Mouse and rat reproduction: manipulating the mouse embryo

Prof. Irene Athanassakis
Department of Biology,
University of Crete

Rats


kangaroo rat


cotton rat


Norway rat


black rat


African pouched rat


naked mole rat


wood rat


pack rat

Mice


house mouse


field mouse


deer mouse


smoky mouse


spiny mouse


dormice mouse


kangaroo mouse


white-footed mouse

Laboratory rats and mice refer to:

• Norway rats (*Rattus norvegicus*)

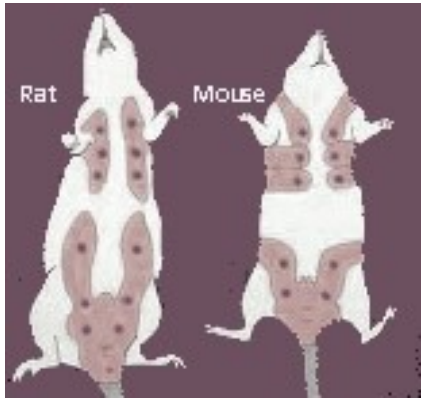
•house mice (*Mus musculus*)

which

•belong to different species

•cannot 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

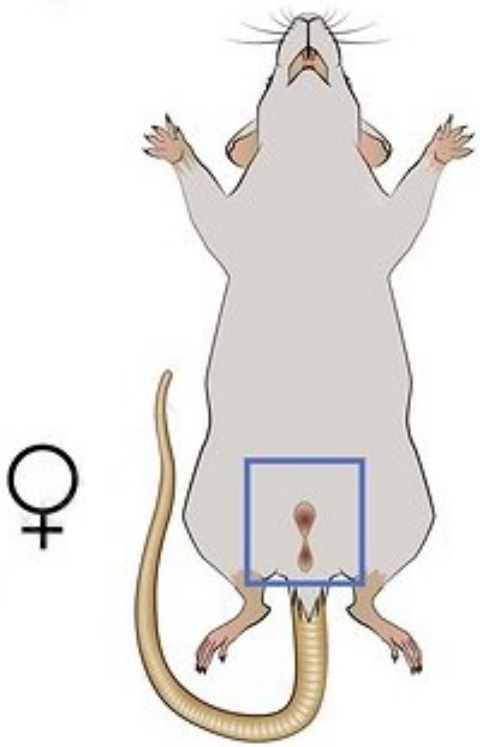
- Female reproductive organs
    - Pregnancy
    - Manipulations of virgin and pregnant mice
        - Oocyte collection
        - Collection of pre-implantation embryos
        - Post-implantation embryos

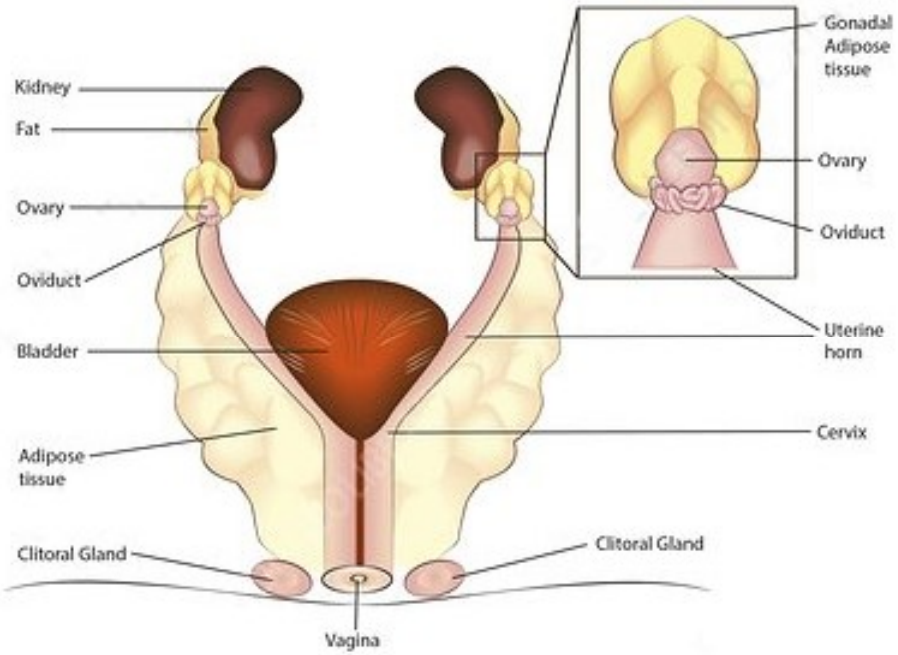
- Male reproductive organs
    - Manipulations of male mice
        - Sperm collection
        - Testis

- Superovulation
- Embryo transfer
- Pseudopregnant females
- Vasectomy
- IVF

Gender

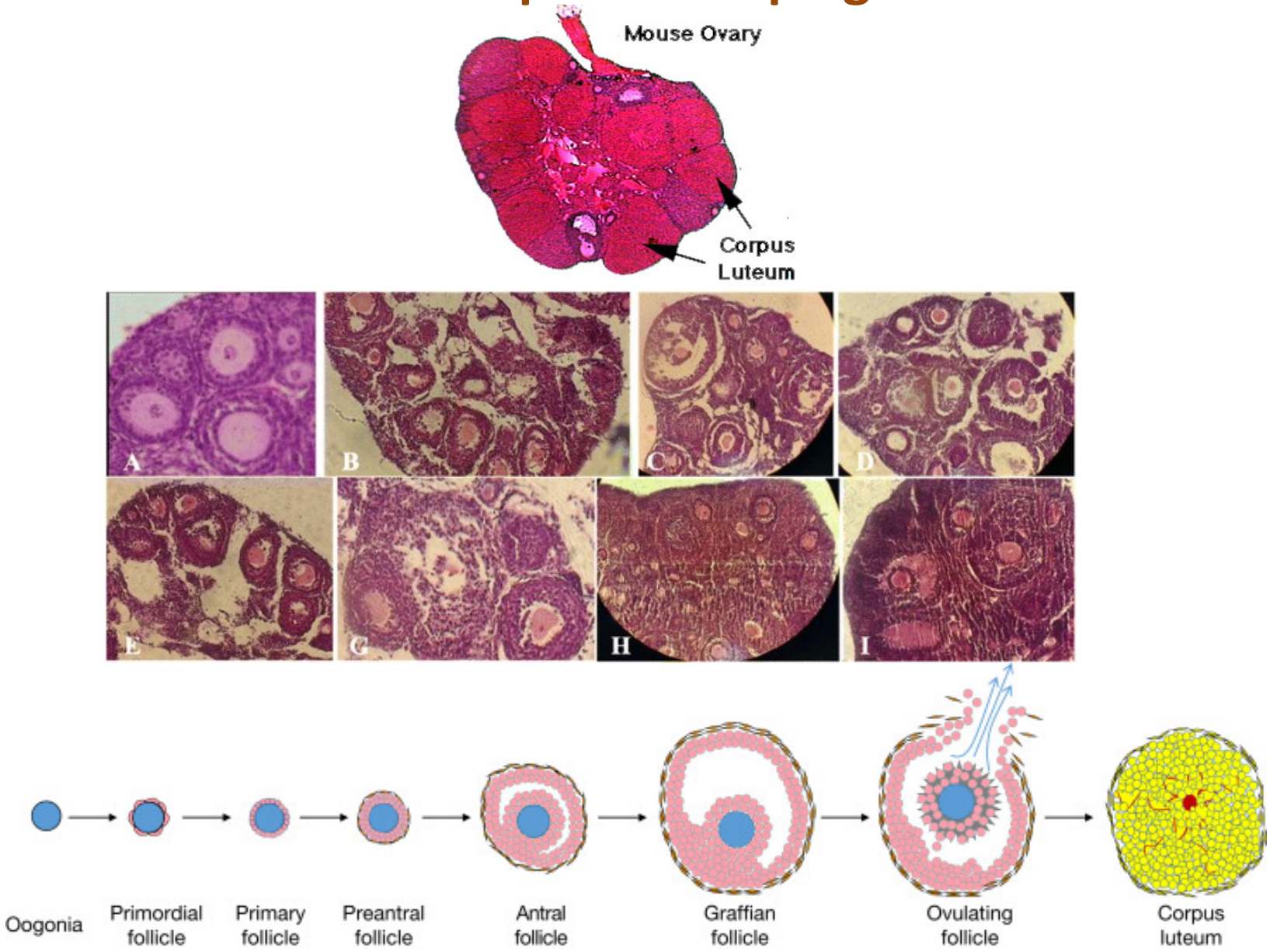
Female reproductive organs





https://www.sciencephoto.com/media/1057773/view/mouse-female-reproductive-system-illustration

