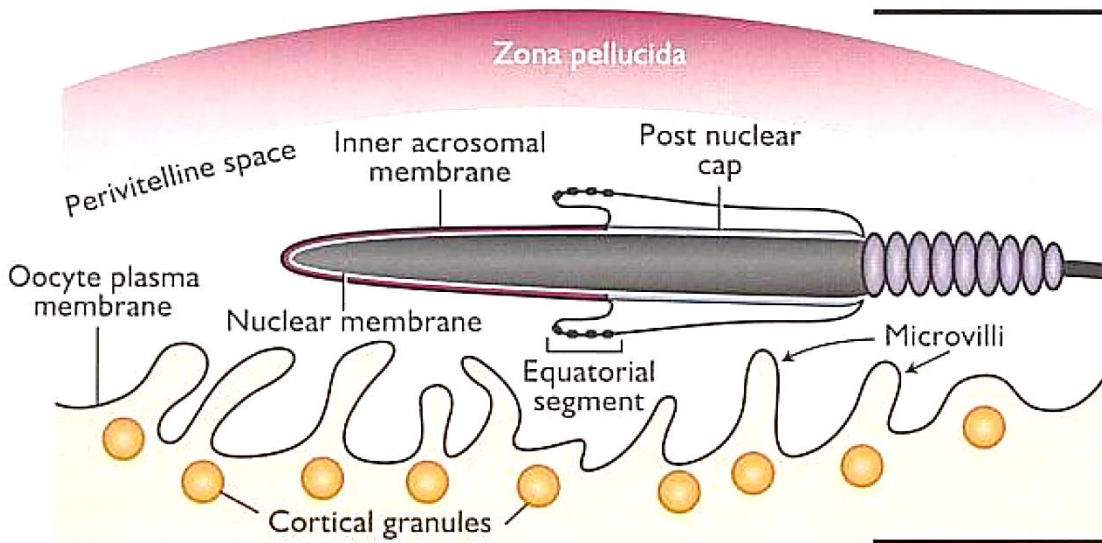
During Acrosomal Reaction

During the reaction, the plasma membrane overlying the acrosomal membrane begins to fuse with the outer acrosomal membrane. The fusion of the two membranes leads to vesiculation that creates pores through which the acrosomal enzymes can pass. This allows the sperm to penetrate through the zona pellucida.

After Acrosomal Reaction

After the reaction, the vesicles are sloughed, leaving the inner acrosomal membrane, the equatorial segment and the post nuclear cap intact.

