



Mouse and rat reproduction: manipulating the mouse embryo

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**R
a
t
s**



kangaroo rat



cotton rat



Norway rat



black rat



African pouched rat



naked mole rat



wood rat



pack rat

**M
i
c
e**



house mouse



field mouse



deer mouse



smoky mouse



spiny mouse



dormice mouse



kangaroo mouse



white-footed mouse

Rodents: 2.000 different species

Laboratory rats and mice refer to:

- Norway rats (*Rattus norvegicus*)
- house mice (*Mus musculus*)



Rat



Mouse

Do not interbreed.

Norway rats have **22 chromosome pairs**

House mice have **20 chromosome pairs**

Norway rats have **6 pairs of nipples**

Mice have **5 pairs of nipples**

Norway rat **gestation is 21-24 days**

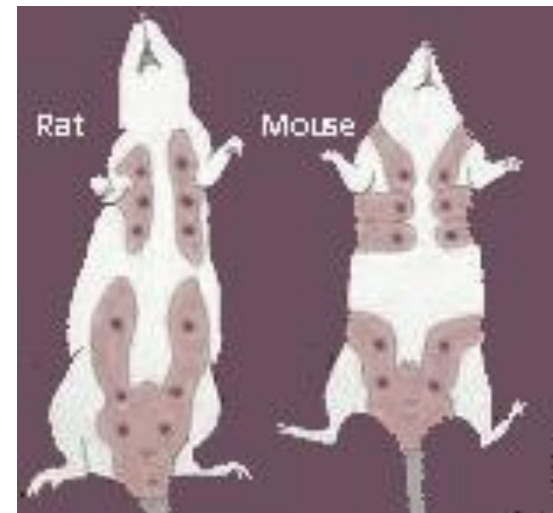
Mouse **gestation is 19-20 days.**

Norway rats **lactate for 3 weeks**

Mice **lactate for 2 weeks.**

Norway rats **open their eyes at 6 days** and have fur at 15 days.

Mice **open their eyes at 3 days** and have fur at 10 days

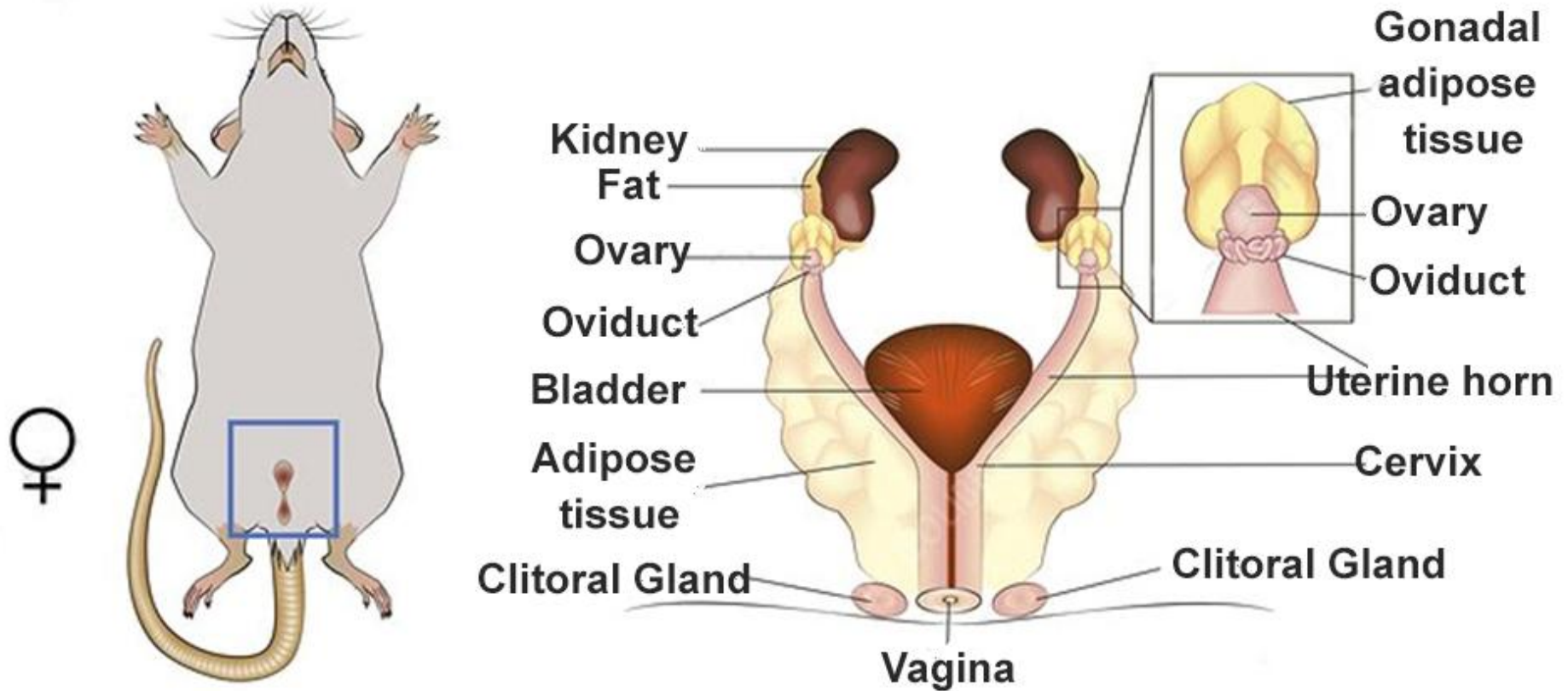


Mouse Reproduction

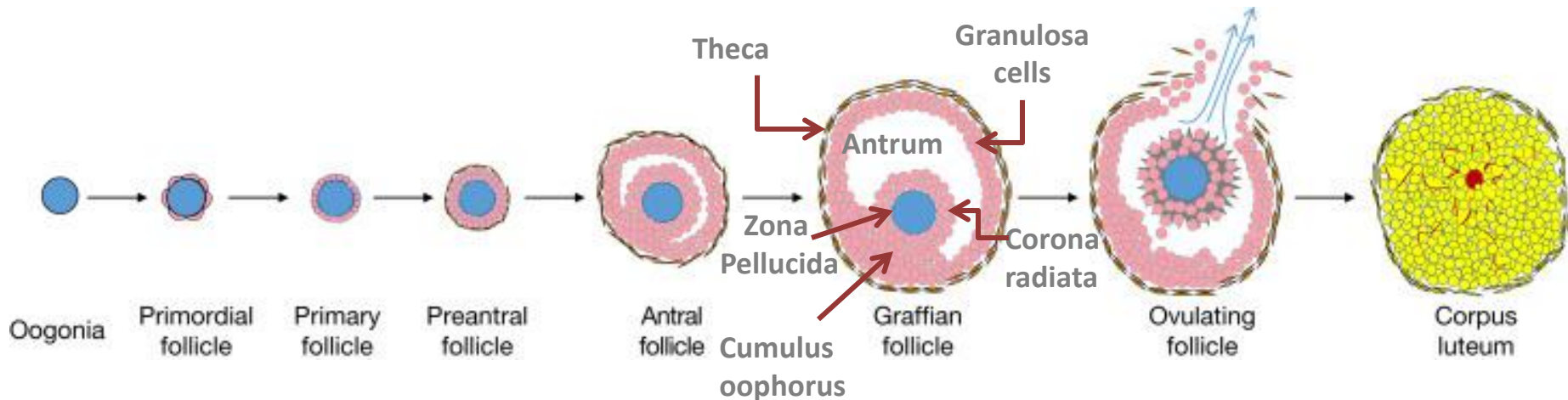
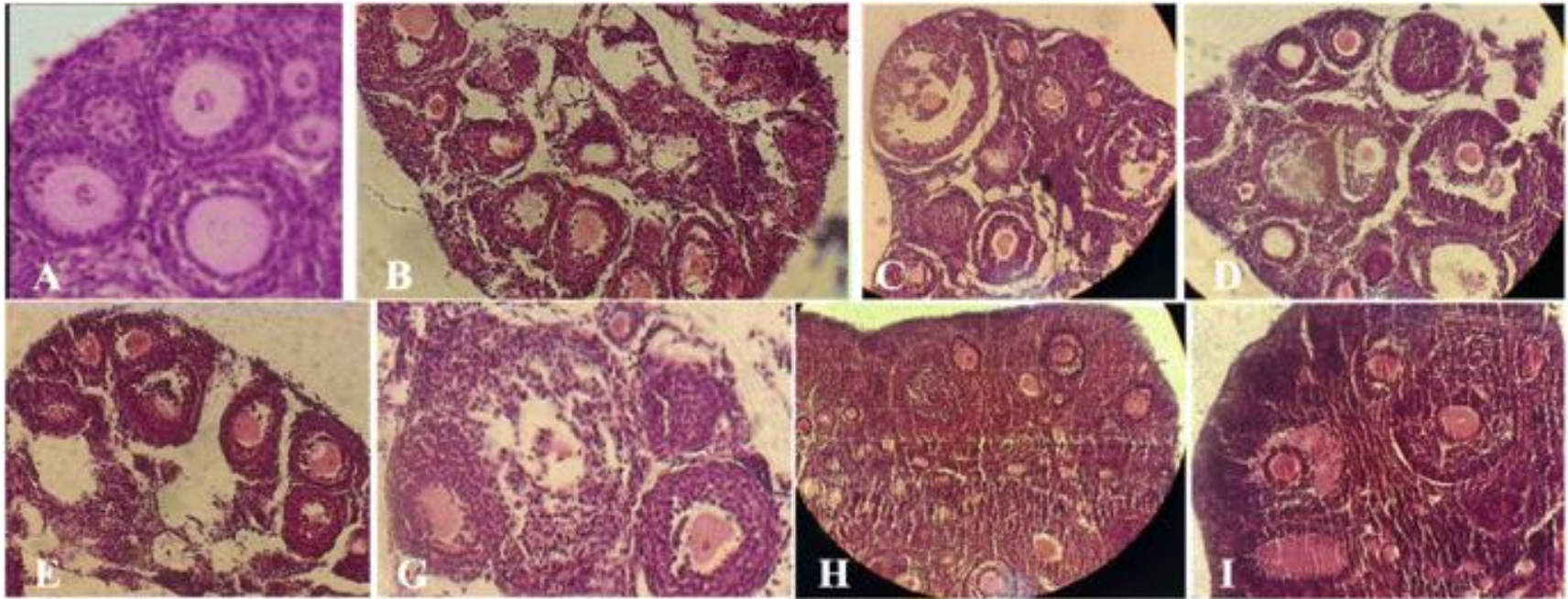
- **Female Reproductive organs**
 - From estrus to pregnancy
 - Oocyte and embryo manipulations
 - Oocyte collection
 - Collection of pre-implantation embryos
 - Post-implantation embryos
 - mid-gestation
- **Male Reproductive organs**
 - Manipulations of male mice
 - Sperm collection
 - Testis
- Superovulation
- Embryo transfer
- Pseudopregnant females
- Vasectomy
- IVF



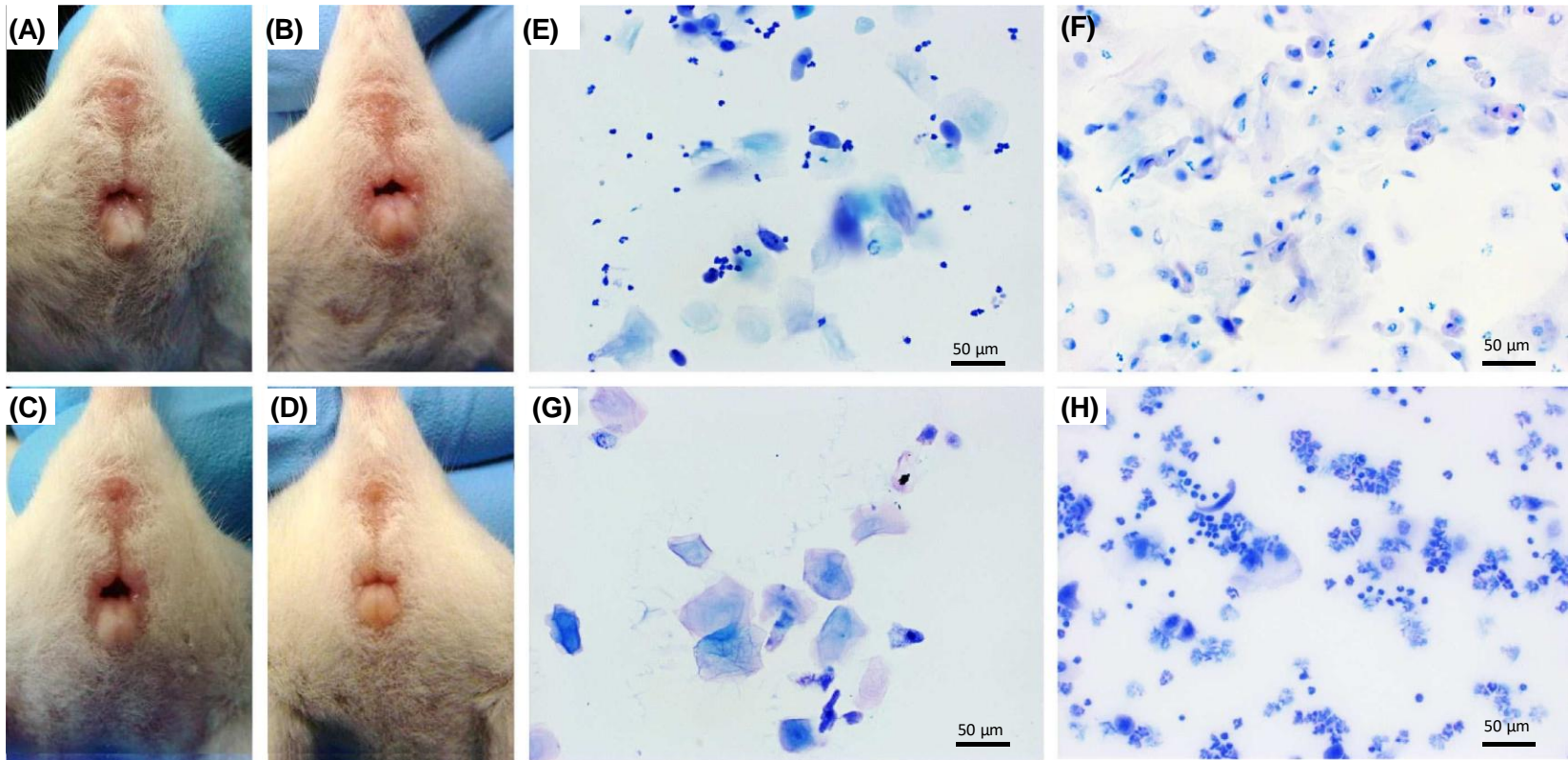
Female reproductive organs



OVARY: Multiple developing follicles



From the ovaries to the vagina and vaginal smear cytology



Usual cycle length in the mouse is 4-5 days.