OVARY: Multiple developing follicles



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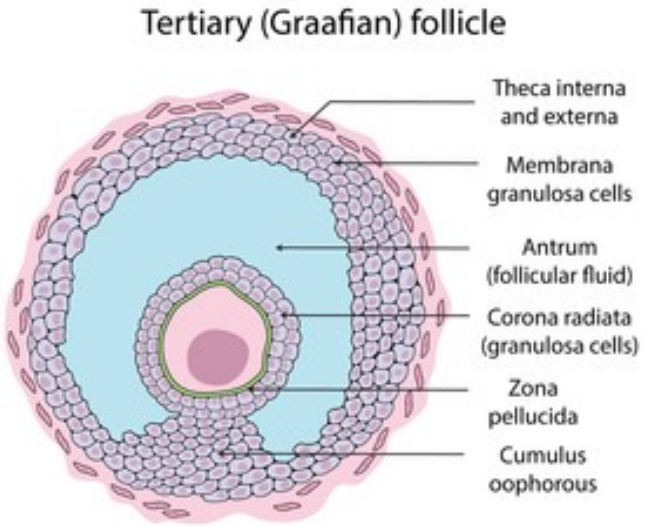
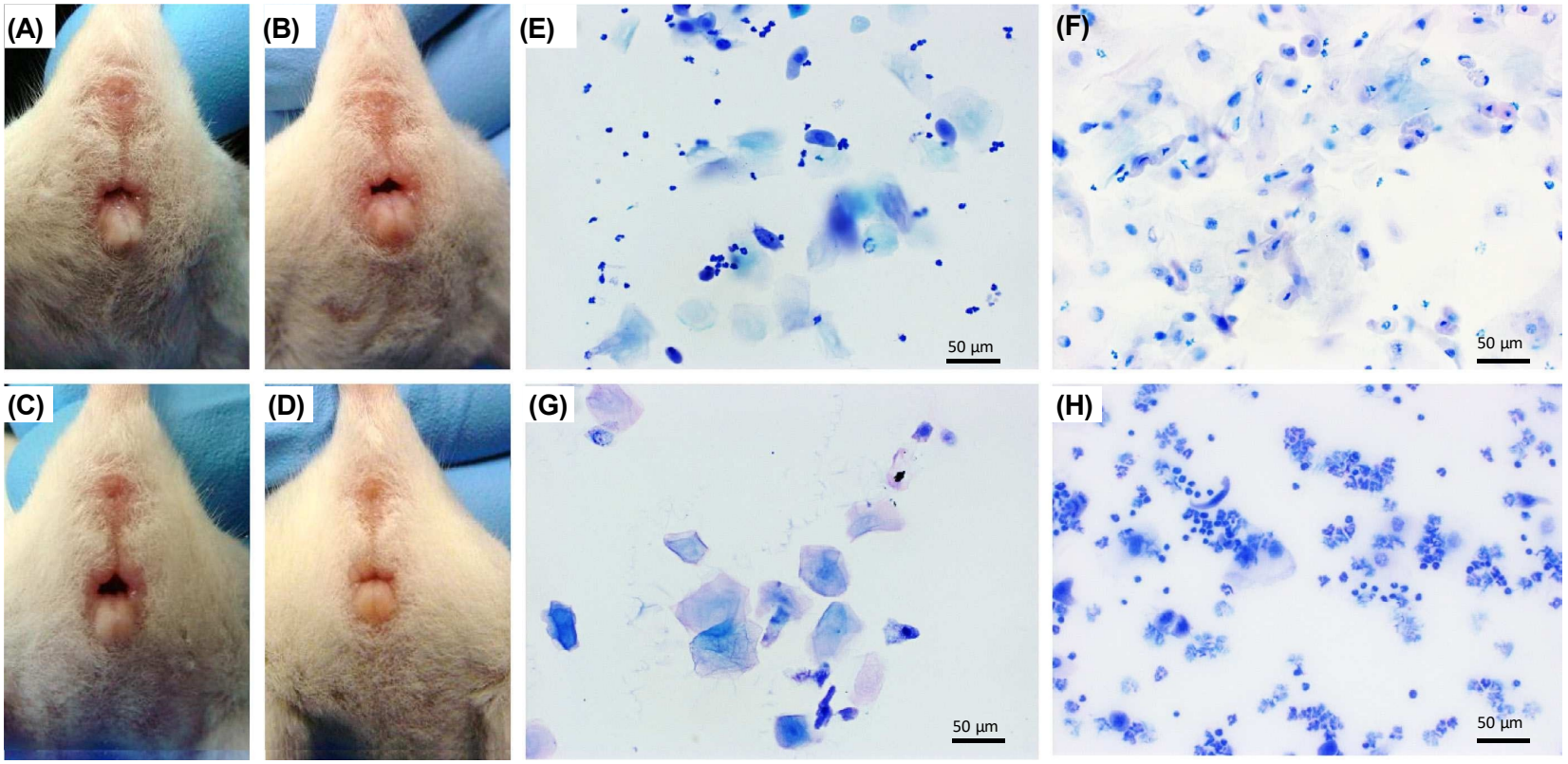


Different stages of mouse oocyte in vitro maturation: (A) Germinal vesicle (GV, arrow; nucleolus, triangular symbol). (B) Germinal vesicle breakdown (GVBD). (C) Extruding the first polar body (arrow) of mouse oocytes (PBI).

https://www.researchgate.net/publication/259201313/figure/fig2/AS:667781531959317@1536222932555/Different-stages-of-mouse-oocyte-in-vitro-maturation-A-Germinal-vesicle-GV-arrow.ppm

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.

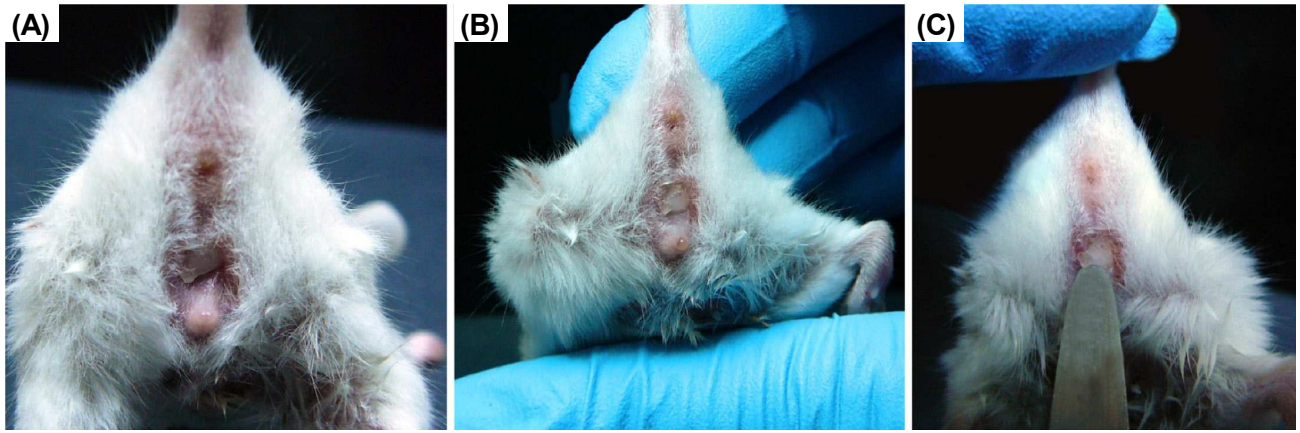
Estrous cytology is characterized by the predominance of cornified epithelial cells.

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.

http://dx.doi.org/10.1016/B978-0-12-394445-0.00001-1

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.

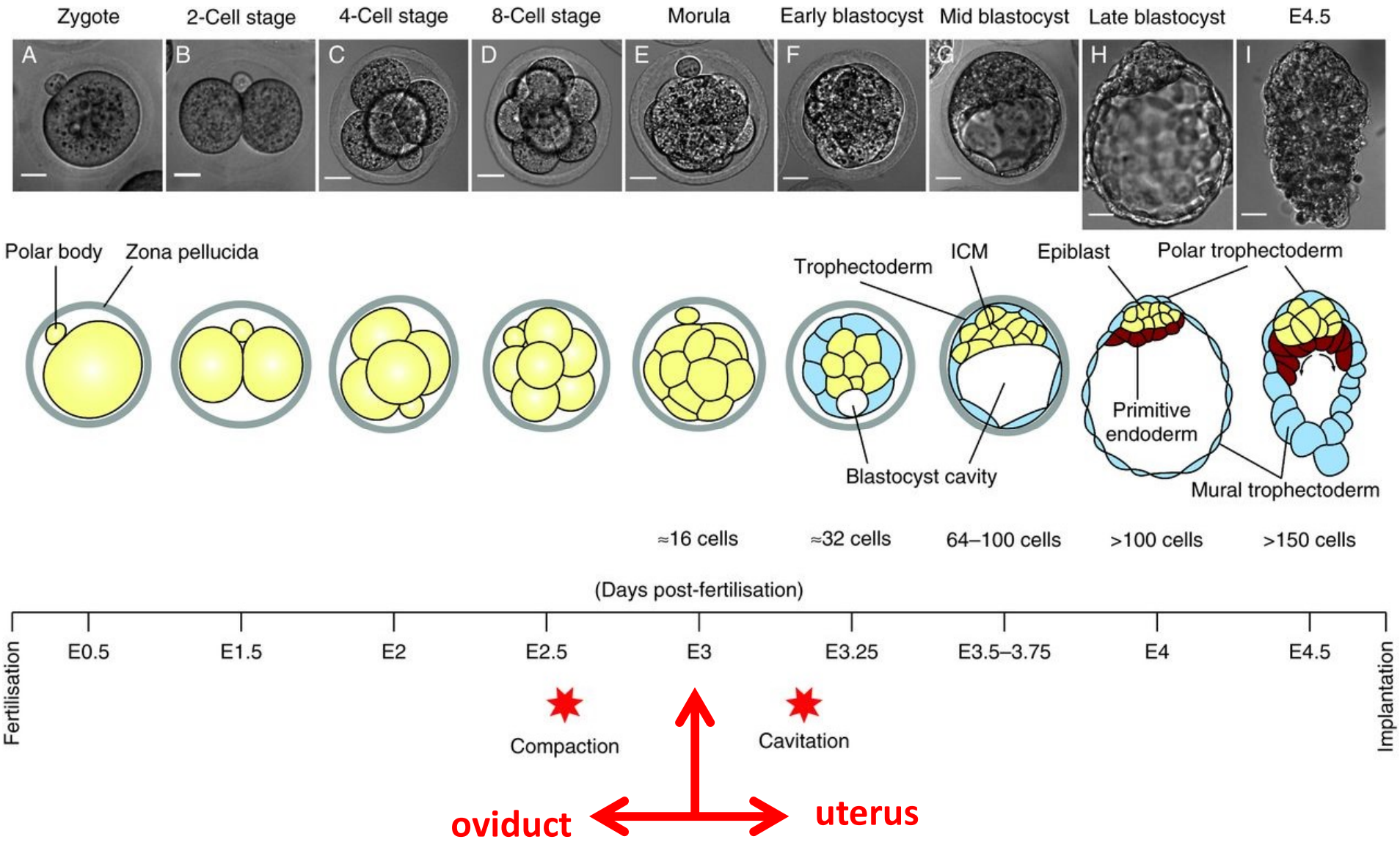
The **color** of the plug can vary from somewhat translucent to opaque and from white to yellow.