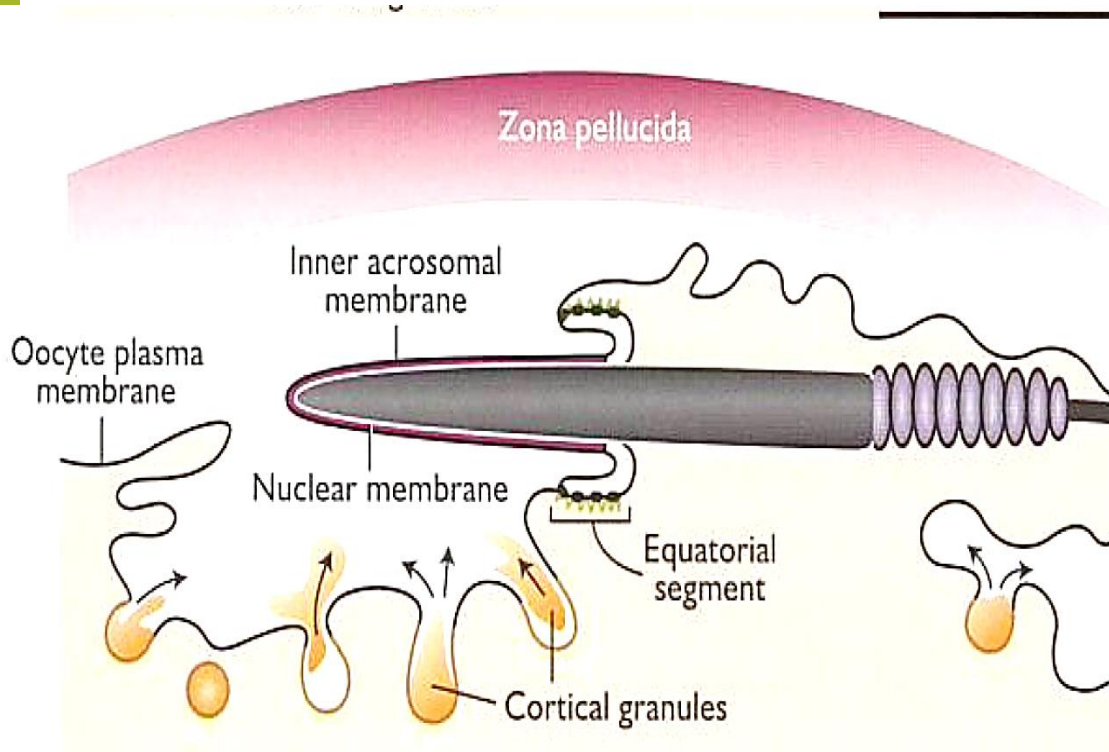
Sperm - oocyte fusion



**Before
membrane
fusion**

When the spermatozoon completely penetrates the zona and reaches the perivitelline space, it settles into a bed of microvilli formed by the oocyte plasma membrane. The cortical granules have migrated to the periphery of the oocyte.

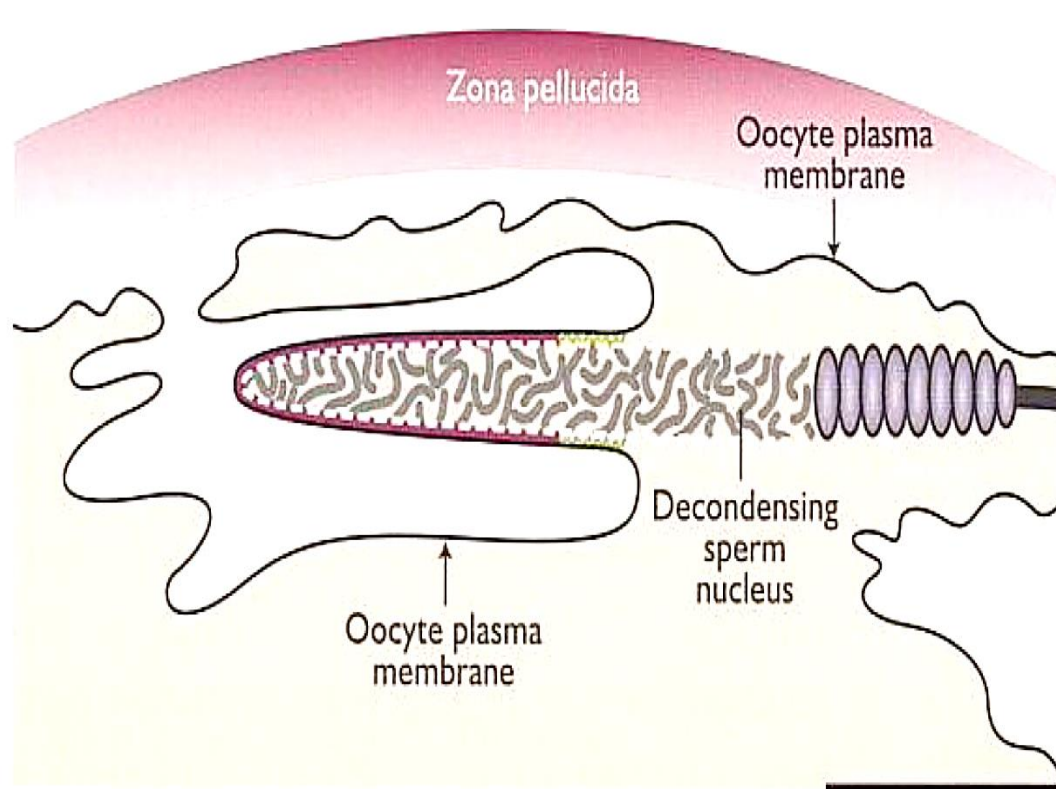
Sperm – oocyte fusion



During membrane fusion

The plasma membrane of the oocyte fuses with the equatorial segment and the fertilizing spermatozoon is engulfed. The cortical granule membrane fuses with the oocyte plasma membrane and the cortical contents are released into to perivitelline space by exocytosis.