A and E: Diestrus: the vaginal opening is small, and labia of the vulva are not swollen.

Diestrous cytology is characterized by **smaller polymorphonuclear leukocytes** and a **few larger epithelial cells**.

B and F: Proestrus: the vaginal opening (labia and dorsal commissure of the vulva) is swollen and moist.

Proestrous cytology is characterized by a **predominance of epithelial cells** that are nucleated. A **few cornified epithelial cells** may be present.

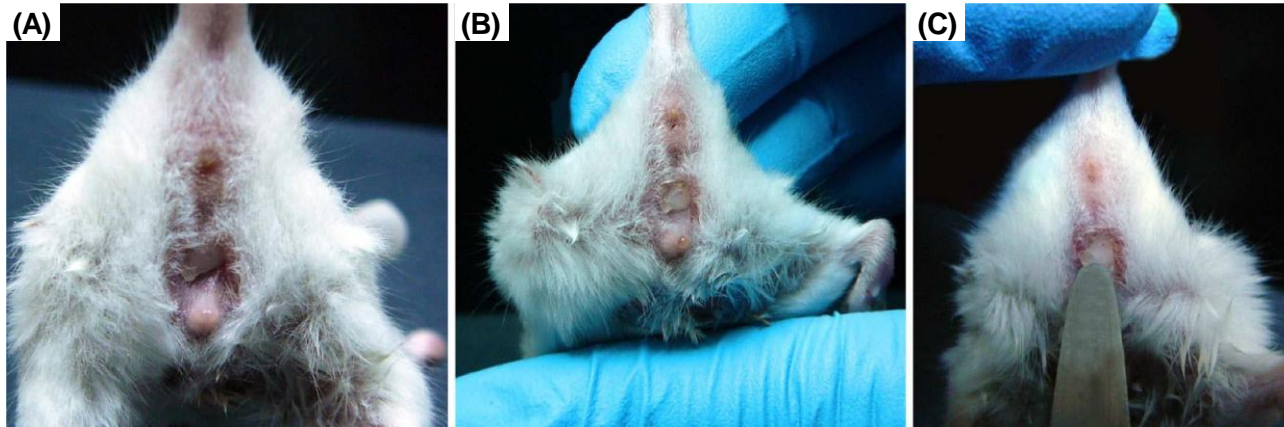
C and G: Estrus: the vaginal opening (lateral labia and dorsal commissure of the vulva) is swollen and the vaginal mucosa is paler and dryer than at proestrus. If whitish soft debris is present on the vaginal mucosal surface, estrus has passed and the female is not receptive to breeding.

Estrous cytology is characterized by the **predominance of cornified epithelial cells**.

D and H: Metestrus: the vaginal opening is small to closed, and the labia of the vulva are not swollen.

Metestrus cytology is characterized by the presence of a **few epithelial cells** and increasing **numbers of polymorphonuclear leukocytes**.

The copulation plug



Following mating, secretions from the reproductive tract of the male laboratory mouse harden in the female's reproductive tract. It is indicative of mating but not of conception or pregnancy.

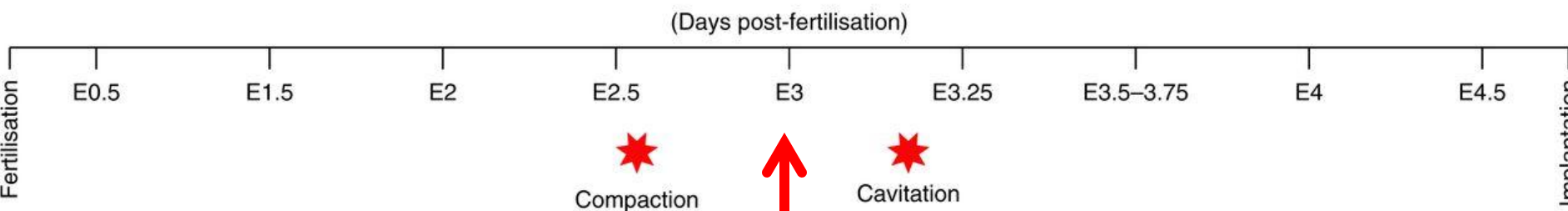
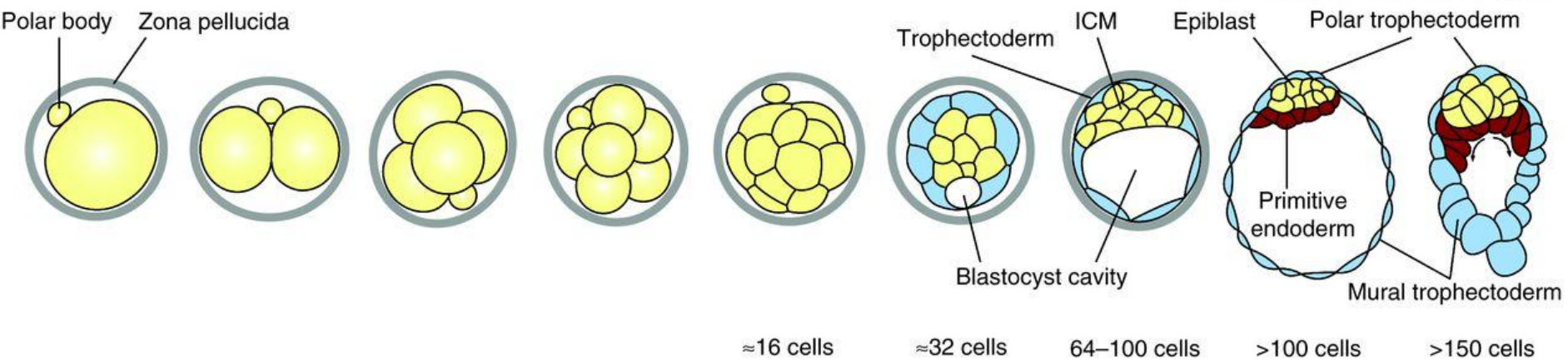
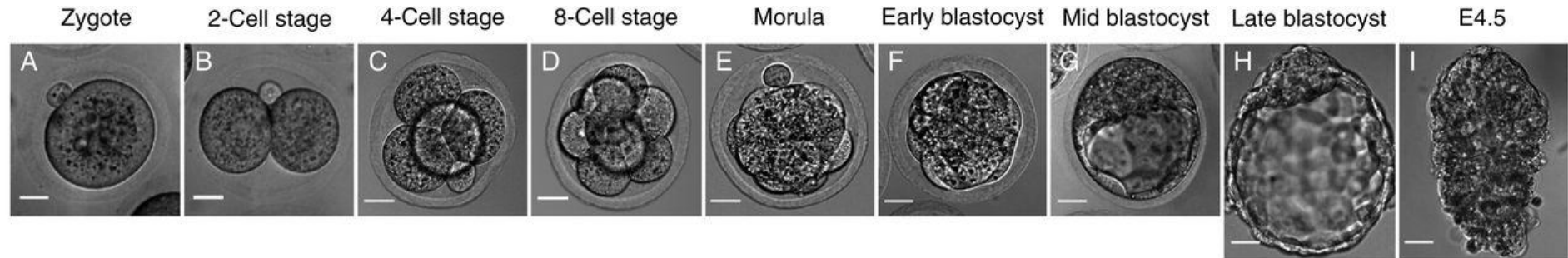
Copulation plugs are transient in the vagina and vary in position. Plugs fall out naturally during the day of mating and are most reliably detected within the first **12 h** after copulation. If the room is dark from 7 pm to 7 am, one would estimate that matings occurred at 1 am.

The **position** of the plug can range from being very deep in the vagina to very superficial.

The **color** of the plug can vary from somewhat translucent to opaque and from white to yellow.

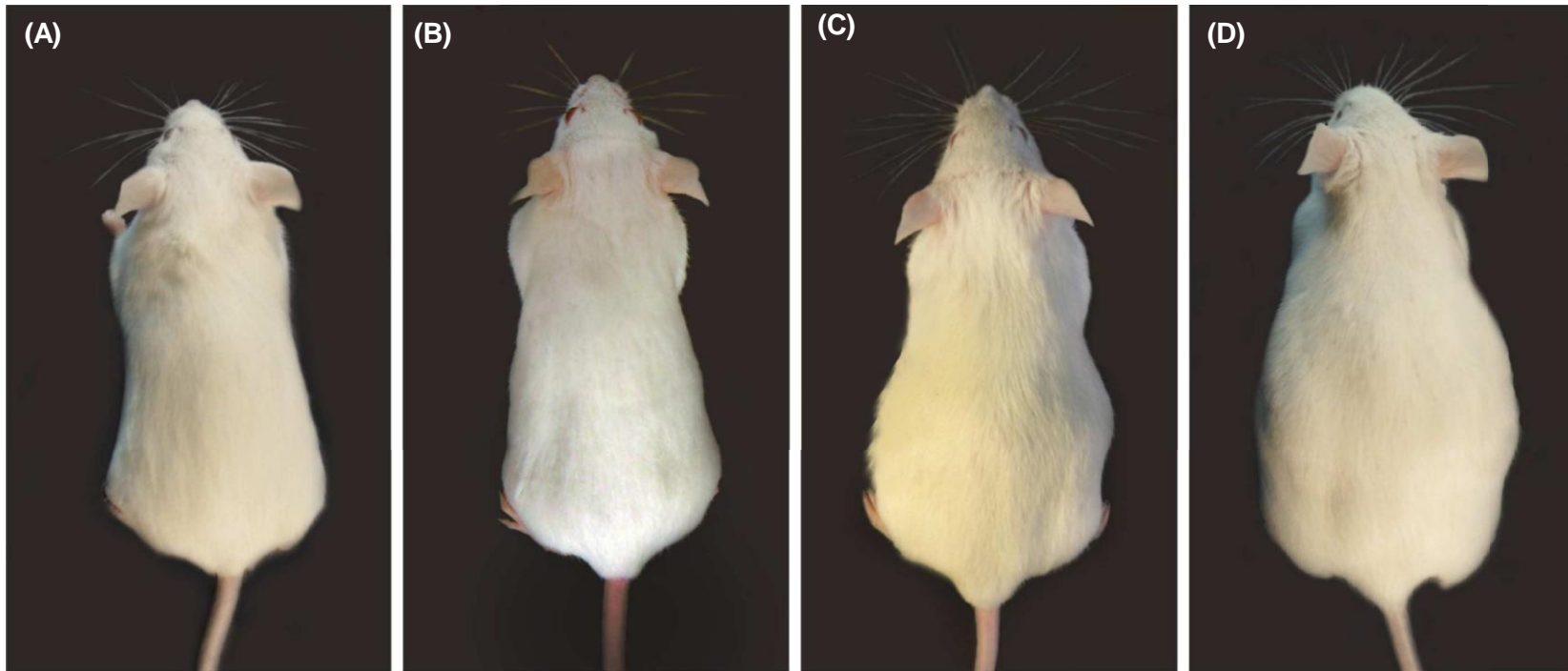
Copulation plugs are **hard** and have a rough "pebbled" or "ground glass-like" external surface.

Pre-implantation embryos (zygotes to blastocyst)



oviduct ← **uterus**

Physical changes in body appearance during a mouse pregnancy



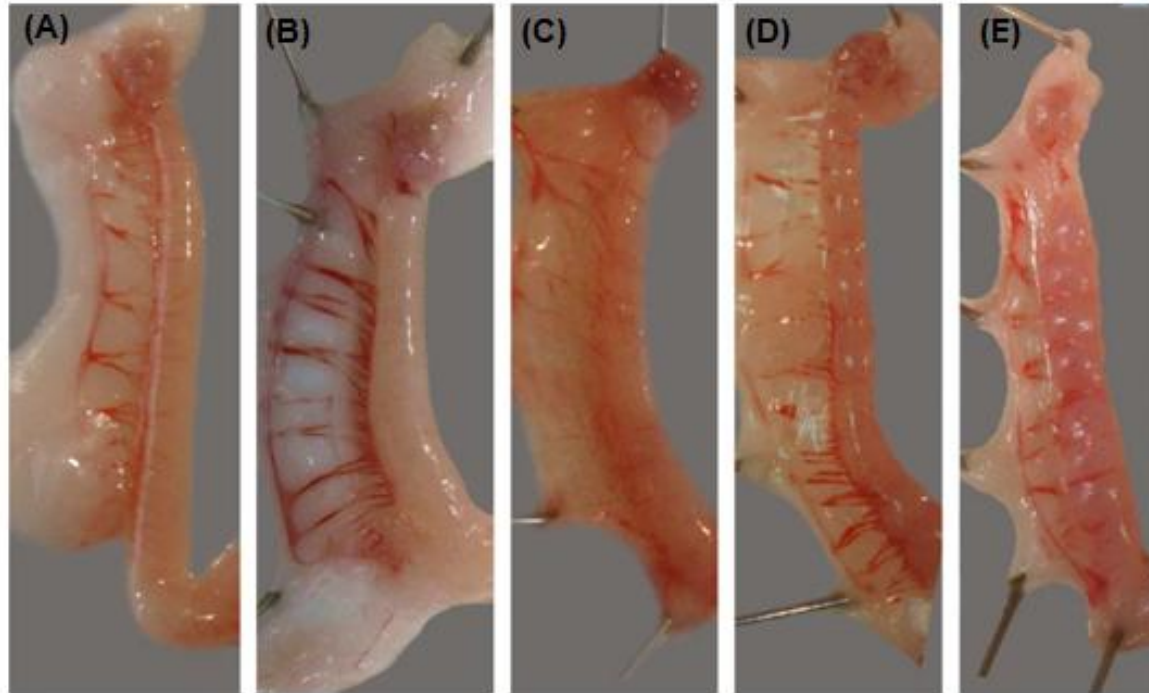
(A) Normal, unmated, young adult female mouse.