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

To visualize the plug well, it is recommended that the mouse be picked up by the tail and examined in a vertical position, rather than examined in her natural, horizontal standing position. To assess the texture of the plug and to ensure that it is not placed deeply against the cervix, use of a round-edged microspeculum is always recommended. It is important to clean the speculum between mice to prevent the spread of microorganisms. Sterile saline or water is recommended for this since contact with chemical disinfectants, including alcohol, is highly irritating to the delicate vaginal mucosa.

http://dx.doi.org/10.1016/B978-0-12-394445-0.00001-1

Pre-implantation embryos (zygotes to blastocyst)



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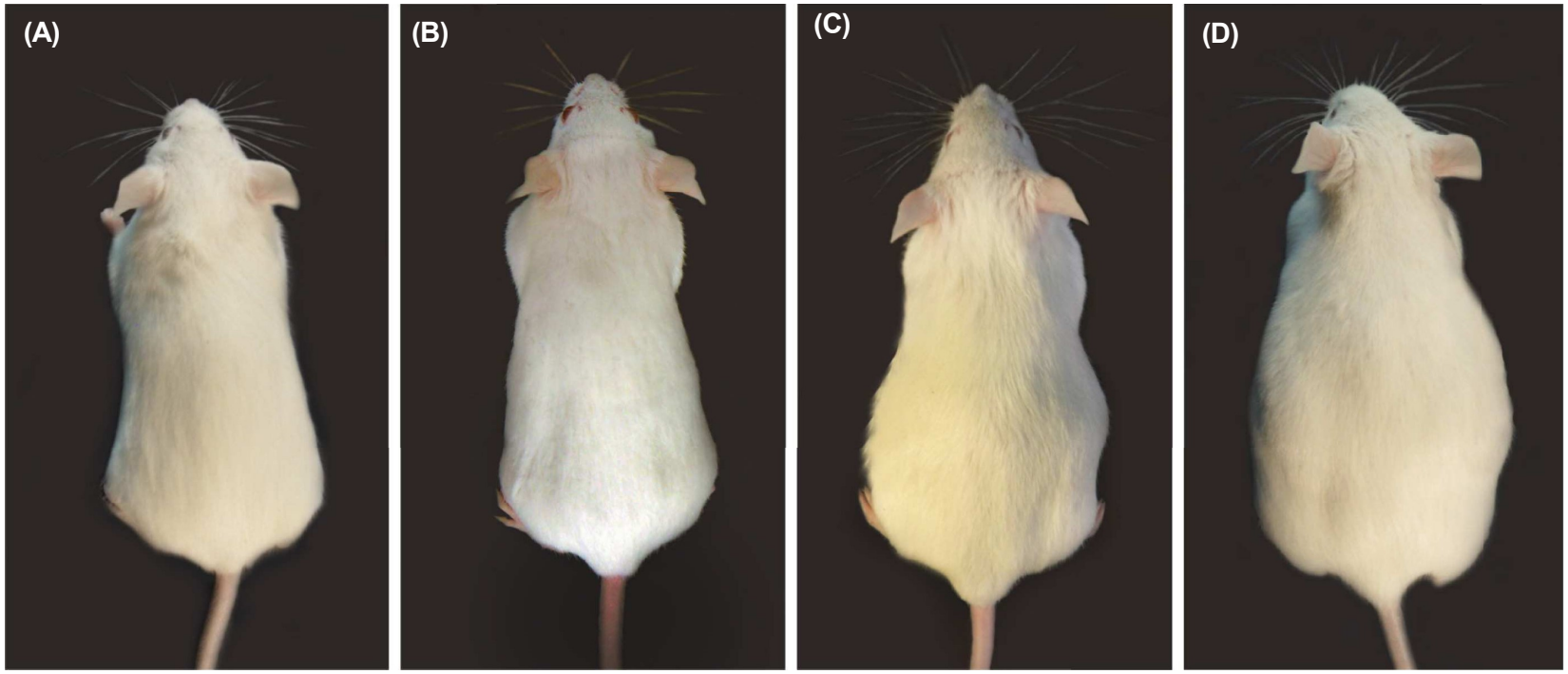
Physical changes in body appearance during a mouse pregnancy



(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.

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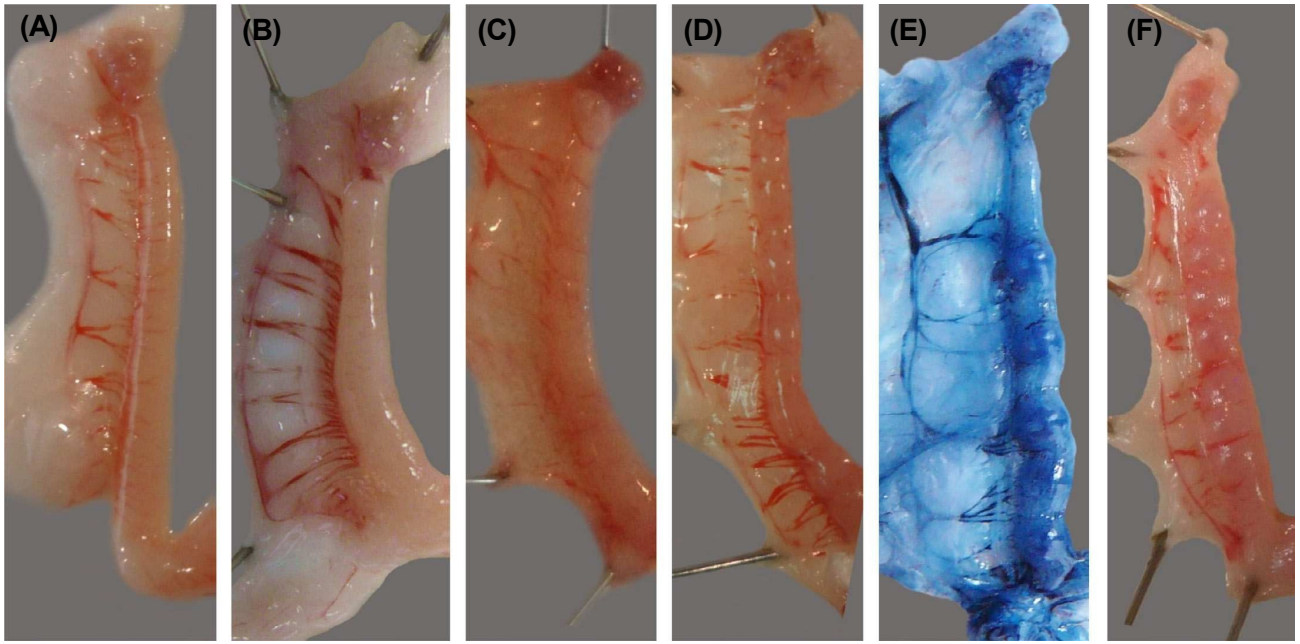
Early post-implantation uterus (gestational days 4.5–6.5)



(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 5.5 having received an external jugular vein injection of 0.2 ml of 10% (wt/vol) Chicago Sky Blue 6B solution in saline 2 min before euthanasia. Five darker blue sites can be identified between the ovary and cervix.

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

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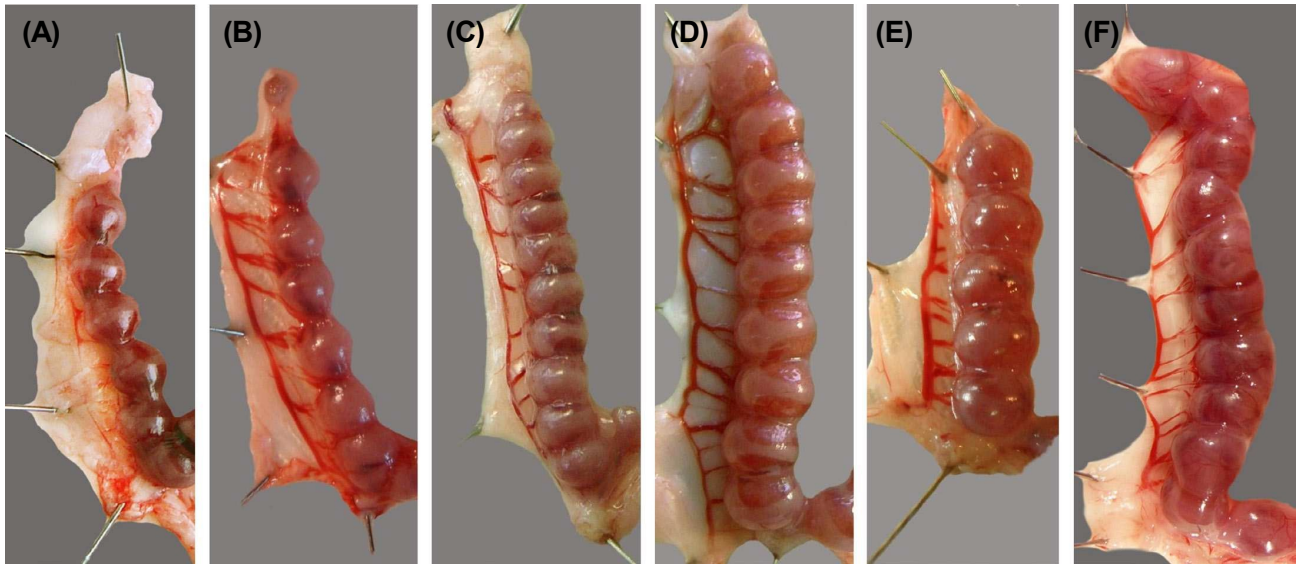
Gravid uterus from gestational days 7.5–12.5



(A) **Gestational day 7.5** implantation sites are oval in shape and contain a cylindrically shaped, primitive streak embryo that is supported by invading trophoblasts and other membranes. Mesometrial trophoblasts, which are the primordia of the placenta, form the *ectoplacental cone*. Ectoplacental cone invasion results in a hemorrhagic center within each implantation site.