Sperm –oocyte fusion



**After
membrane
fusion**

After the fusion between the membrane of the equatorial segment and the oocyte plasma membrane occurs, the nucleus of the spermatozoon is within the cytoplasm. The sperm nuclear membrane disappears and the nucleus of the sperm decondenses.

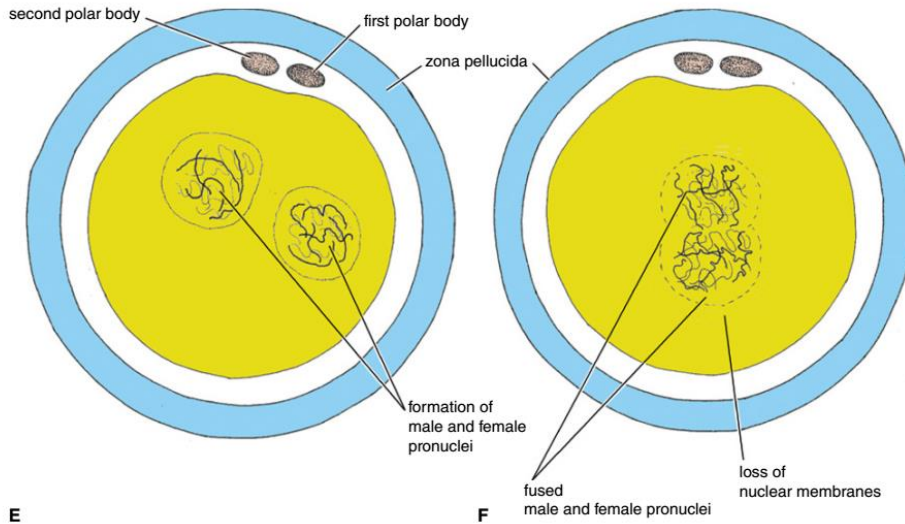
Cortical reaction

- Calcium-triggered reaction of the oocyte cell membrane
- Small, dense granules called **cortical granules** undergo exocytosis and their contents are released into the perivitelline space

Block to Polyspermy

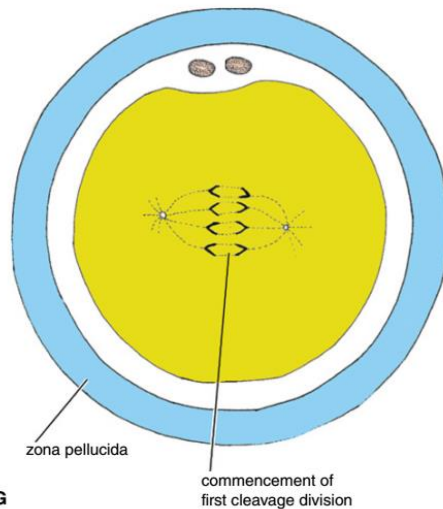
- **ZONA BLOCK**
 - A process whereby the zona pellucida undergoes biochemical changes so that no further sperm can penetrate the zona
- **VITELLINE BLOCK**
 - Reduction of the ability of the oocyte plasma membrane to fuse with additional spermatozoa

Syngamy



E

F



G

The fusion of the male and female pronuclei, fertilization is done

Assisted Reproductive Technology (ART)

- technology used to achieve pregnancy in procedures such as artificial insemination, in vitro fertilization and surrogacy
- Artificial insemination - introduction of sperm into the female's uterus or cervix for the purpose of achieving pregnancy through in vivo fertilization by means other than sexual intercourse

In Vitro Fertilization - union of egg and sperm in vitro

Subsequently the embryos are transferred into the uterus through the cervix for pregnancy to commence.

GAMETE INTRA FALLOPIAN TRANSFER (GIFT) – a tool of assisted reproductive technology against infertility.

Eggs are removed from a female ovaries and placed in one of fallopian tubes , along with the male sperm

ZYGOTE INTRAFALLOPIAN TRANSFER (ZIFT) –used when a blockage in the fallopian tubes prevents the normal binding of sperm to the egg.

Egg cells are removed from a female ovaries, and in vitro fertilized.

The resulting zygote is placed into the fallopian tube by the use of laparoscopy.