(B) Gestational day 7.5. This stage of gestation is not recognized grossly. The imaged mouse was carrying a litter of 15 conceptuses.

(C) Gestational day 15.5. This stage of gestation is easily recognized by an increase in abdominal size. The conceptuses can also be detected by abdominal palpation. The imaged mouse was carrying a litter of eight conceptuses.

(D) Term pregnancy at gestational day 19.5. The imaged mouse was carrying a litter of nine conceptuses.

Early post-implantation uterus (gestational days 4.5–6.5)



(A) The **diestrous uterine horn** is long and narrow. The stage of estrus of this donor mouse was confirmed by use of a vaginal smear

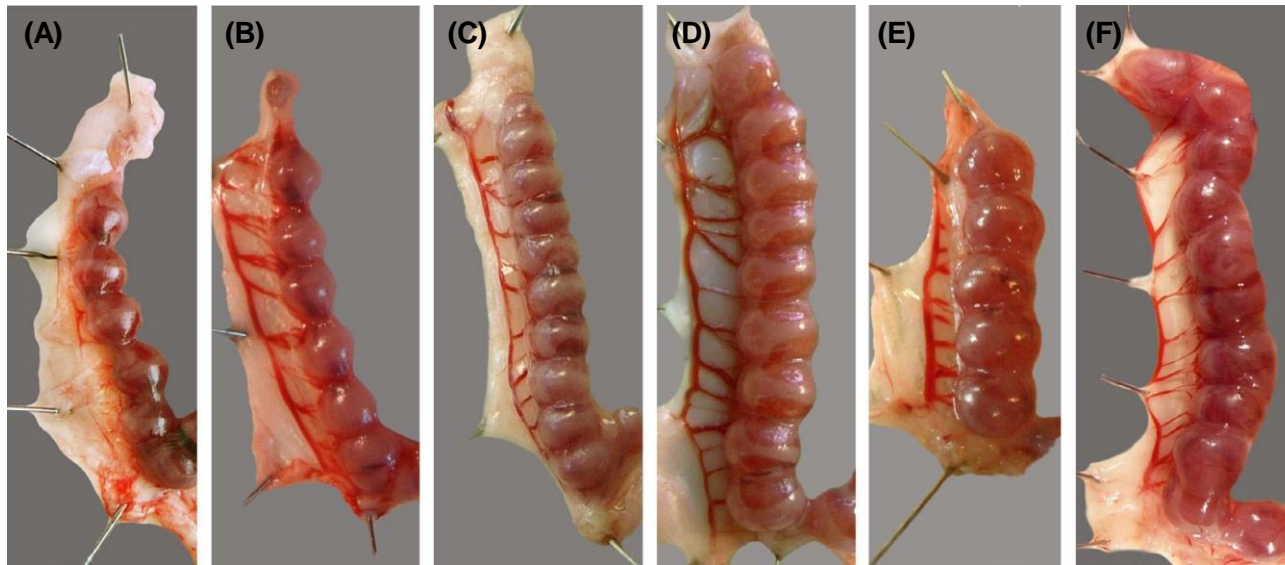
(B) The **estrous uterine horn** is noticeably swollen. Its wall is thin and pale in color. It is usually shorter in length than a nonestrous horn. The stage of estrus of this donor mouse was confirmed by use of a vaginal smear.

(C) **Gestational day 4.5**: Implantation sites are not visible to the naked eye.

(D) **Gestational day 5.5**: Implantation site swelling is apparent.

(E) **Gestational day 6.5**: Individual implantation sites are clearly visible.

Gravid uterus from gestational days 7.5–12.5



(A) **Gestational day 7.5** implantation sites are oval in shape and contain a cylindrically shaped, primitive streak embryo. Mesometrial trophoblasts, form the *ectoplacental cone*.

(B) **Gestational day 8.5** implantation sites have a distinct round appearance and contain an embryo that has become globular in shape as the somites differentiate, the allantois grows toward the placental primordium, and the *embryo rotates*.

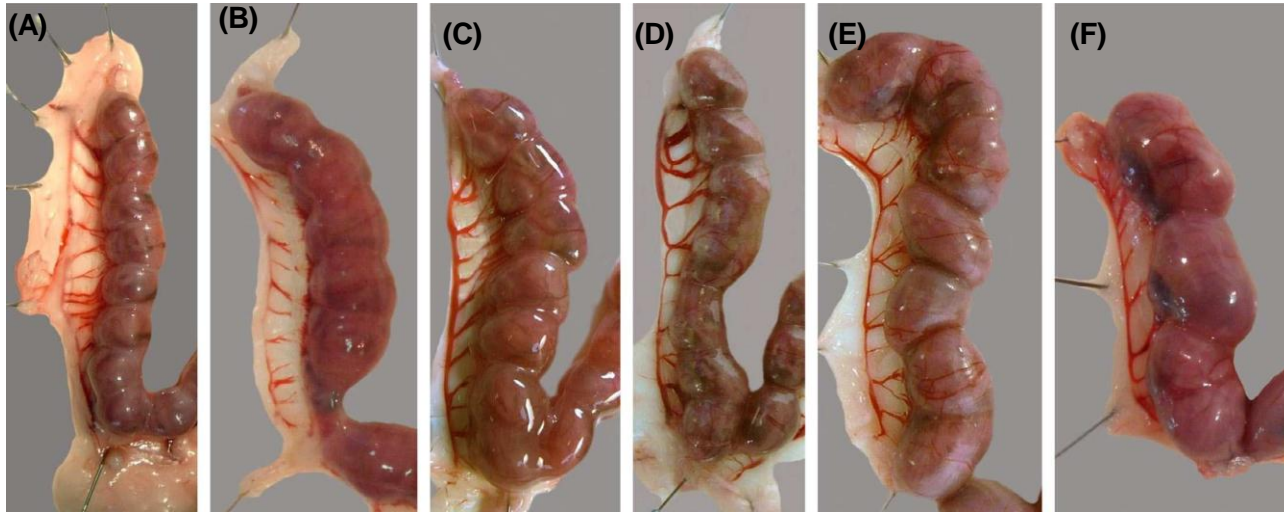
(C) **Gestational day 9.5** implantation sites show continued enlargement, with little or no space remaining between adjacent sites in horns holding large litters.

(D) **Gestational day 10.5** implantation sites are larger. The placenta has a functional blood supply.

(E) **Gestational day 11.5** implantation sites show continuing growth of both the placenta and the fetus. The placental position is clearly apparent from external examination of the uterus.

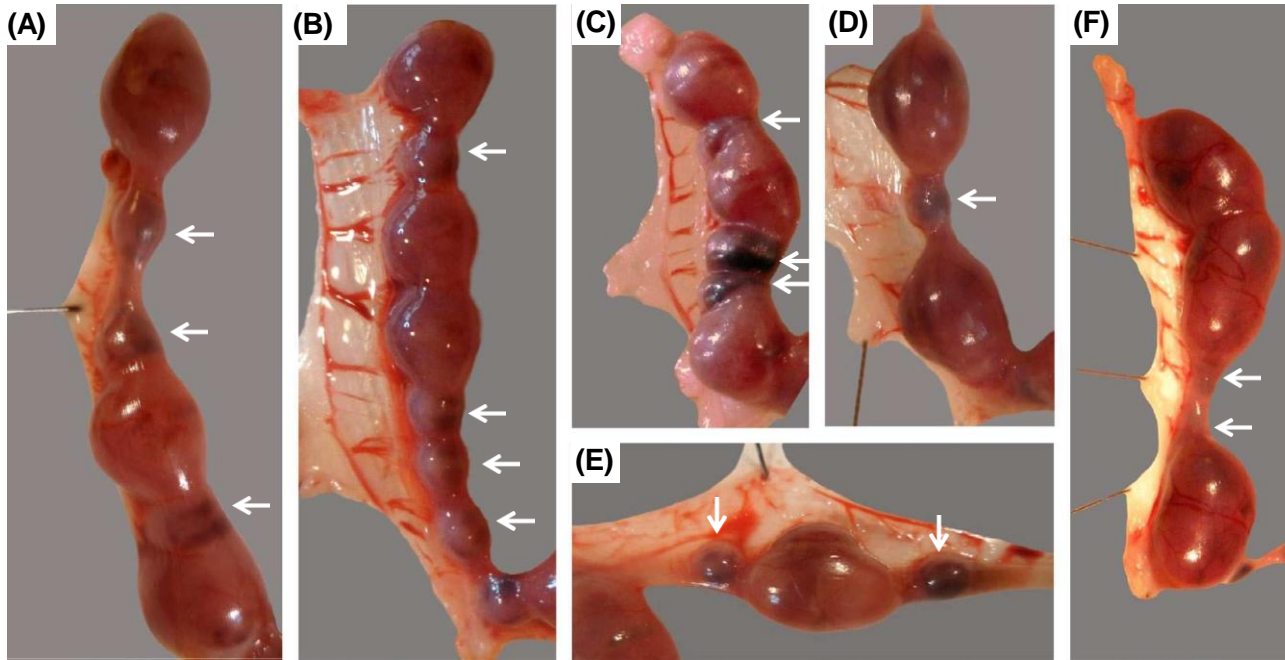
(F) **Gestational day 12.5** implantation sites show increasing size of the fetus and placenta. The placenta appears darker red in color as gestation continues.

Gravid uterus from gestational days 13.5–18.5



- (A) **Gestational day 13.5** uterine horn containing eight implantation sites.
- (B) **Gestational day 14.5** uterine horn containing five viable implantation sites. A sixth implantation site proximal to the cervix is in an advanced state of *resorption*.
- (C) **Gestational day 15.5** uterine horn containing seven implantation sites.
- (D) **Gestational day 16.5** uterine horn containing eight implantation sites.
- (E) **Gestational day 17.5** uterine horn containing seven implantation sites.
- (F) **Gestational day 18.5** uterine horn containing three implantation sites.

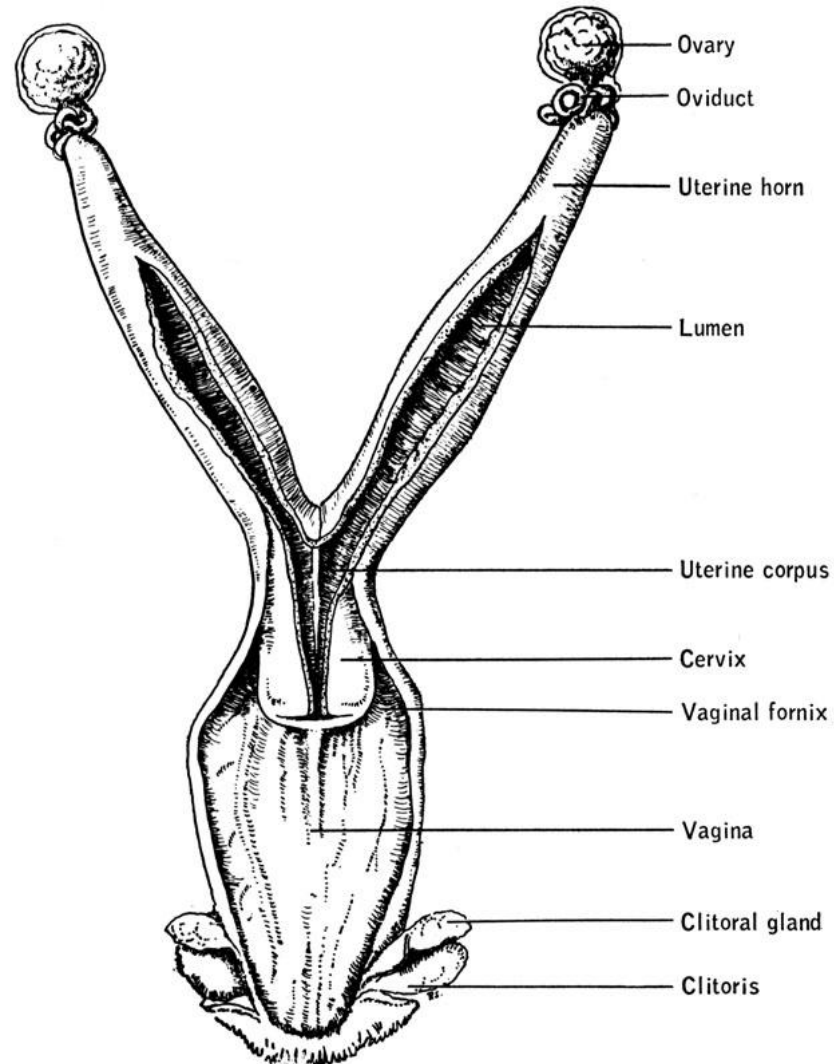
Process of fetal death and implantation site resorption