(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. The most hyperemic area is central and is thought to represent the limit of trophoblast invasion.

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

(D) **Gestational day 10.5** implantation sites are larger. The placenta now 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 now 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.

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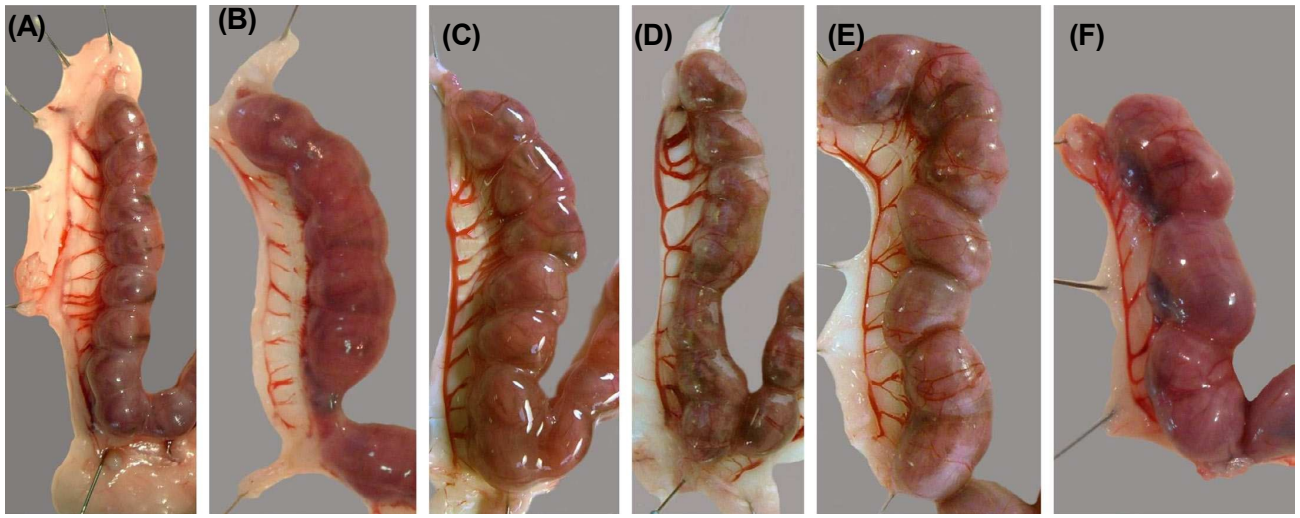
Gravid uterus from gestational days 13.5–18.5



(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.

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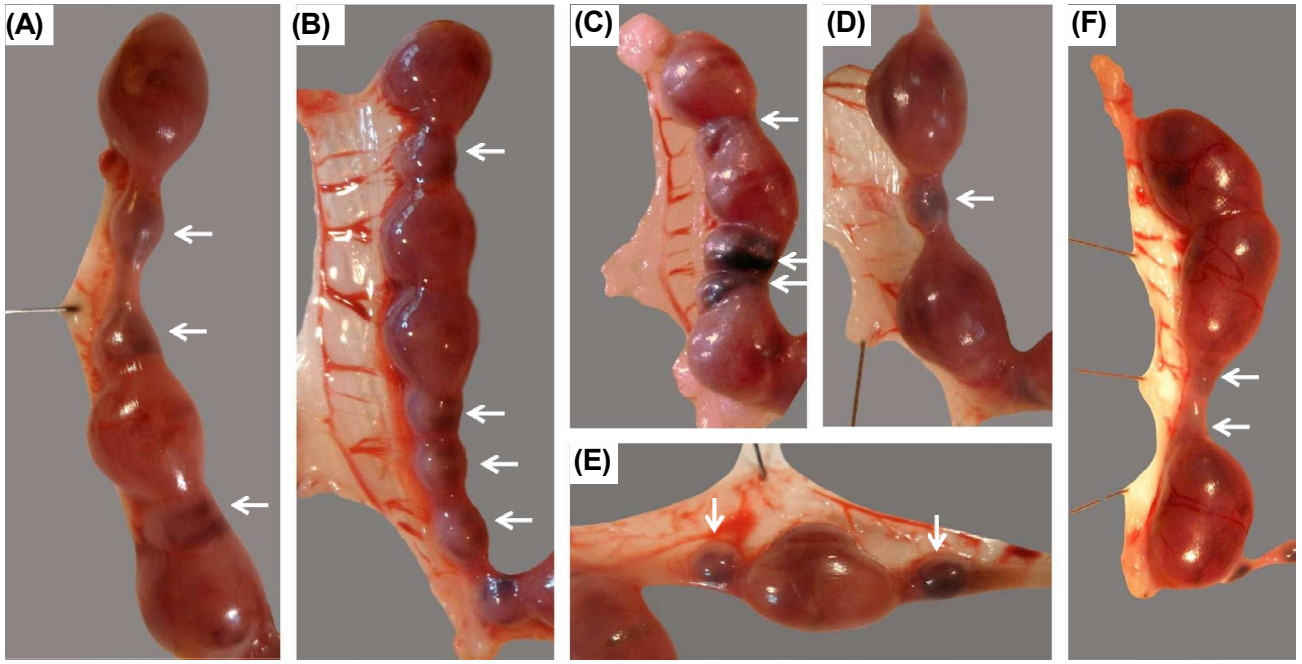
Process of fetal death and implantation site resorption



(A) **Uterine horn at gestational day 11.5** showing three resorbing (arrow) and three viable implantation sites.

(B) **Uterine horn at gestational day 12.5** showing four resorbing (arrow) and three viable implantation sites.

(C) **Uterine horn at gestational day 12.5** showing three resorbing (arrow) and three viable implantation sites.

(D) **Uterine horn at gestational day 15.5** showing one resorbing (arrow) and two viable implantation sites.

(E) **Uterine horn at gestational day 13.5** showing two resorbing (arrow) and one viable implantation sites.

(F) **Uterine horn at gestational day 17.5** showing two resorbing (arrow) and three viable implantation sites.

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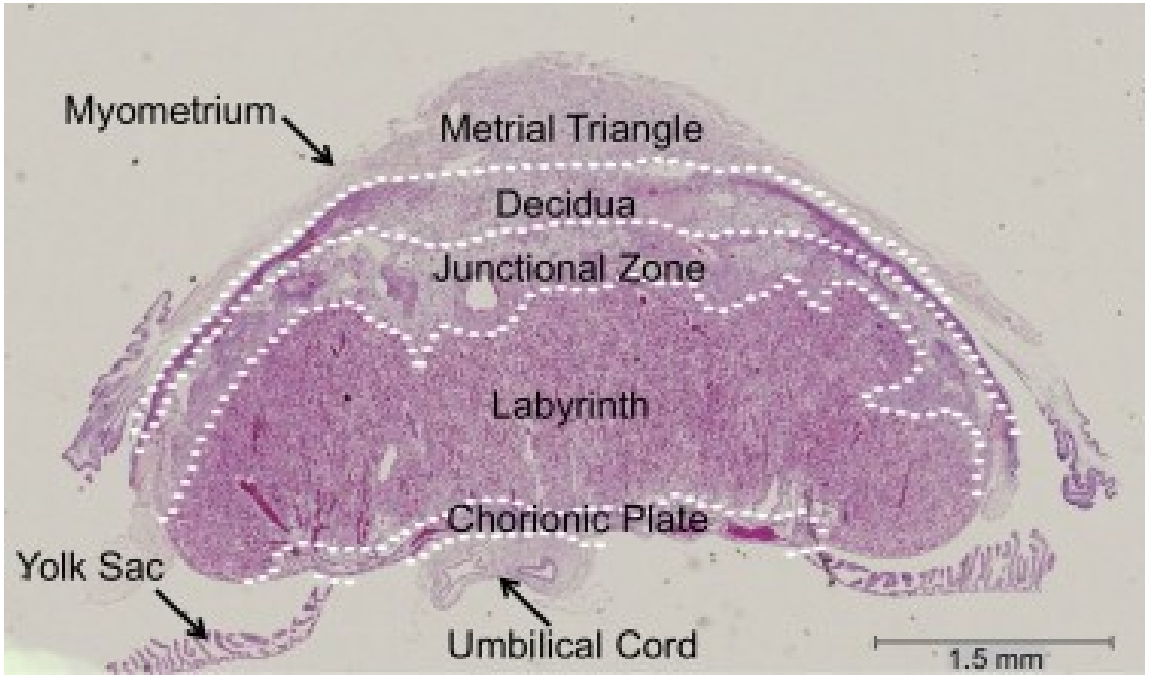
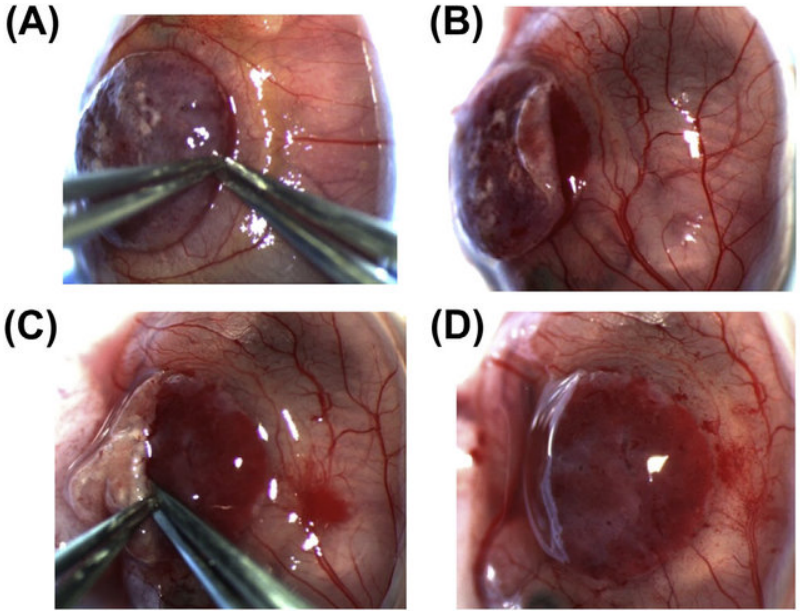
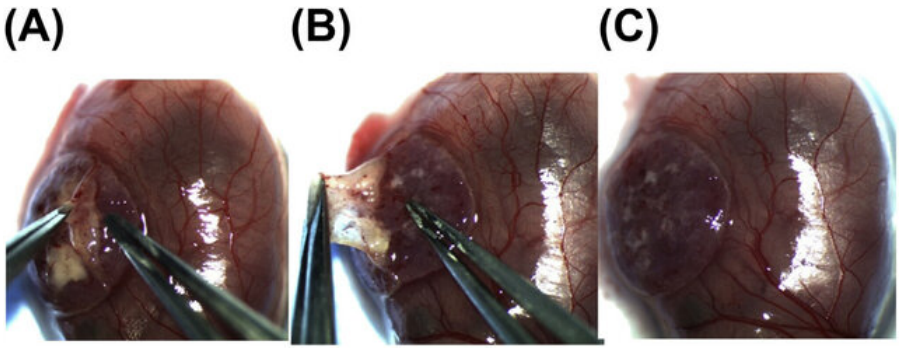


Histological section of mouse placenta (H&E staining). Dashed white lines indicate borders between adjacent layers of the placenta.