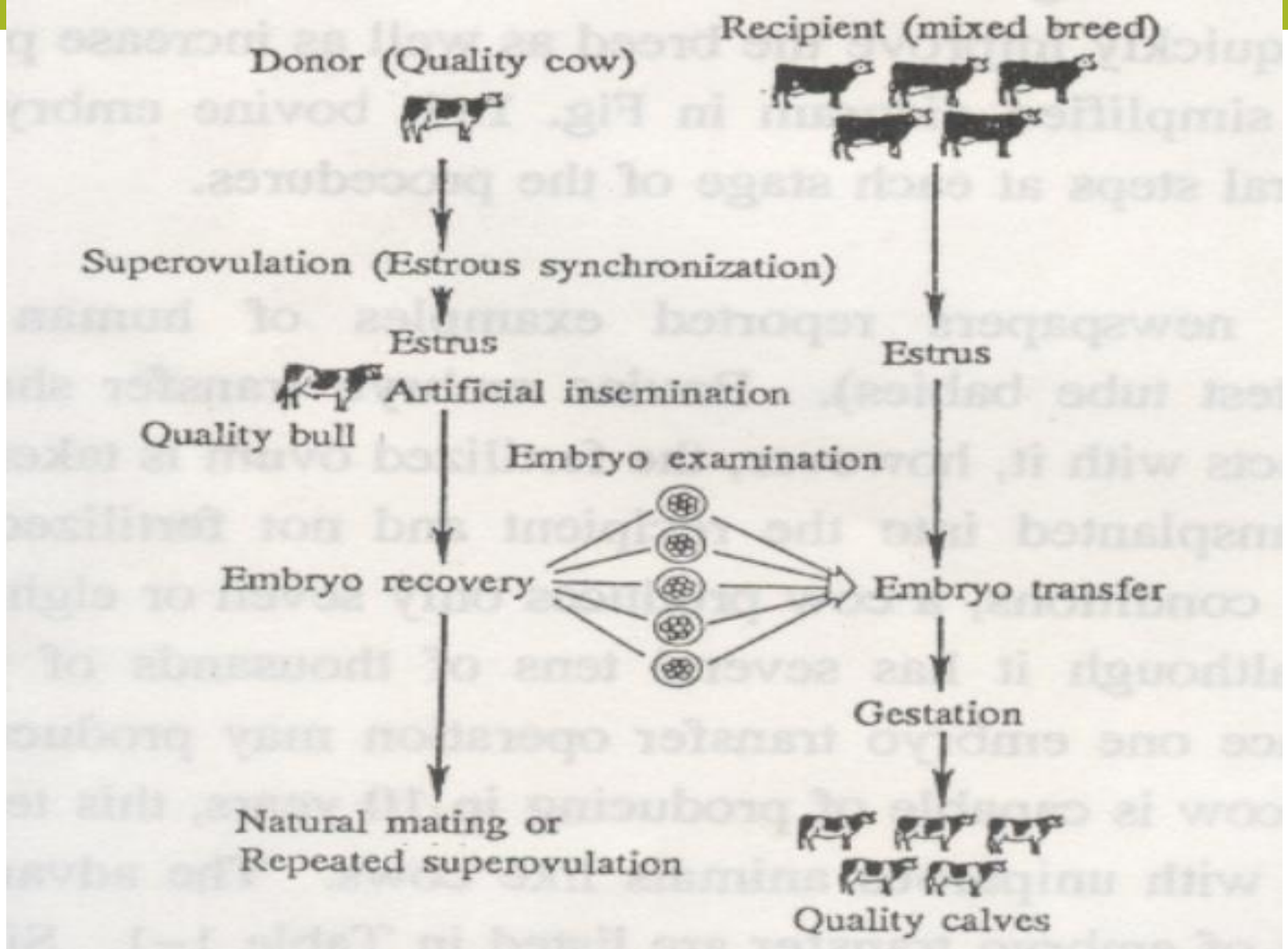
INTRA CYTOPLASMIC SPERM INJECTION (ICSI) – Sperm is injected directly into the eggs in a laboratory.

Used if infertility originates from the male such as: – Low numbers of sperm – Low sperm motility

Single spermatozoan is directly injected into the cytoplasm of the oocyte through a micropuncture of zona pellucida..