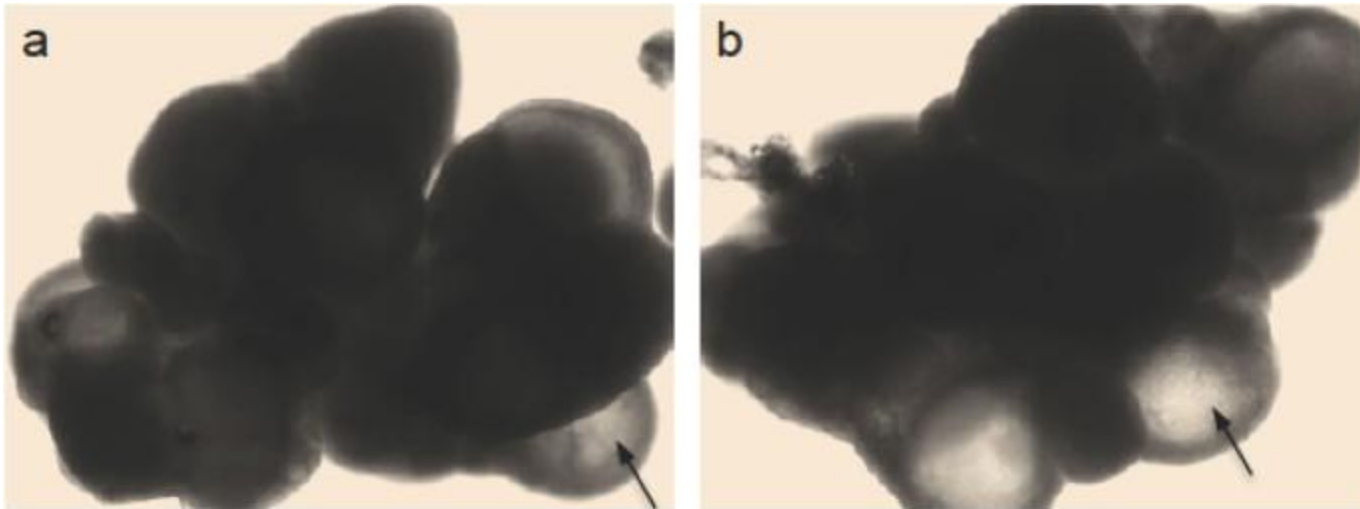


- (A) **Uterine horn at gestational day 11.5** : 3 resorbing (arrow) and three viable implantation sites.
- (B) **Uterine horn at gestational day 12.5**: 4 resorbing (arrow) and three viable implantation sites.
- (C) **Uterine horn at gestational day 12.5**: 3 resorbing (arrow) and three viable implantation sites.
- (D) **Uterine horn at gestational day 15.5**: 1 resorbing (arrow) and two viable implantation sites.
- (E) **Uterine horn at gestational day 13.5**: 2 resorbing (arrow) and one viable implantation sites.
- (F) **Uterine horn at gestational day 17.5**: 2 resorbing (arrow) and three viable implantation sites.

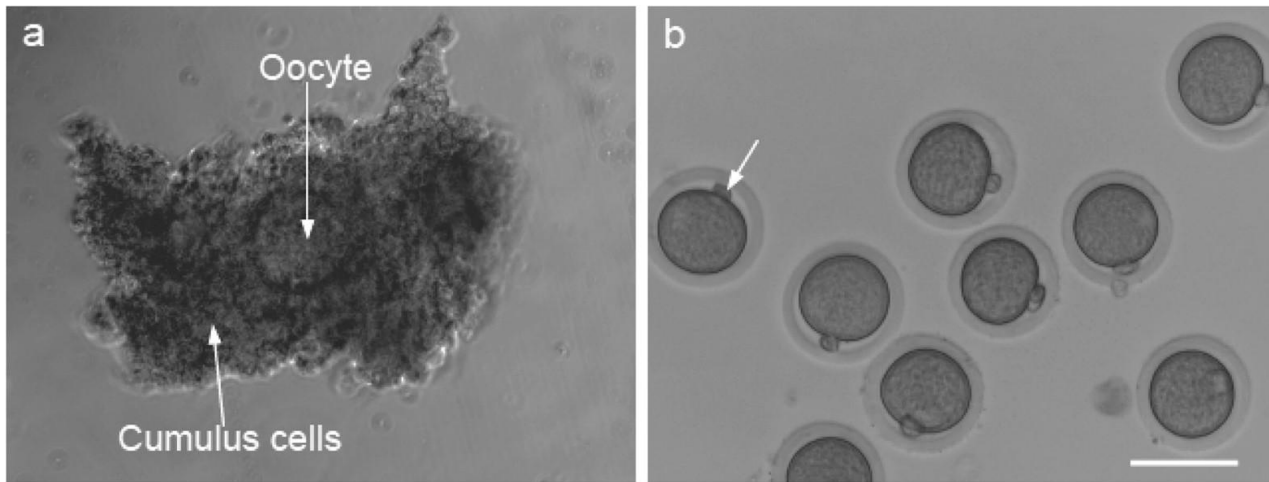
Pre-implantation embryo manipulation

- Isolate oocytes from the ovaries or oviducts
- Isolate up to 16-cell embryos from the oviducts
- Isolate blastocysts from the uterine horns

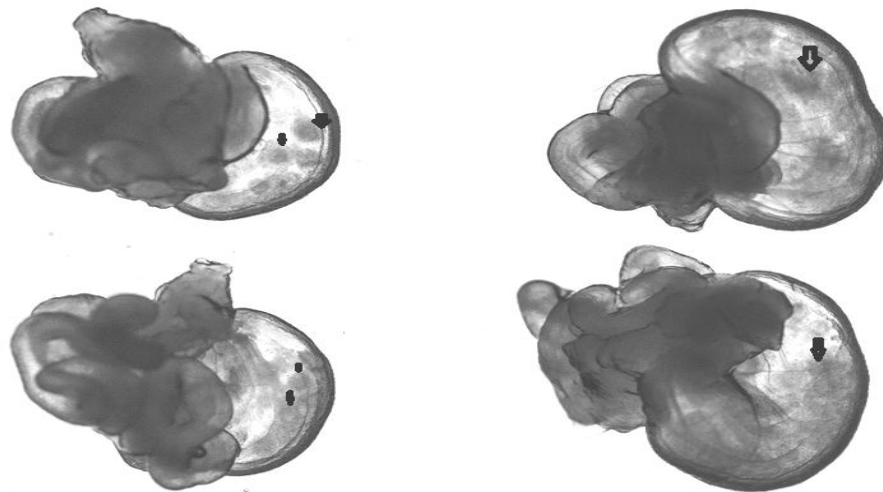




Typical images of ovaries collected from female deer mice following injection with either PMSG only (one dose, a) or PMSG & hCG (one PMSG and one hCG at 56 h after PMSG administration, b). Arrows indicates antral follicles.



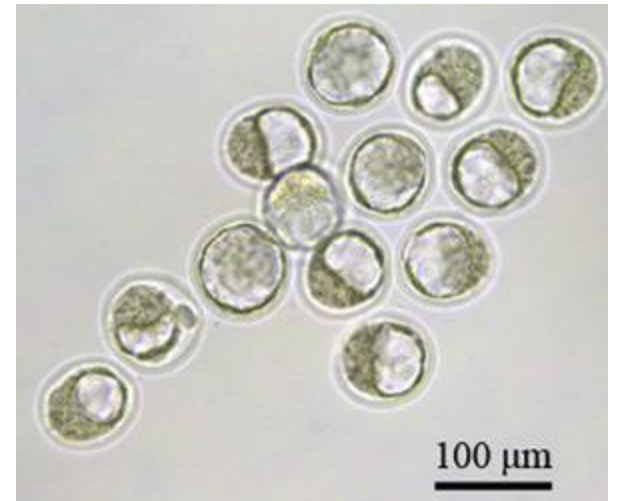
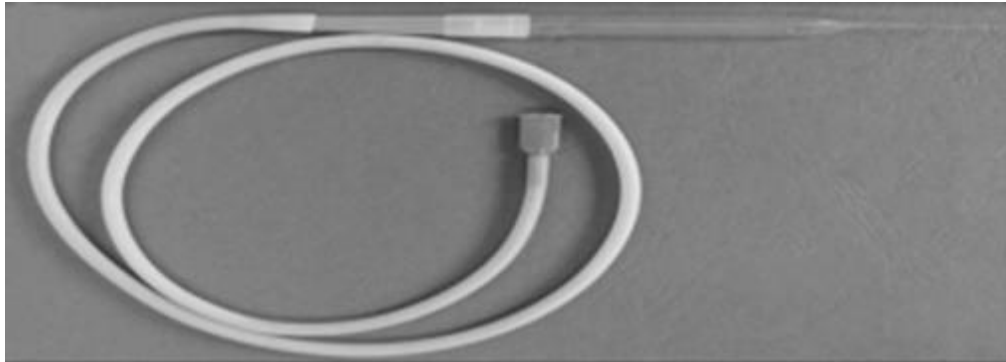
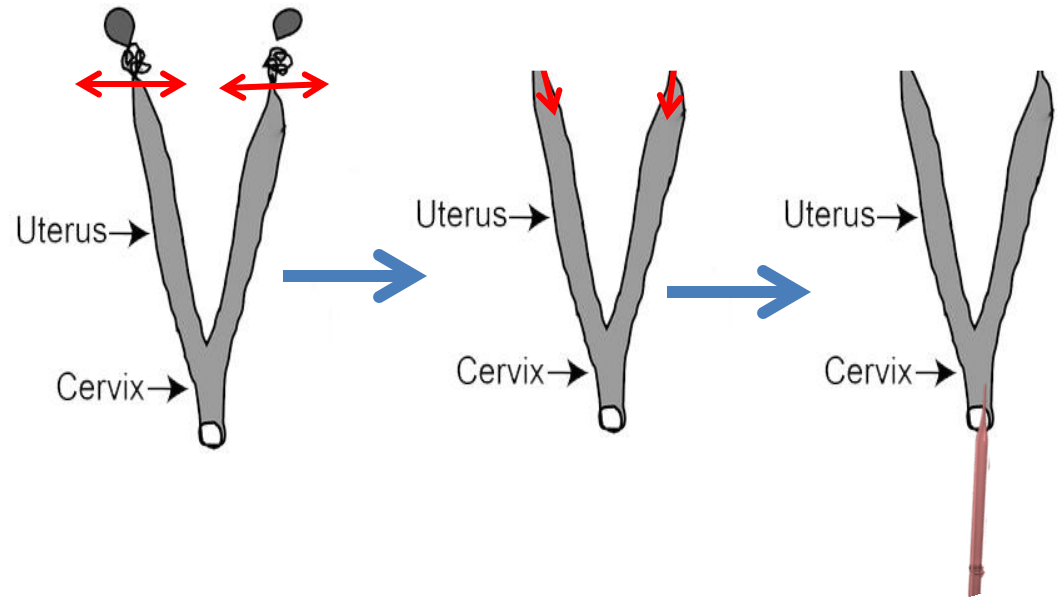
a) isolated from ovary **(b)** with a first polar body (arrow)



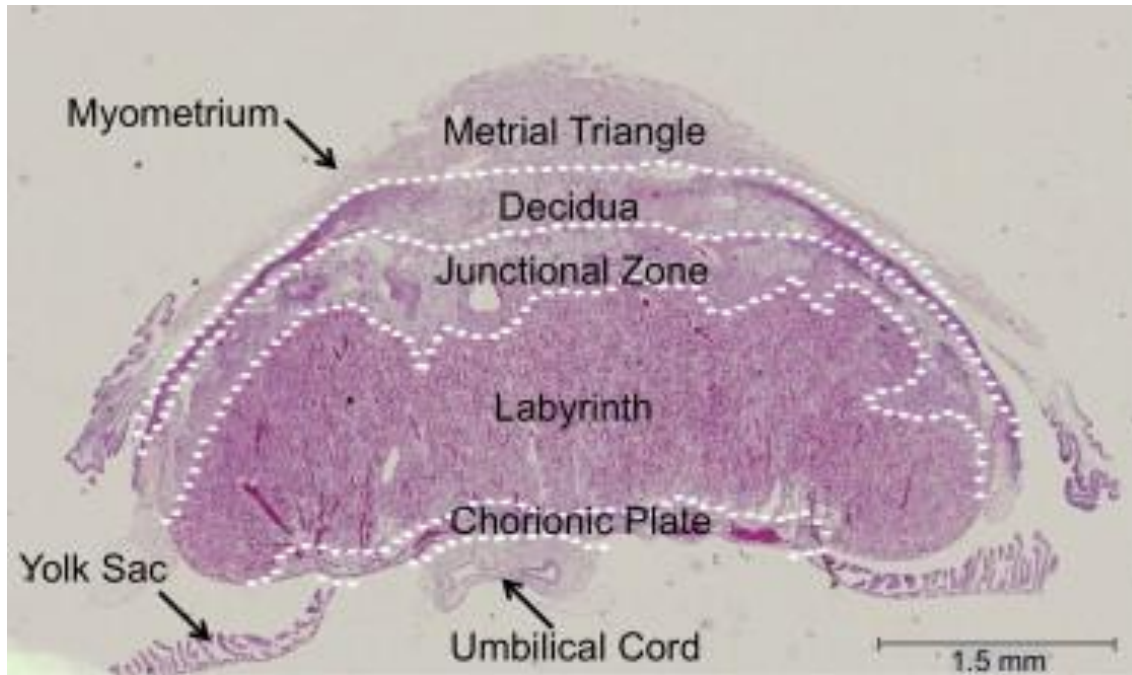
Oviduct isolation- isolate cumulus-oocyte complexes from the swollen ampulla (arrows)