Dissection of the junctional zone layer (spongiotrophoblast)



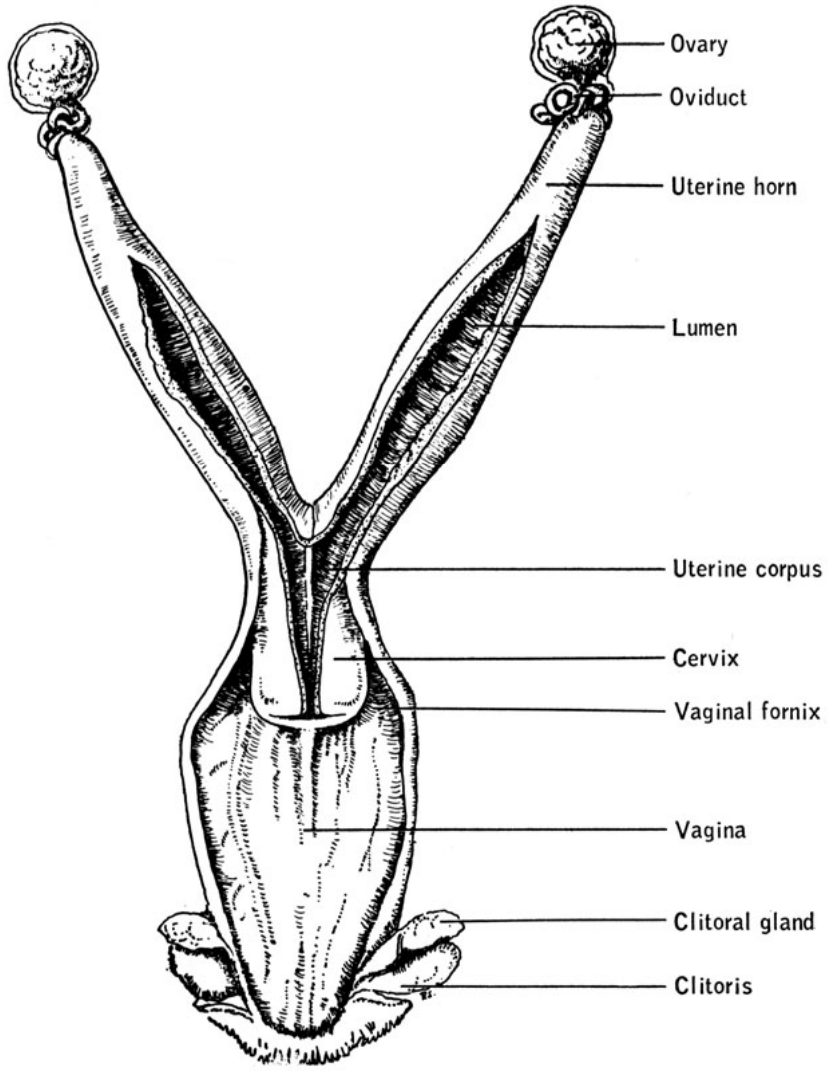
Dissection of the decidual layer



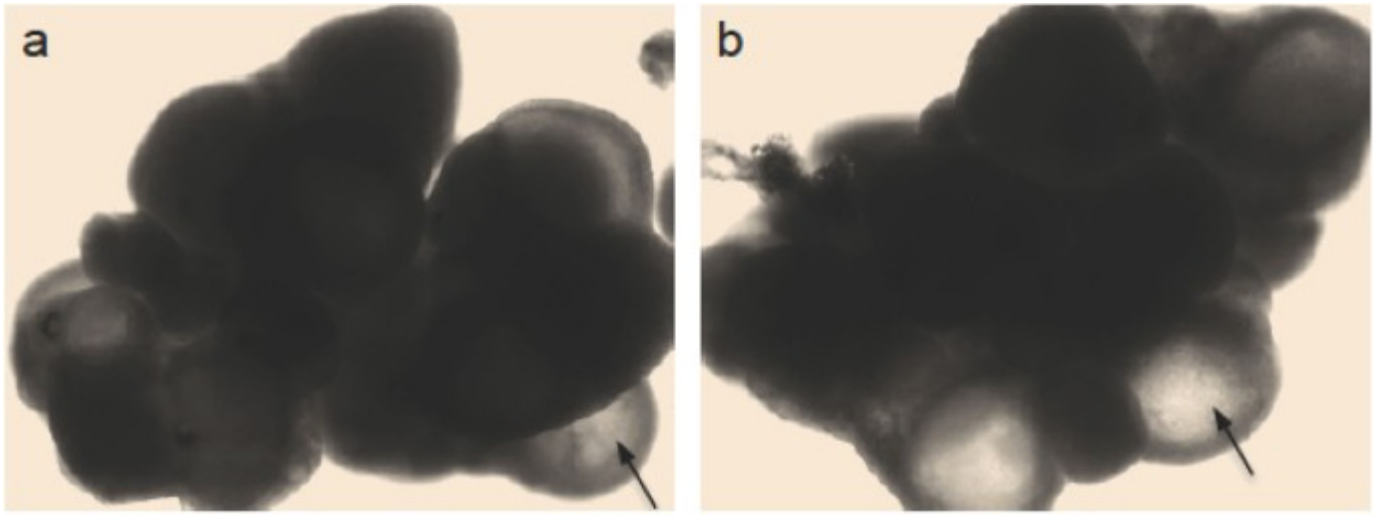
https://doi.org/10.1016/B978-0-12-394445-0.00044-8

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**

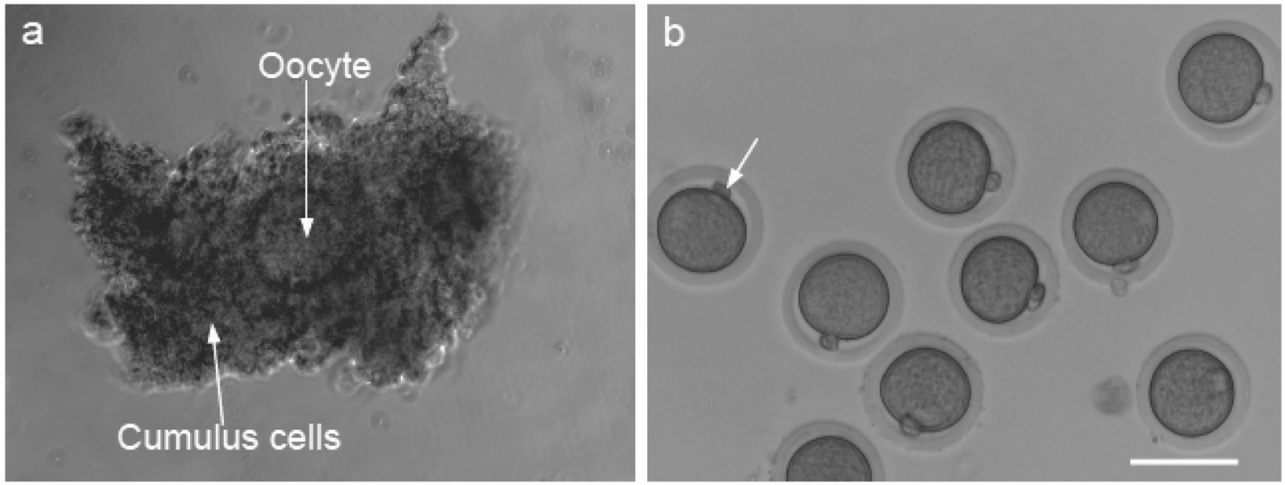


https://www.informatics.jax.org/greenbook/figures/figure13-53.shtml

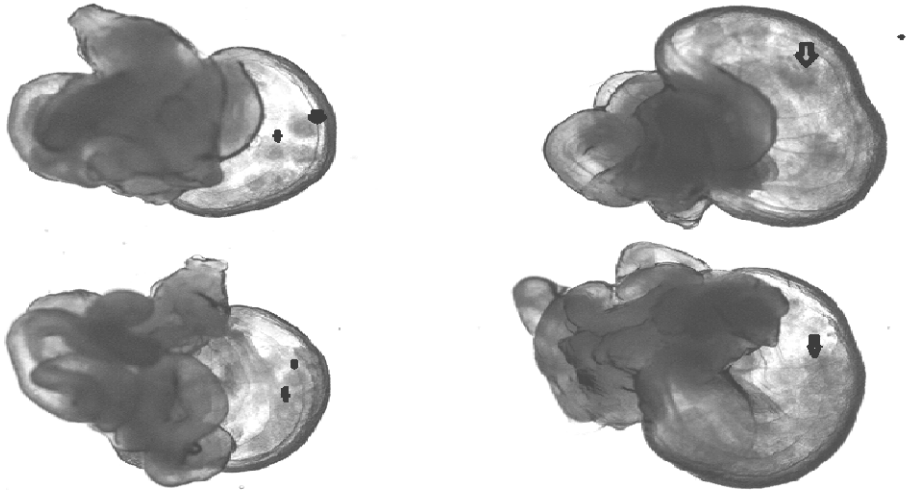


Typical images of ovaries collected from female deer mice following injection with either PMSG only (one dose, **a**) or PMSG & hCG (one PMSG and one hCG at 56 h after PMSG administration, **b**). Arrows indicates antral follicles.

https://doi.org/10.1038/srep12232

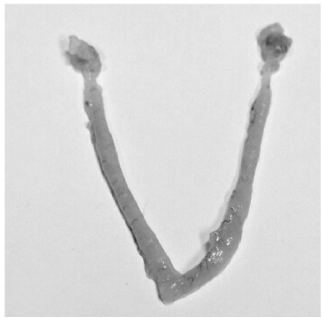


a) isolated from ovary (**b**) with a first polar body (arrow) isolated from in vitro maturation of the cumulus-oocyte complex in culture medium.