Embryo transfer –

- **an** assisted reproduction technology in which **embryos** are placed into the uterus of a recipient female (surrogate) to establish a pregnancy
- Accompanied by hormone treatment to induce multiple ovulation (MOET)



A Multiple Ovulation-Embryo Transfer Scheme

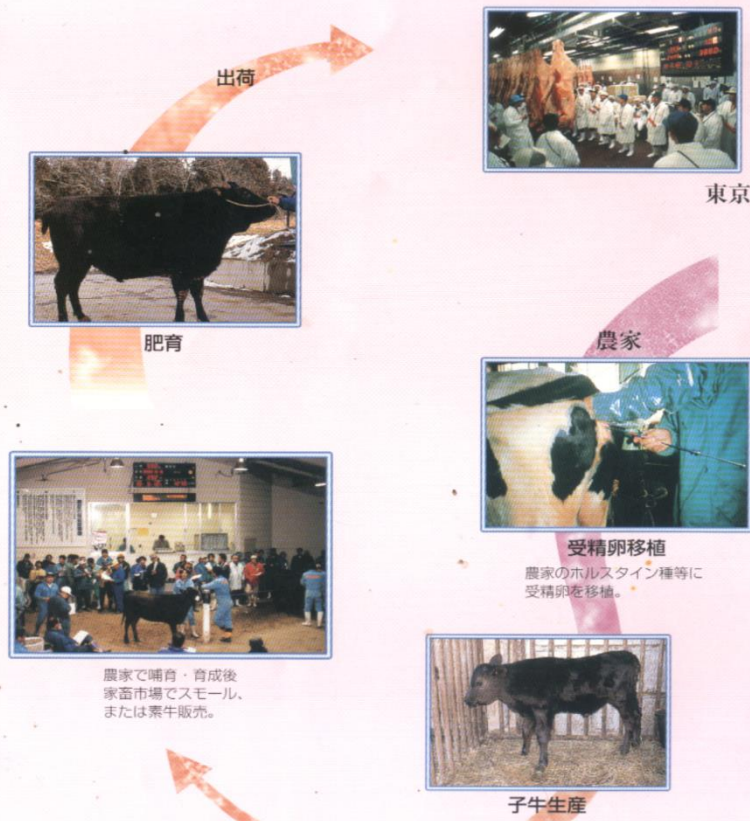
Advantages of MOET

- Shorter period of breed improvement
- Improvement of stock quality
- Preservation of superior gene quality
- Multiplication of rare or endangered species
- Lower transportation/importation cost
- Contribution to the study of sterility or low fertility
- Increase rate of twinning or multiple birth

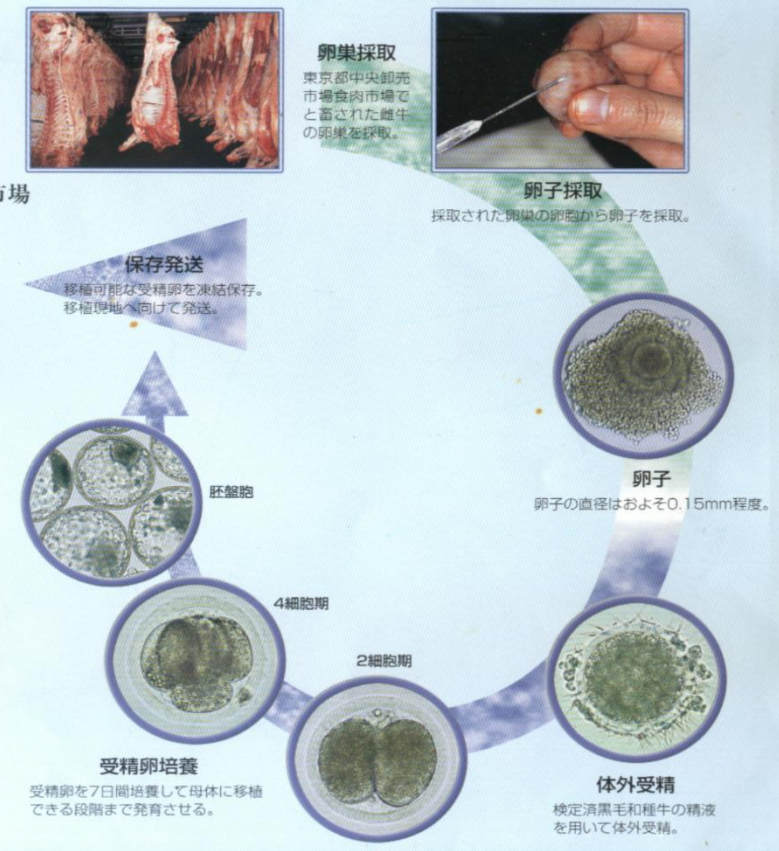
Disadvantages of MOET

- Technique is complex
- Expensive
- Adverse effects of hormones (Ab devt.)
- Dystocia (difficult labor)
- Injury from surgical approach
(adhesion, scarring, death by anesthesia)
- Neglect of animal welfare
- Possibility of international misconduct