Isolate blastocysts by flashing the uterus

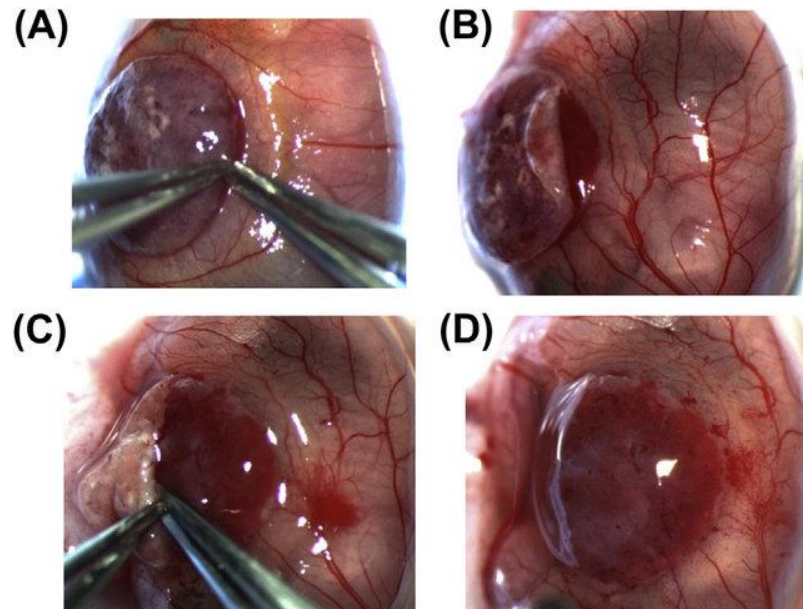


Placental isolation

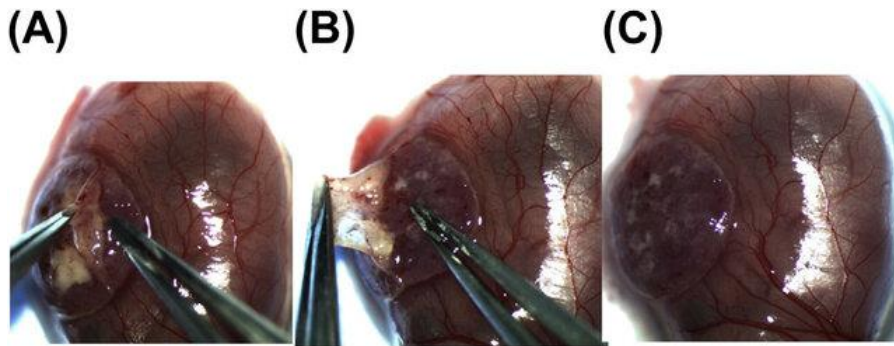


Histological section of mouse placenta (H&E staining). Dashed white lines indicate borders between adjacent layers of the placenta.

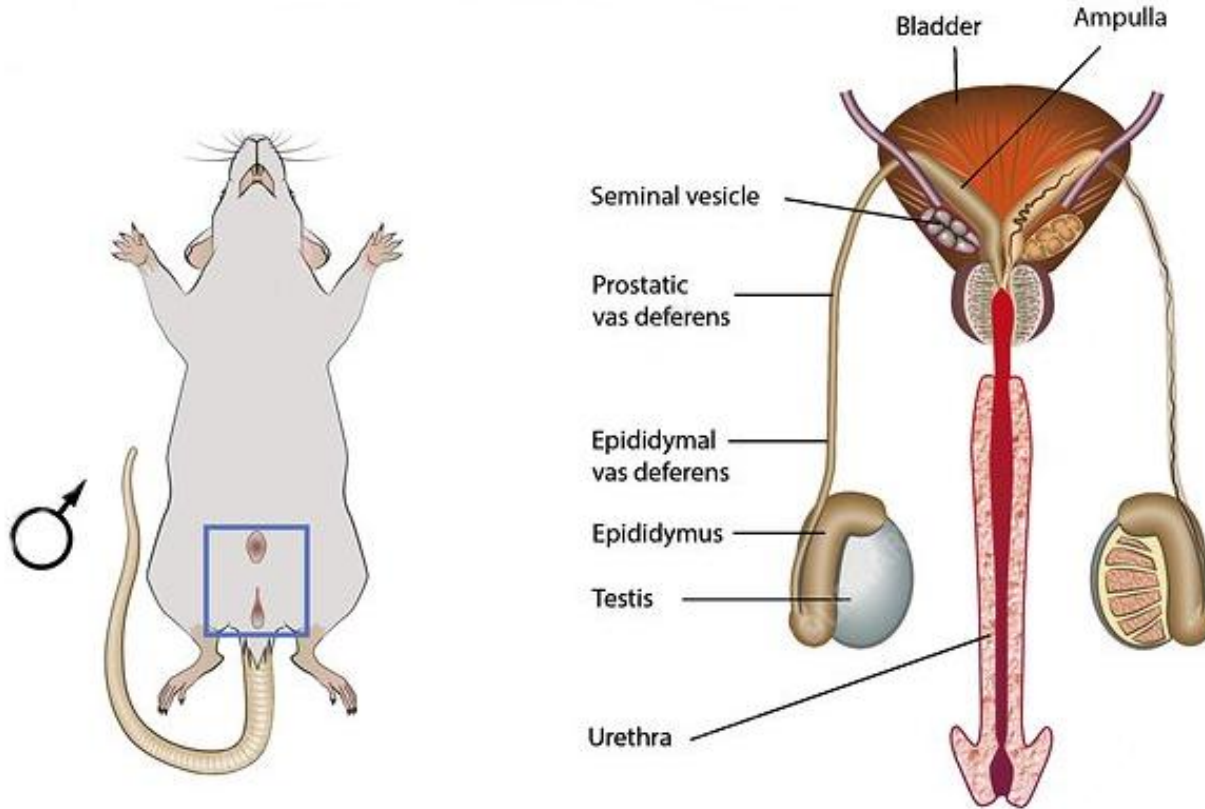
Dissection of the junctional zone layer (spongiotrophoblast)



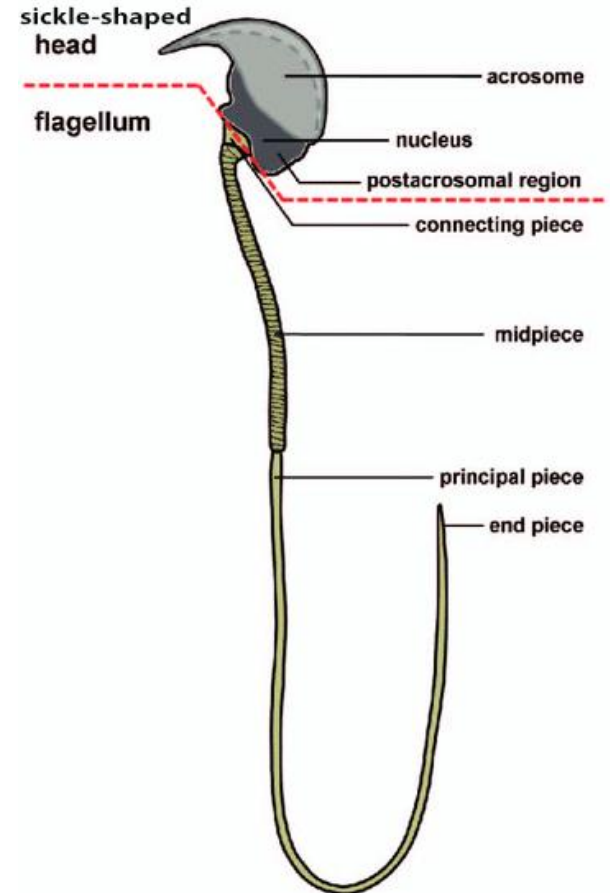
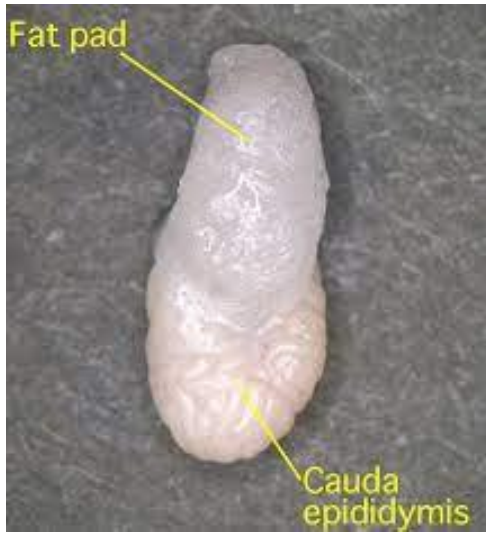
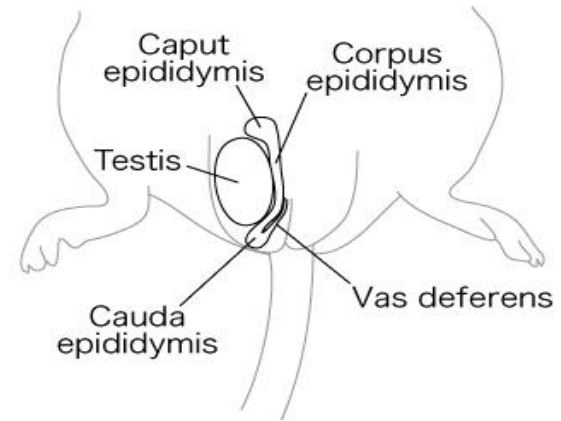
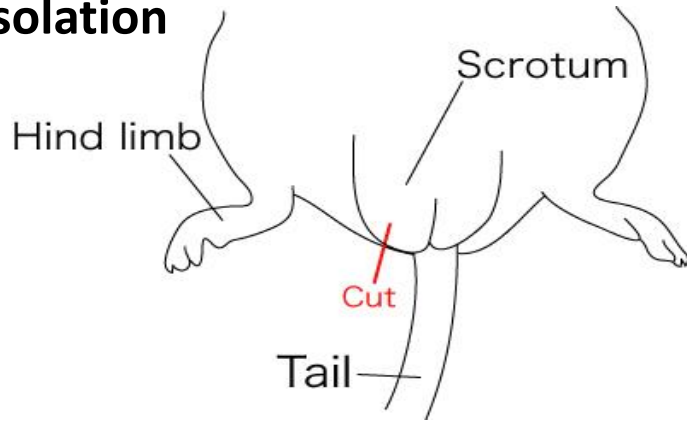
Dissection of the decidual layer

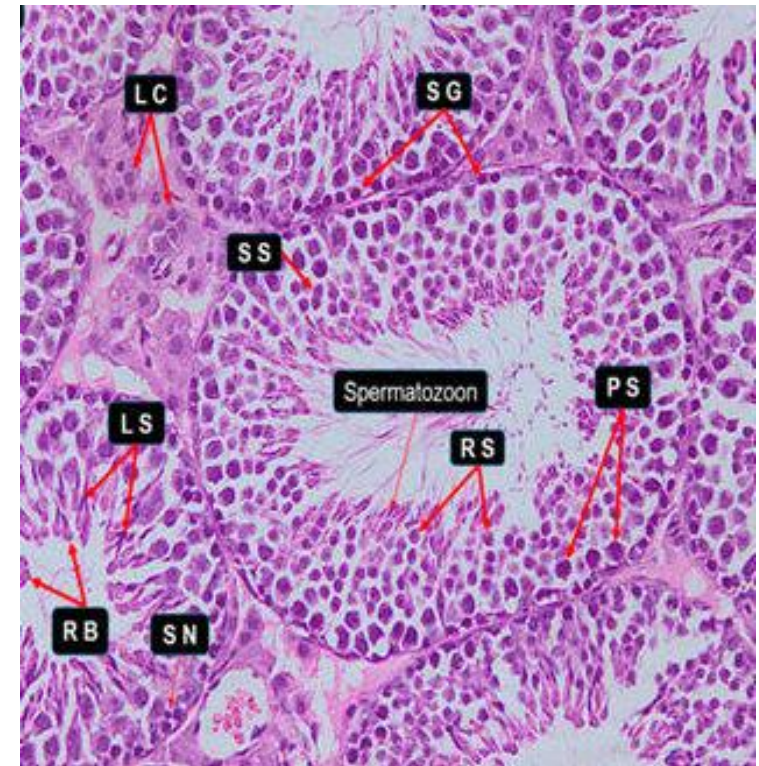
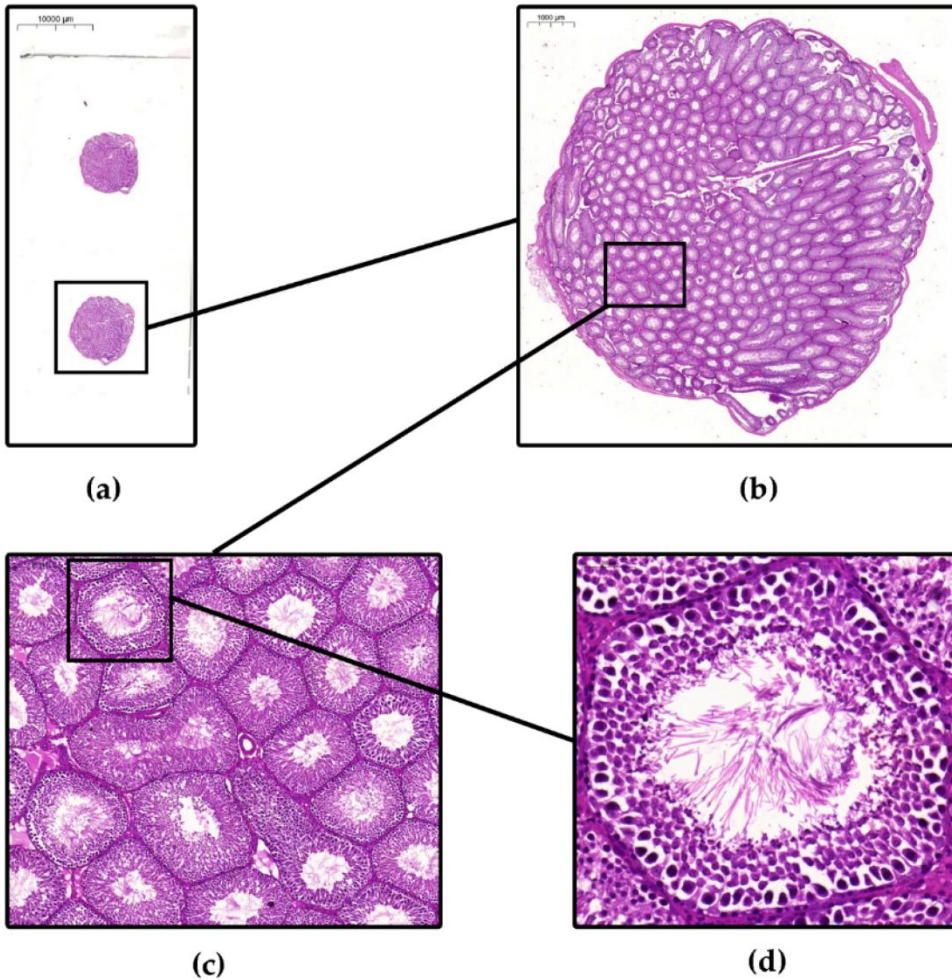