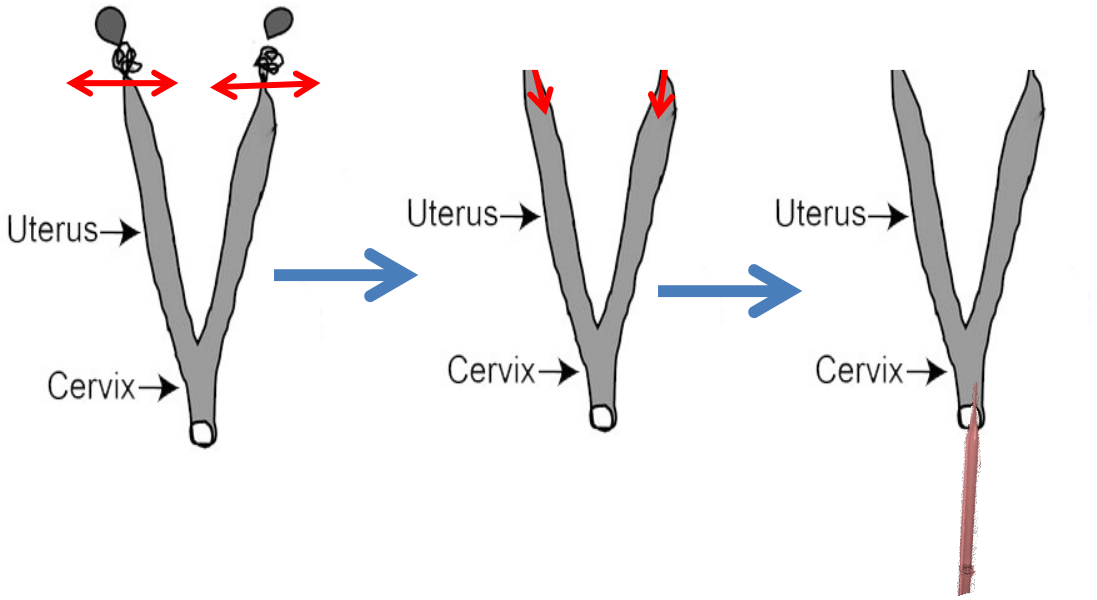


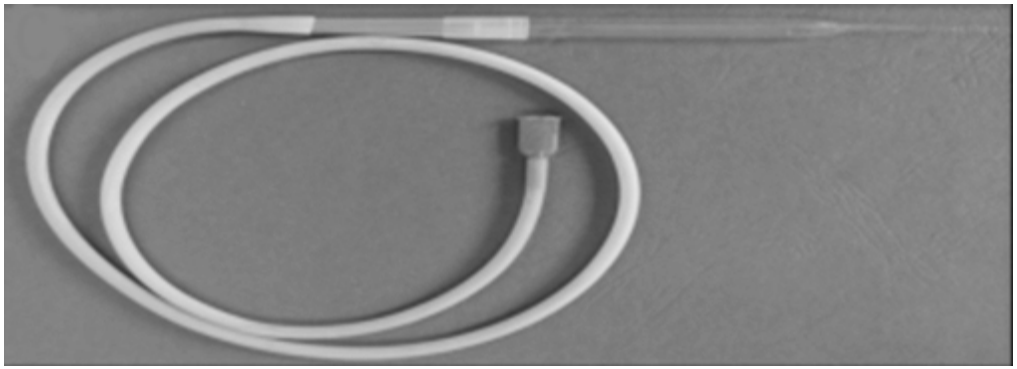
Oviduct isolation- isolate cumulus-oocyte complexes from the swollen ampula (arrows)