体外受精卵産子の生産と肥育



体外受精卵の生産

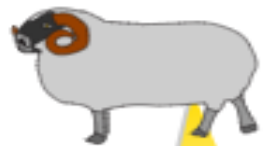


In-Vitro Fertilization- Embryo Transfer (IVF-ET)

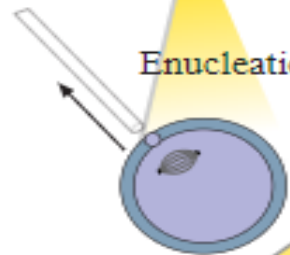
CLONING/Somatic Cell nuclear Transfer (SCNT)

- Removal of the nucleus of the oocyte
- injection/implantation of a diploid nucleus of a somatic cell
- activation of the master genes that have been shut down in the original somatic cell
- Creation of a new but the same organism

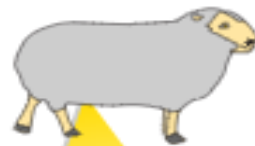
Scottish Blackface
(Cytoplasmic Donor)



Enucleation



Finn-Dorset
(Nuclear Donor)



Mammary Cells

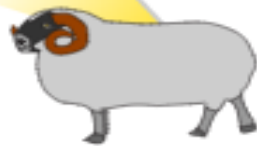