Male reproductive organs



Sperm isolation





- SC: Spermatids Cells
- RB: Residual Bodies
- RS: Round Spermatids
- LS: Long Spermatids
- SS: Secondary Spermatocytes
- PS: Primary Spermatocytes
- SG: Spermatogonia
- LC: Leydig Cells

BL: basal lamina

BTB: blood testis barrier

ES: elongated spermatid

MPC: myoid peritubular cell

PSC: primary spermatocyte

RS: round spermatid

SPG: spermatogonia

SSC: secondary spermatocyte

ST: seminiferous tubule

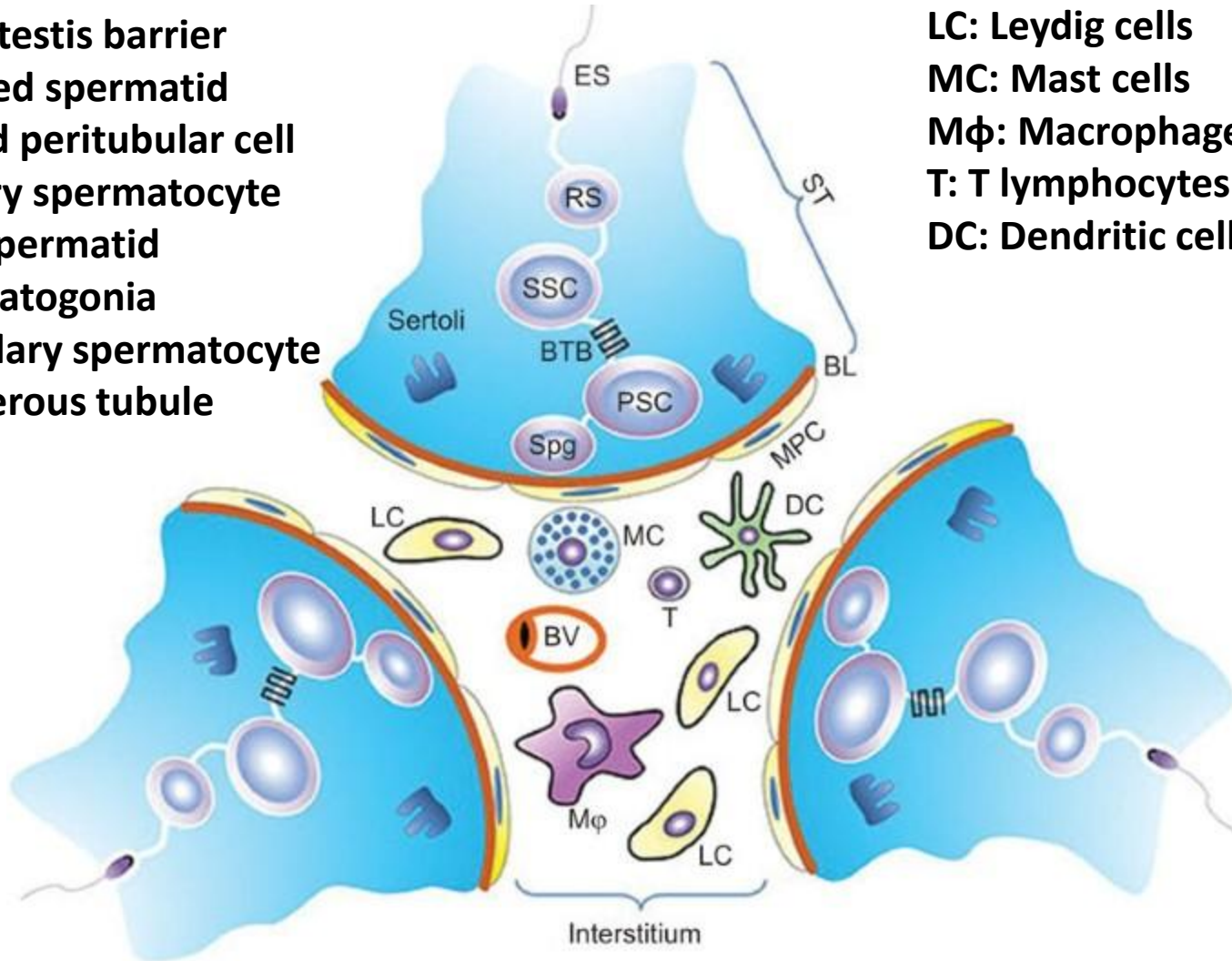
LC: Leydig cells

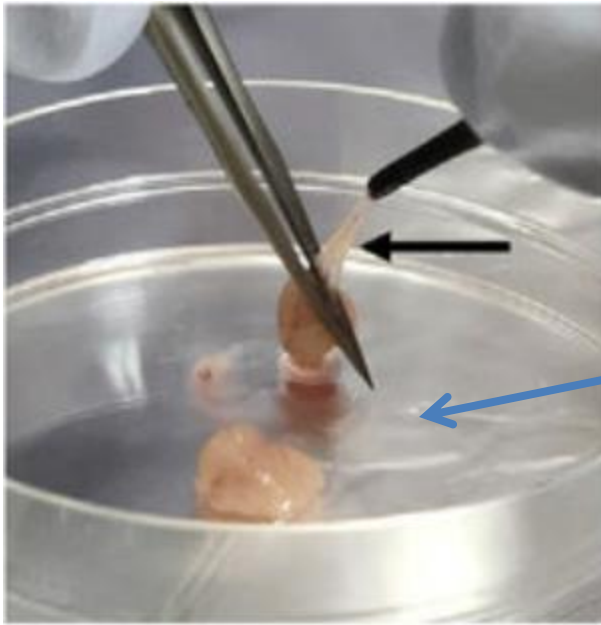
MC: Mast cells

Mφ: Macrophages

T: T lymphocytes

DC: Dendritic cells

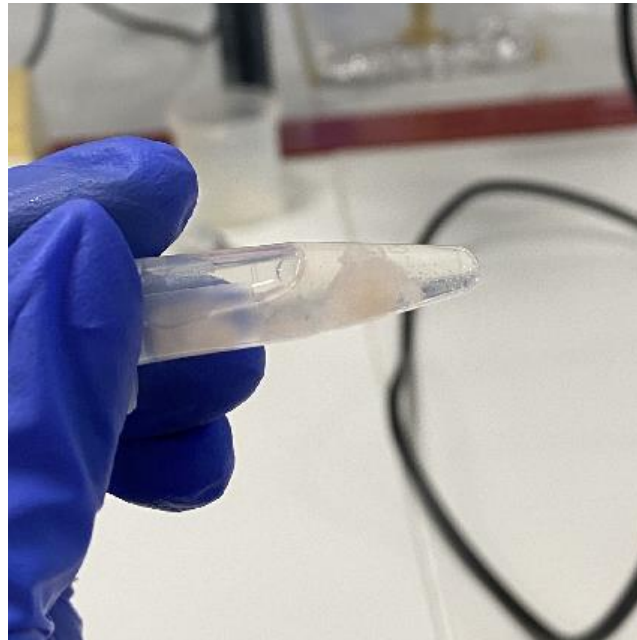




**Medium
containing cells
from interstitial
space**



Removal of tunica albuginea



**Isolate cells from
seminiferous
tubules with
collagenase
treatment**

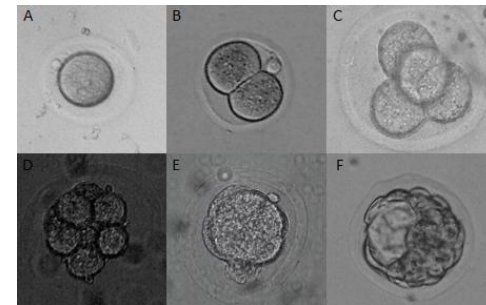
Superovulation

Superovulation is a technique used to get female mice to release a higher number of oocytes.

It includes administration of **PMSG** (Pregnant Mare's Serum Gonadotropin, 5 IU/female) that stimulates ovaries to develop follicles and produce FSH and LH. Human chorionic gonadotropin (**hCG**, 5 IU/female) is administered 46-48 hours after PMSG so that it precedes the wave of endogenous LH production (15-20 hours after the middle of the second dark cycle).

Example: PMSG on Day 1 at 1:00 p.m., hCG on Day 3 at 12:00 p.m. Cage each superovulated female with a fertile male 3-4 hours after the last injection (3-4:00 pm)

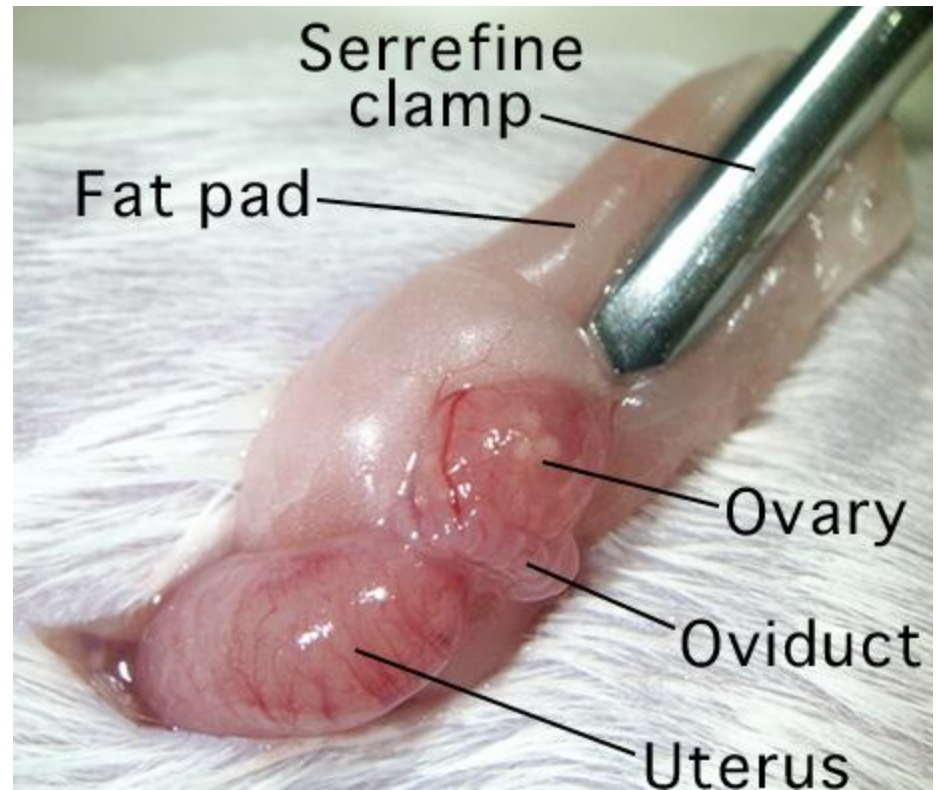
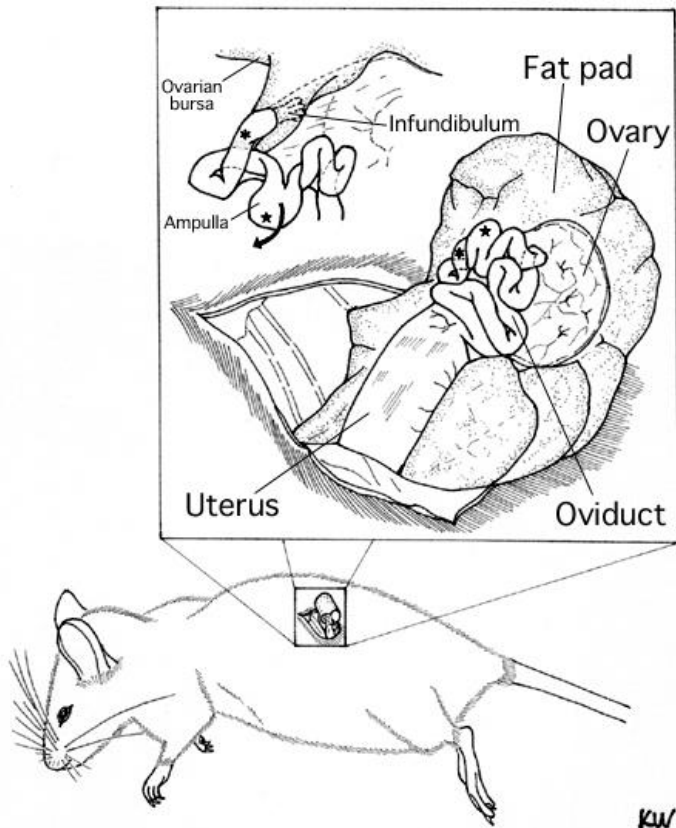
Embryo Stage	Hours post-hCG
Zygote	18-22
2-cell embryo	44-48
4-cell embryo	56-60
8-cell embryo	72-78
Morula	78-96
Blastocyst	94-115