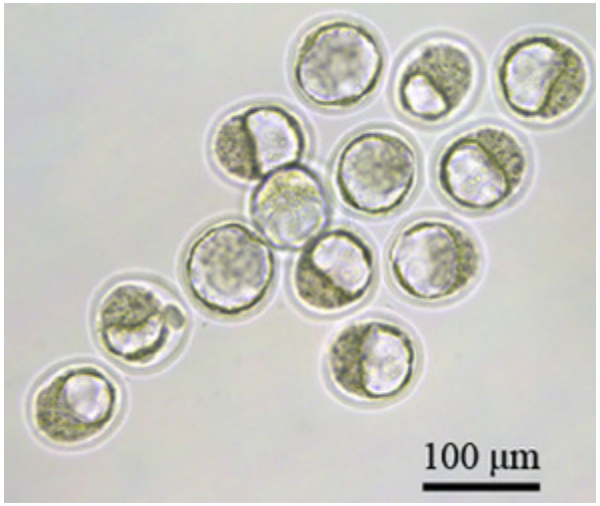
https://doi.org/10.1038/srep12232

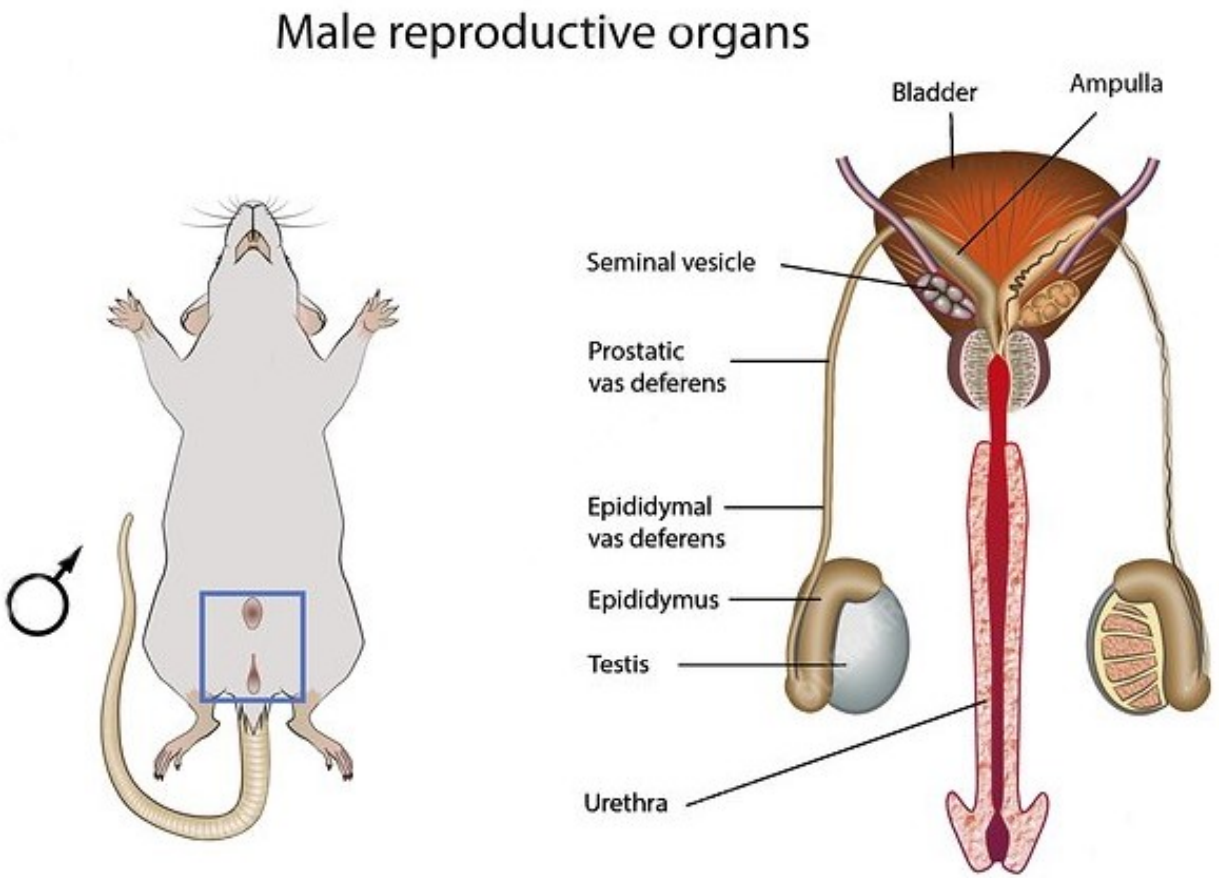


Isolate blastocysts by flashing the uterus

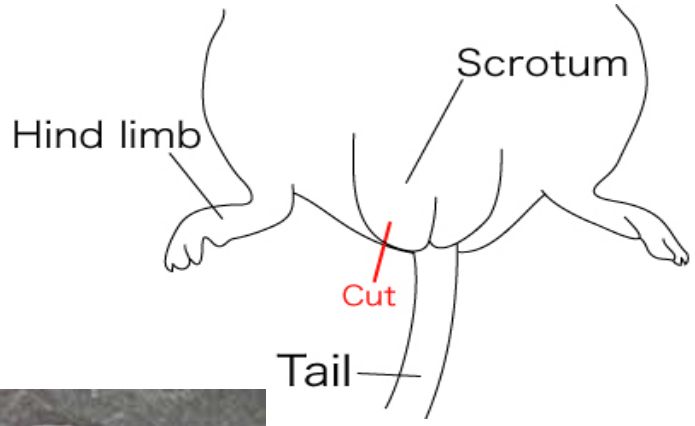


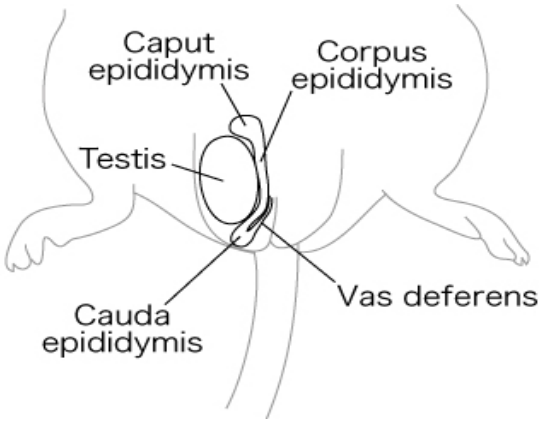


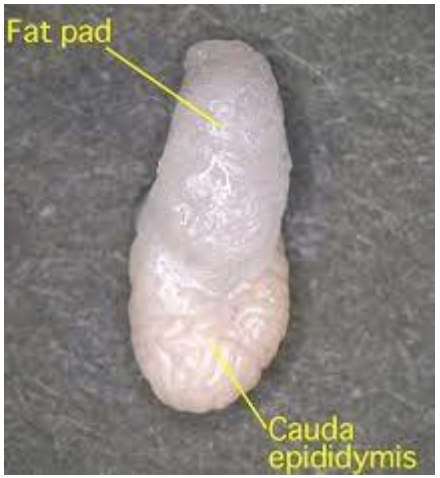


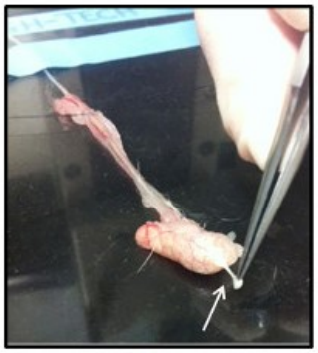


https://www.sciencephoto.com/media/1057775/view/mouse-male-reproductive-system-illustration