Direct Current Pulse

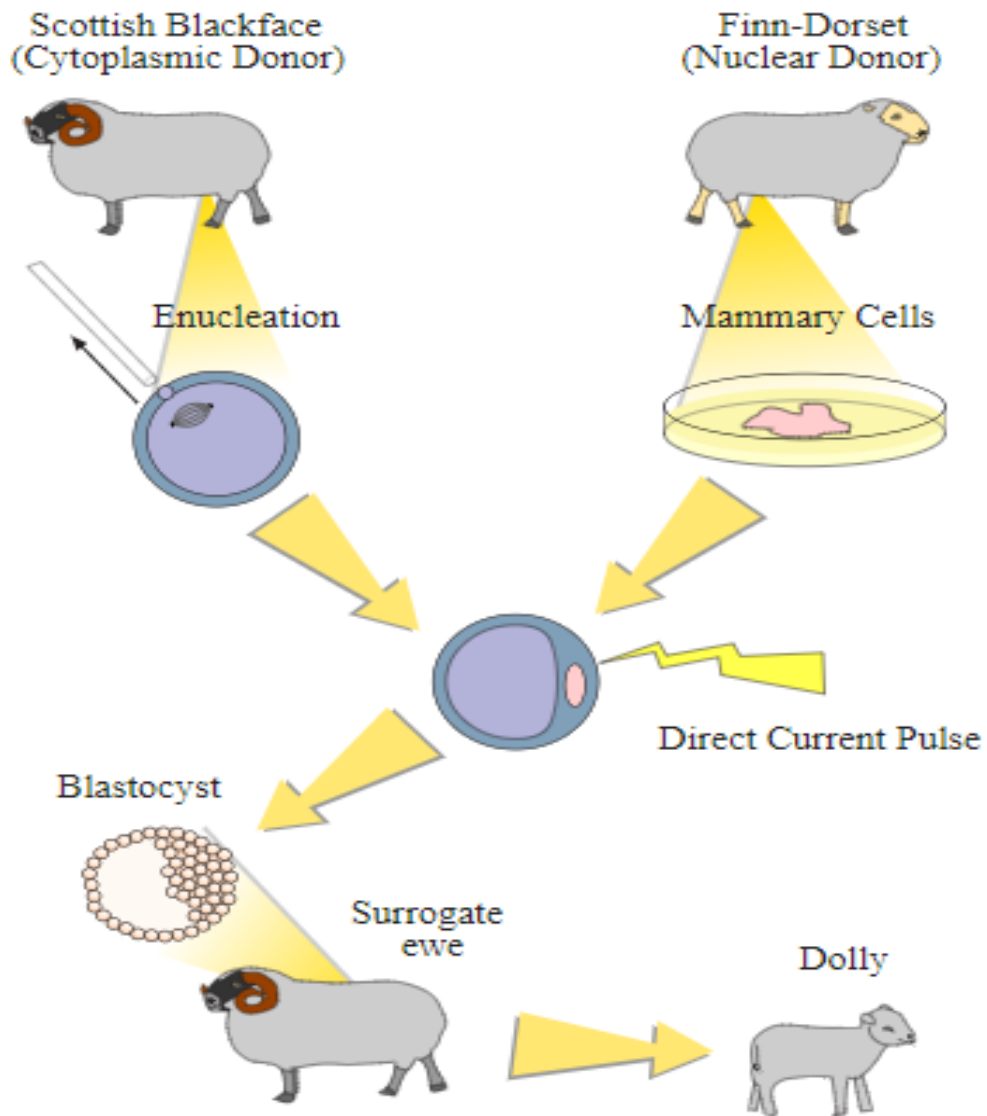
Blastocyst



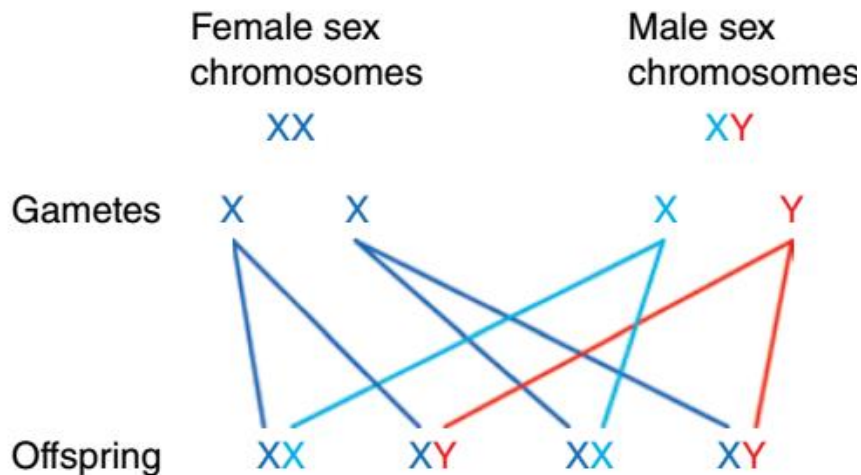
Surrogate
ewe



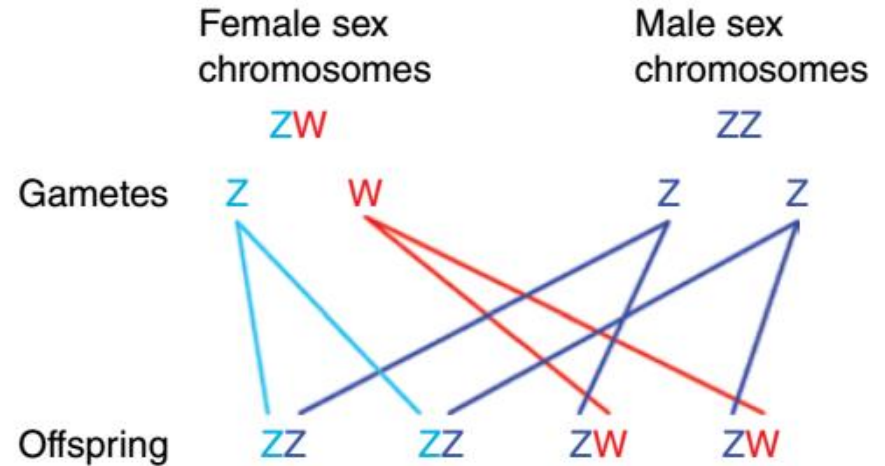
Dolly



Sex determination



Mammals



Avians

- In mammals, the females are homozygous while the males are heterozygous.
- In avians, it's the reverse. The females are heterozygous while the males are homozygous

KARYOTYPING

- A technique used to identify abnormalities in chromosomes
 - Additional autosome (47 chromosomes)-
Down Syndrome
 - XXY- Klinefelter Syndrome
 - XO- Turner Syndrome

end... Thank you!

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