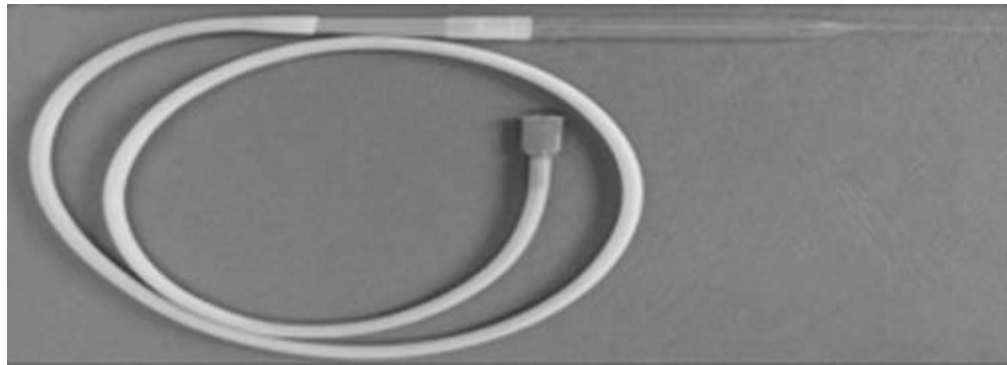
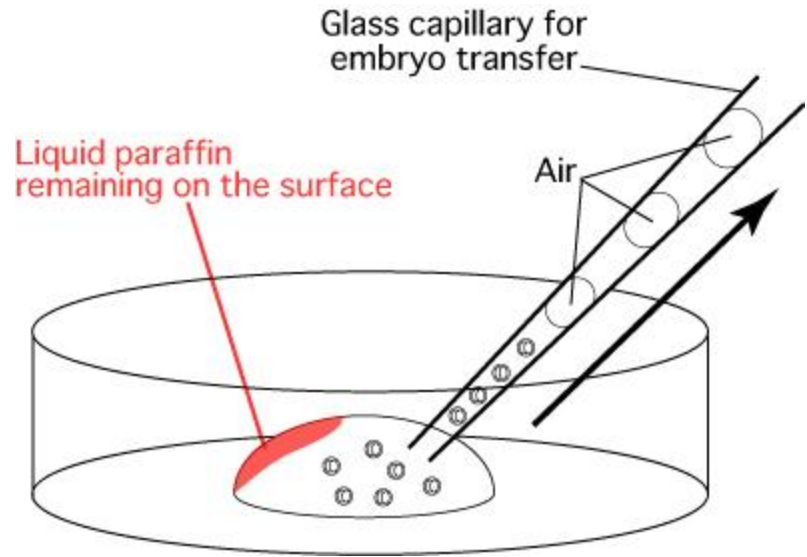
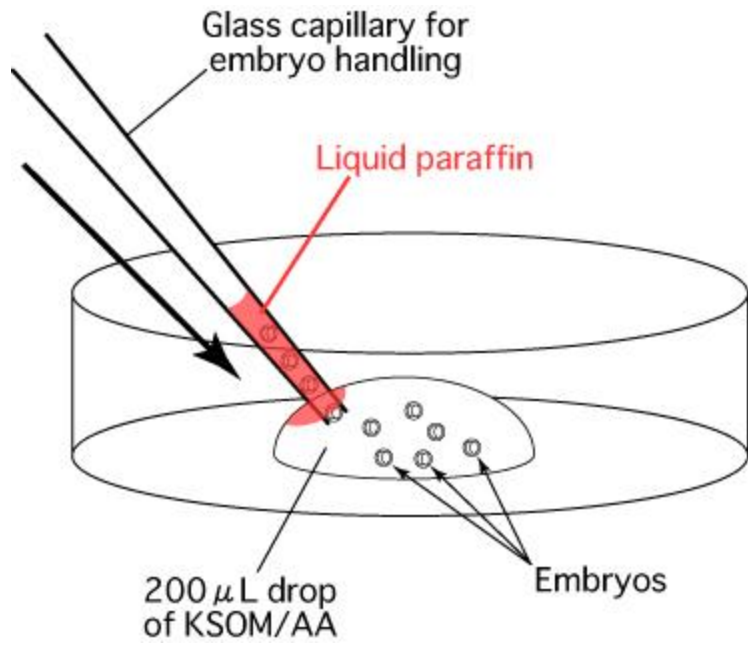


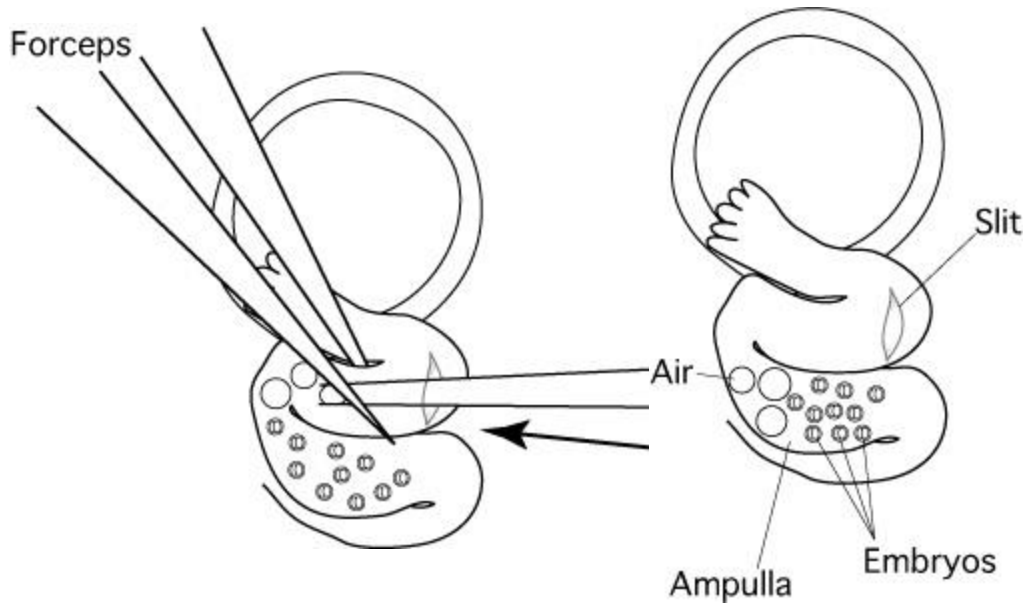
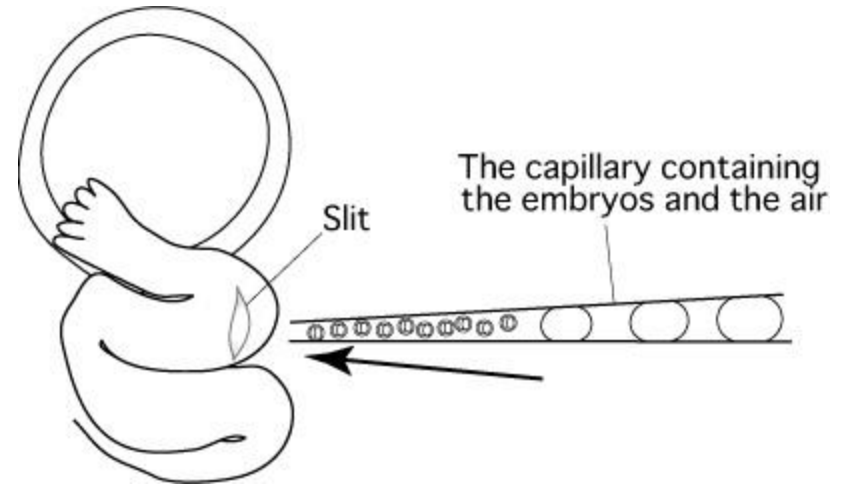
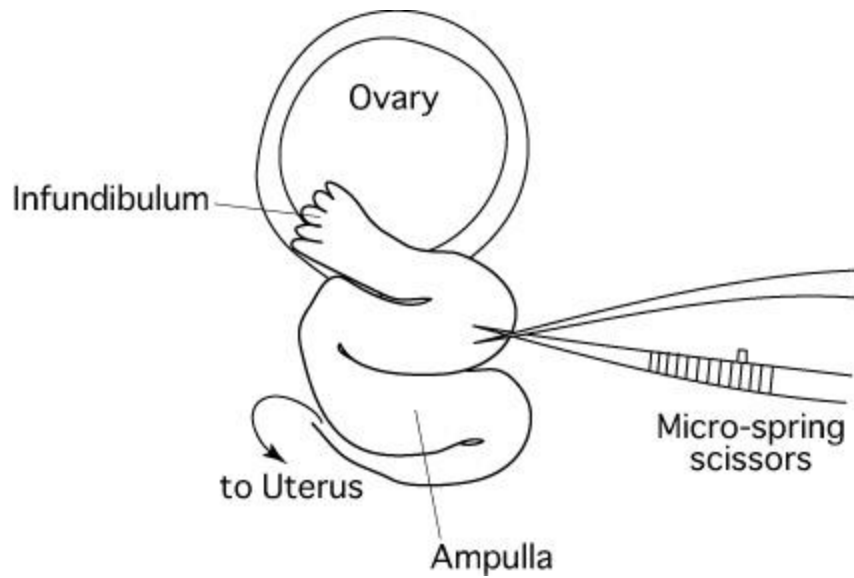
- Pronuclei are formed 6-7 h post- fertilization
- Within 2-3 h the second polar body is formed
- First cleavage occurs about 24 h post-fertilization
- The 8-cell embryos undergo compaction to form a solid ball of cells
- Blastocysts consist of the Inner Cell Mass (ICM), the trophoectoderm and the blastocoel

Embryo transfer

Most commonly **day 3 embryos** (day 0 corresponds to plug day) are transferred to the uterus of **day 2** of pseudopregnant females (females mated with sterile males)







Push the ovary, oviduct and uterine horn back into the abdomen and close the wound using wound clips

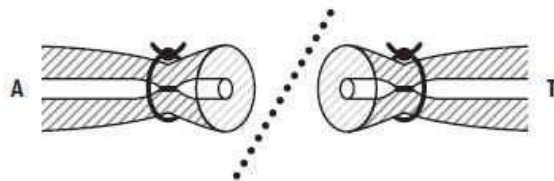
Vasectomy



(a) Incision site for vasectomy. **(b)** A tubular structured vas deferens

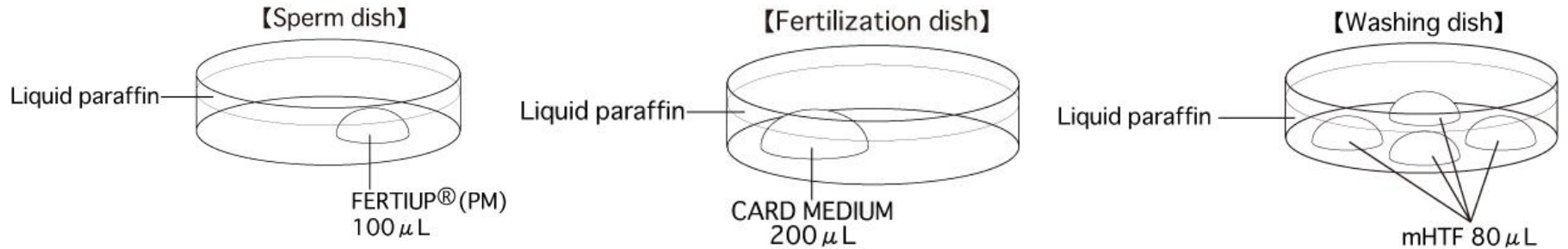
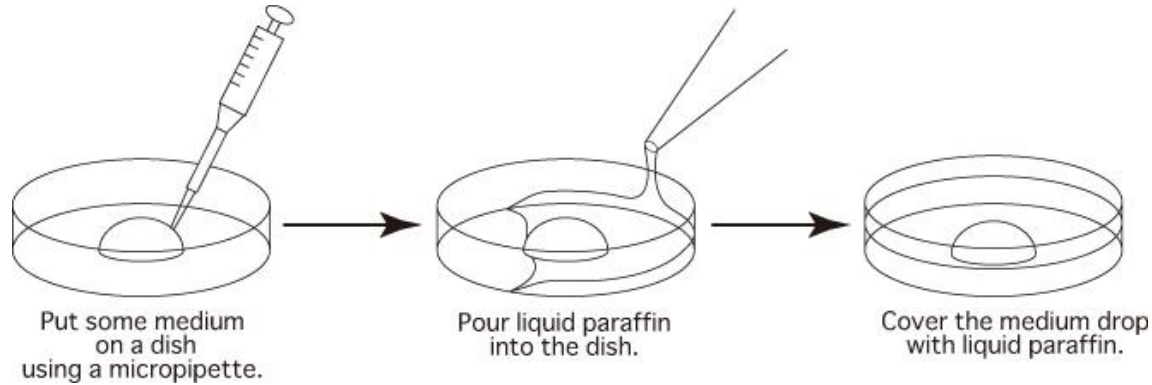


Ligation both ends with FI

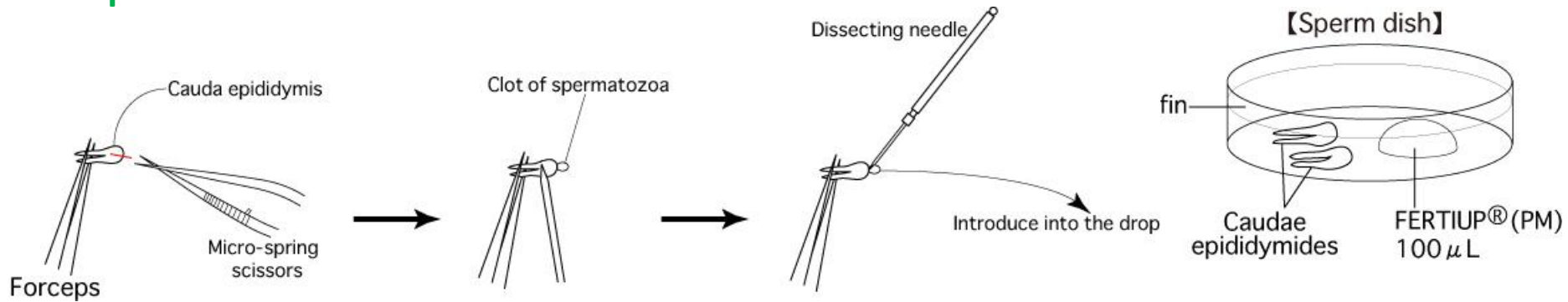


Occlusive Failure Range = 0.0-5.85%

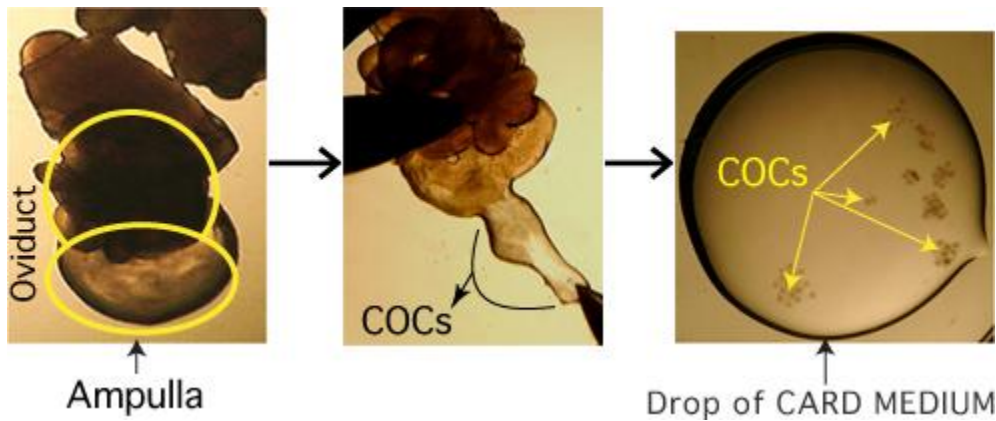
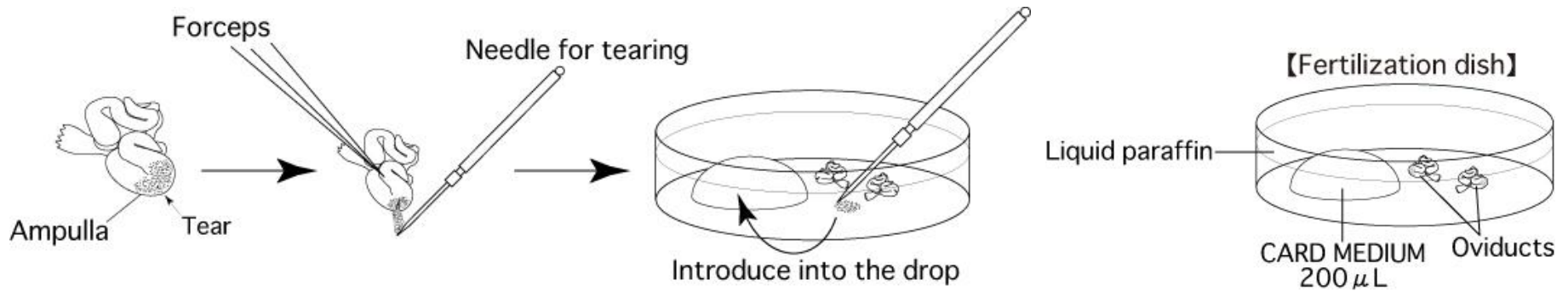
IVF: preheated-equilibrated media- standard CO2

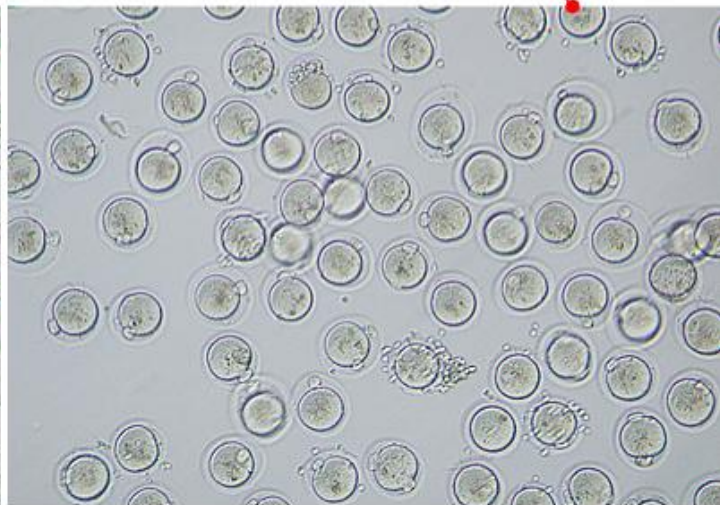
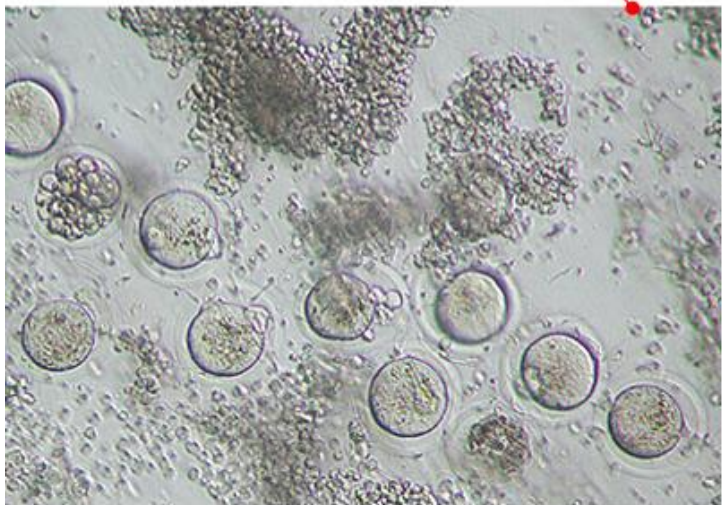
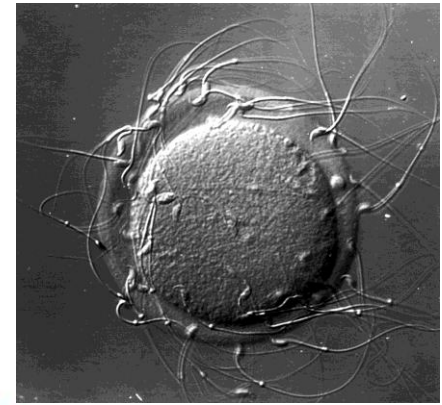
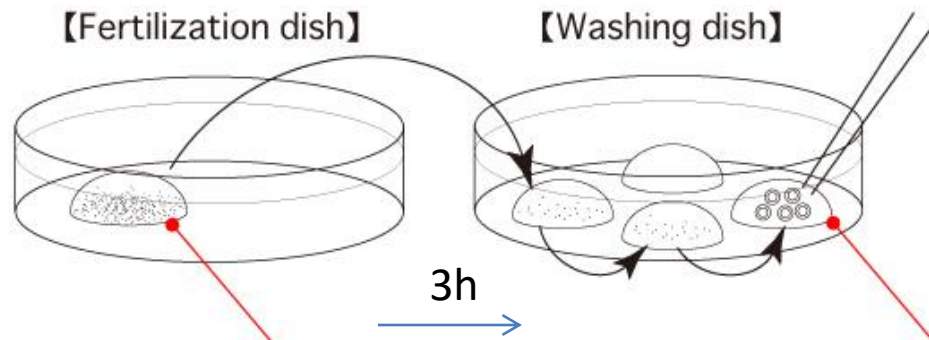
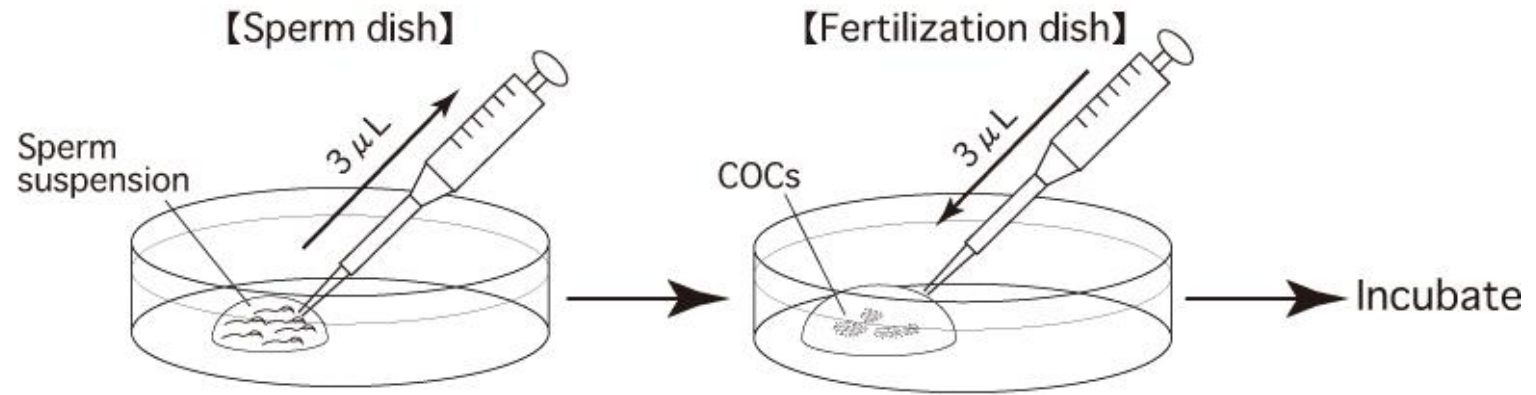


sperm

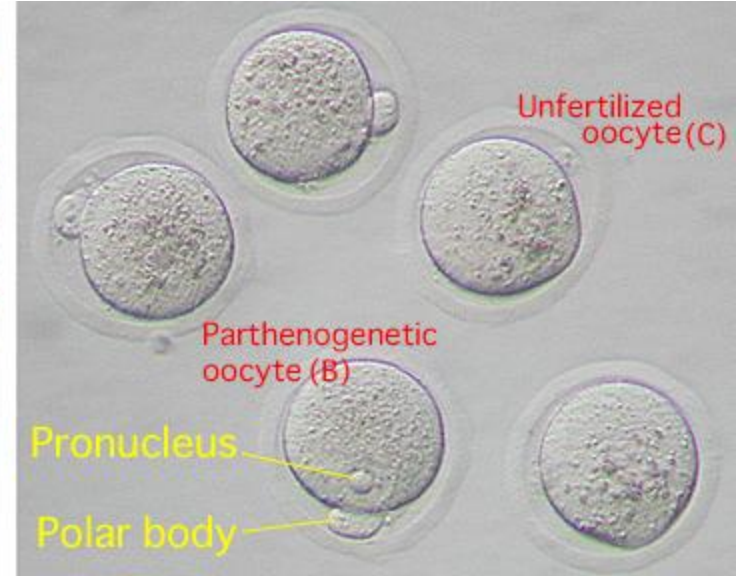
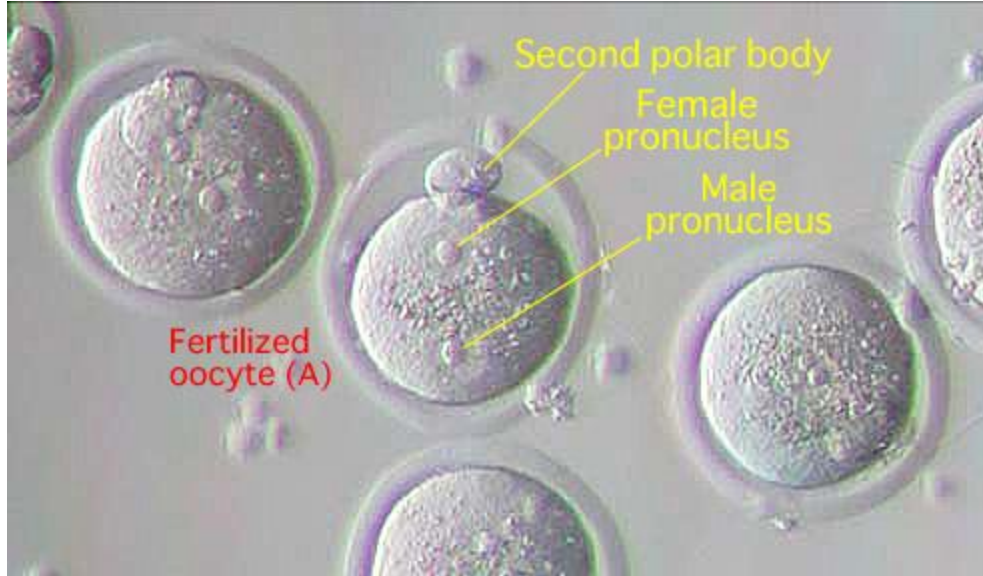


Oocytes-COCs





6h later



Transgenic mice production