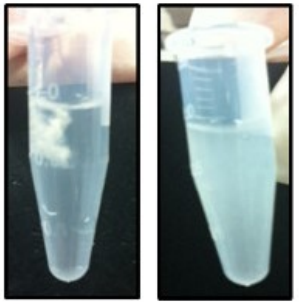


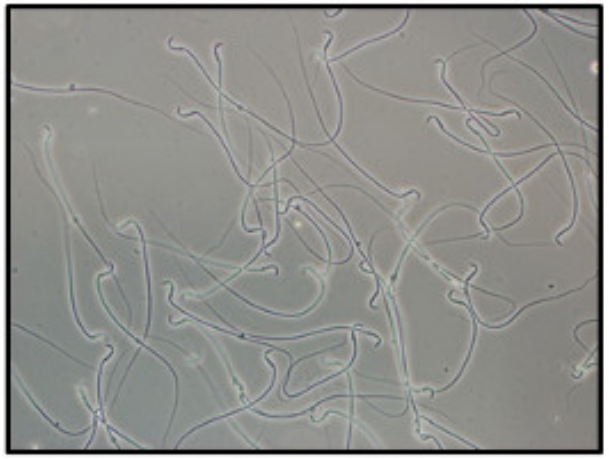


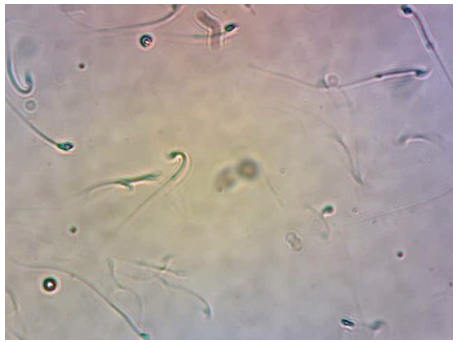


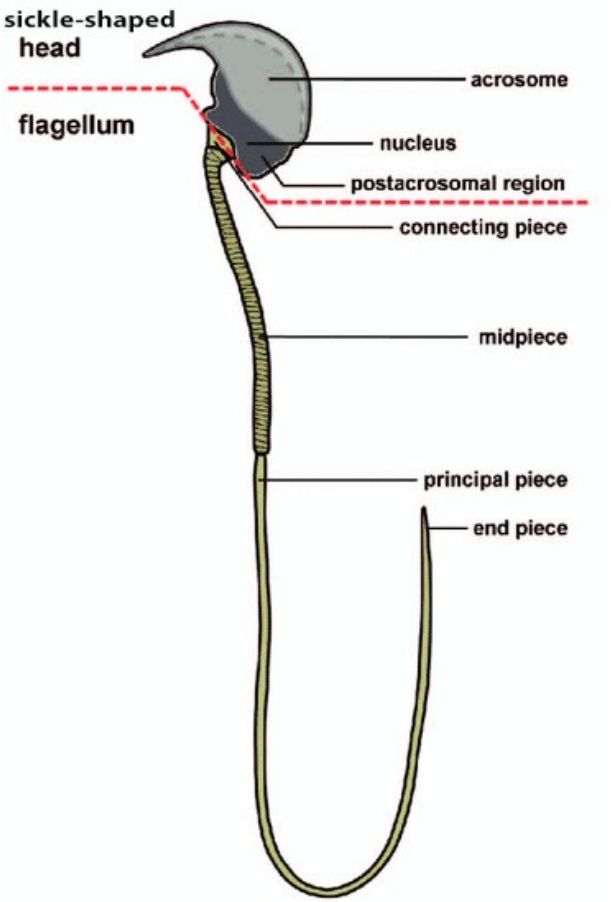


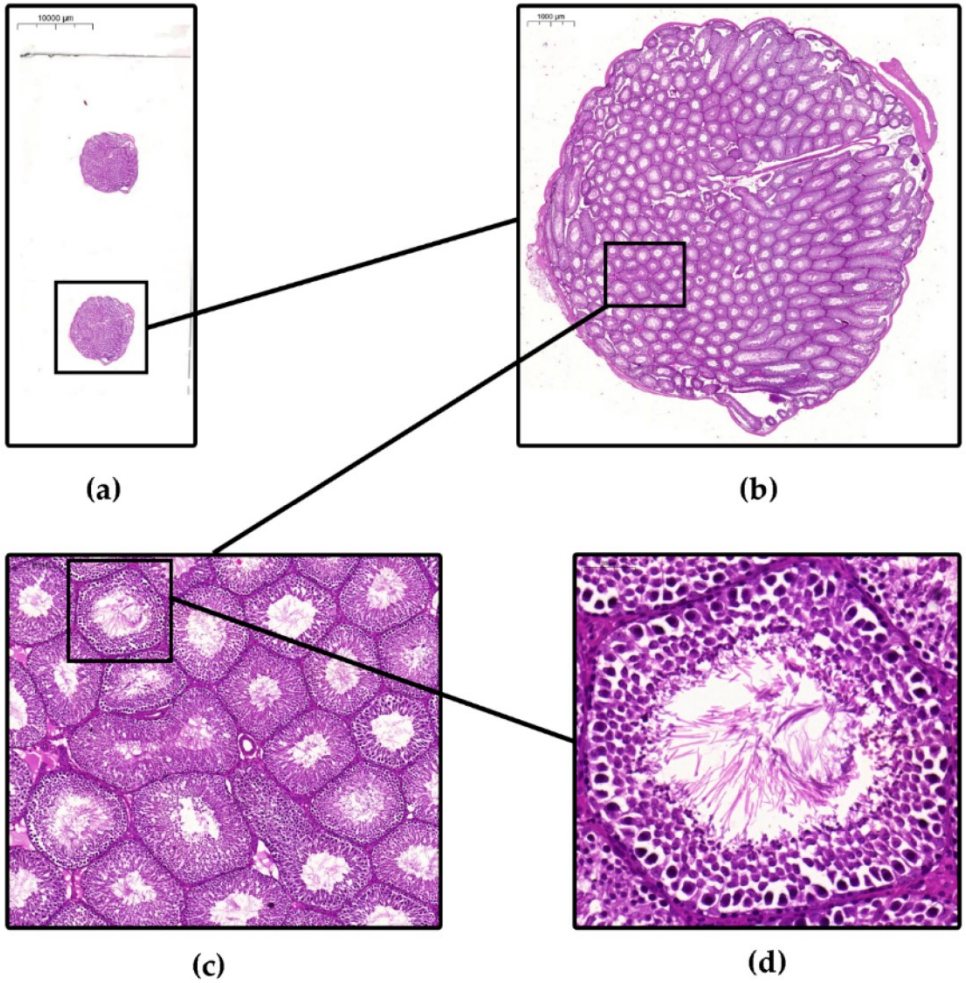
Cauda epididymal tubule

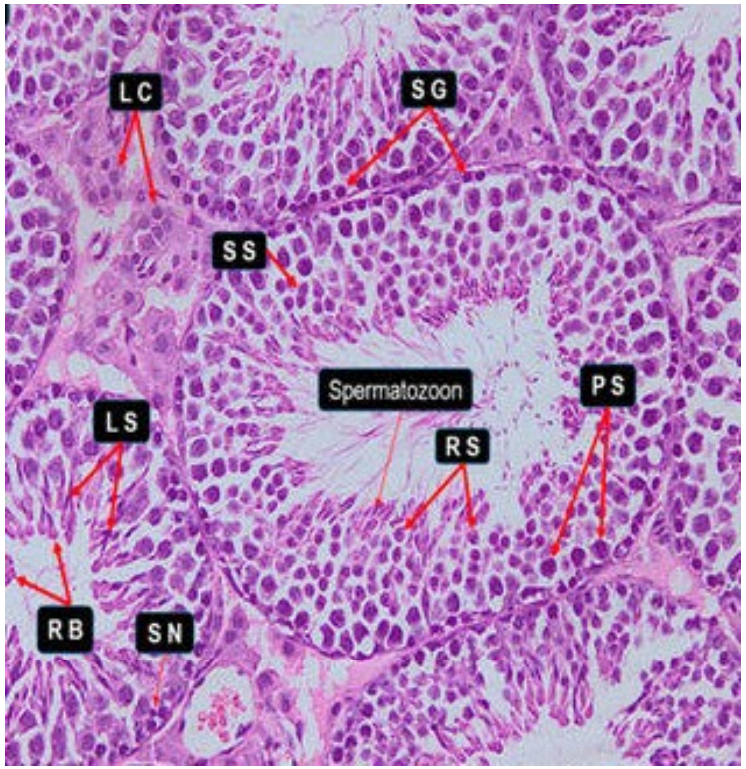






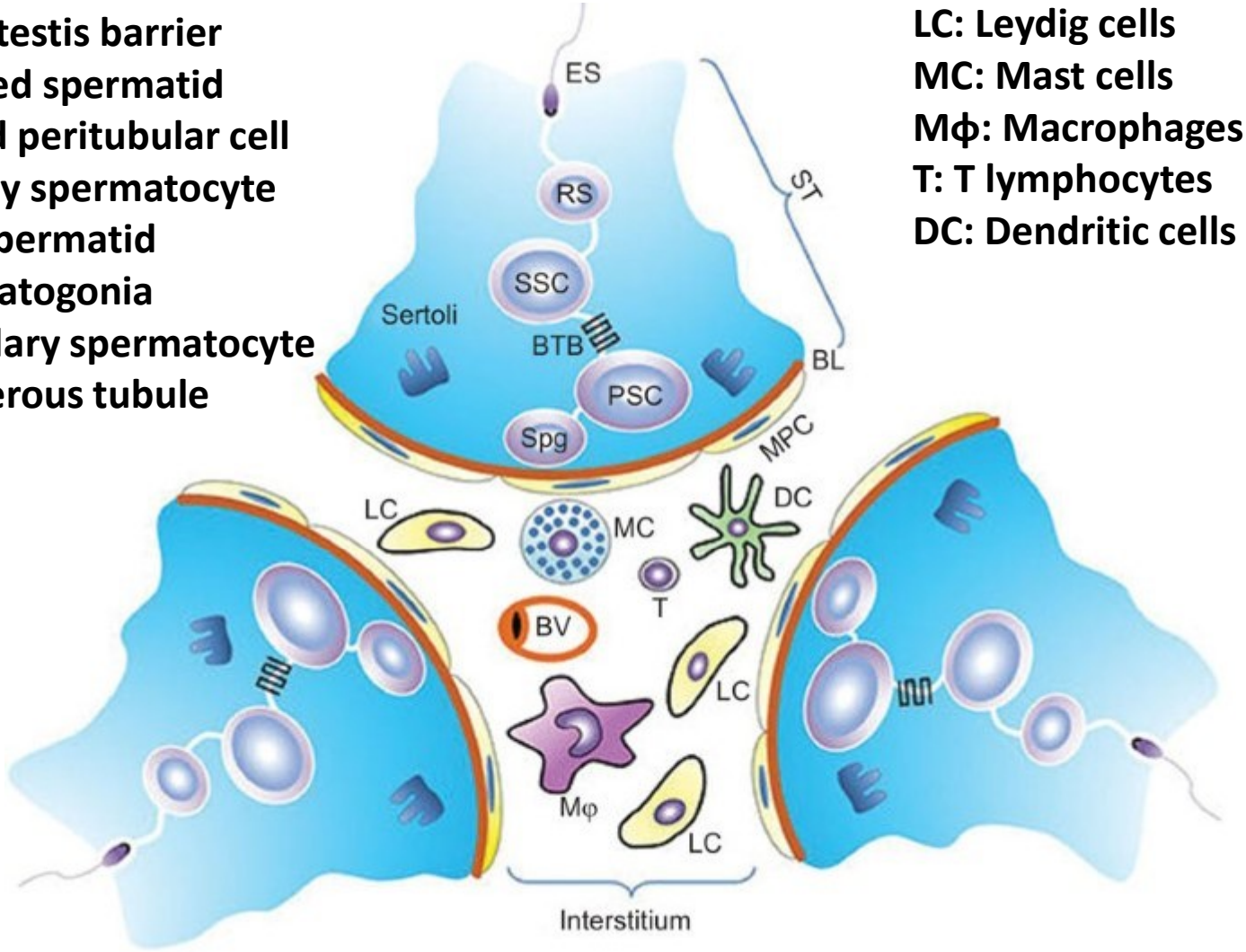




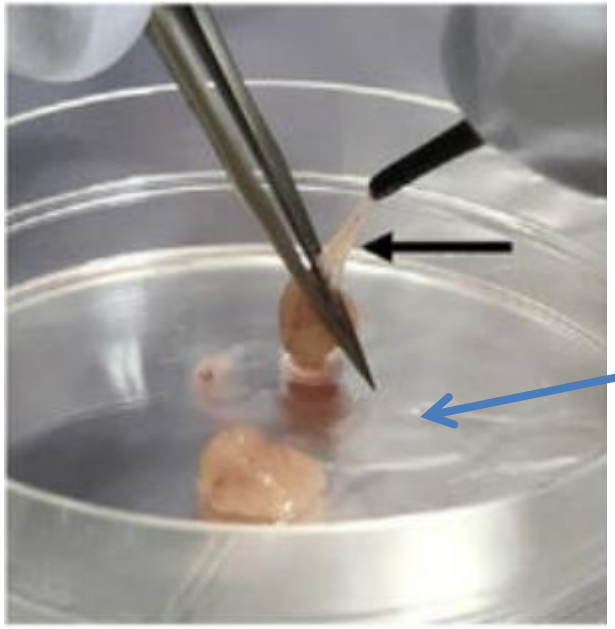


SC: Spermatids Cells
RB: Residual Bodies
RS: Round Spermatids
LS: Long Spermatids
SS: Secondary Spermatocytes
PS: Primary Spermatocytes
SG: Spermatogonia
LC: Leydig Cells

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

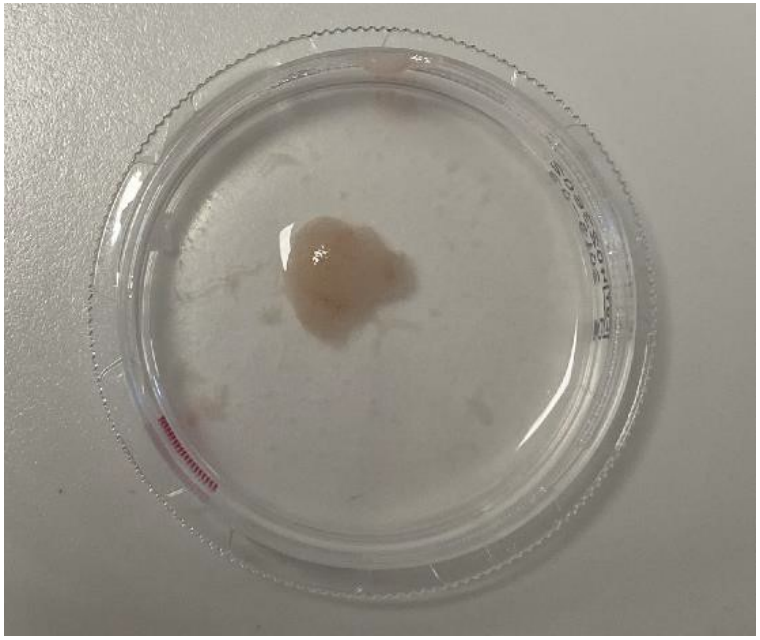


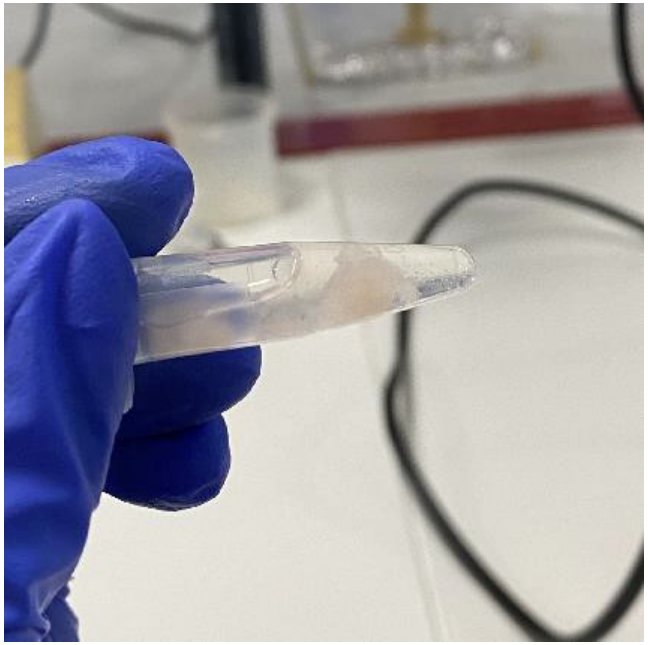
LC: Leydig cells
MC: Mast cells
Mϕ: Macrophages
T: T lymphocytes
DC: Dendritic cells



Removal of tunica albuginea

Medium containing cells from interstitial space





Isolate cells from seminiferous tubules with collagenase treatment

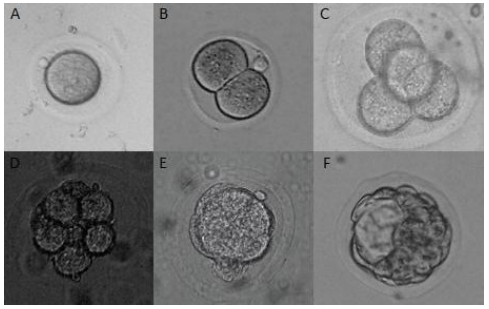
Superovulation

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).

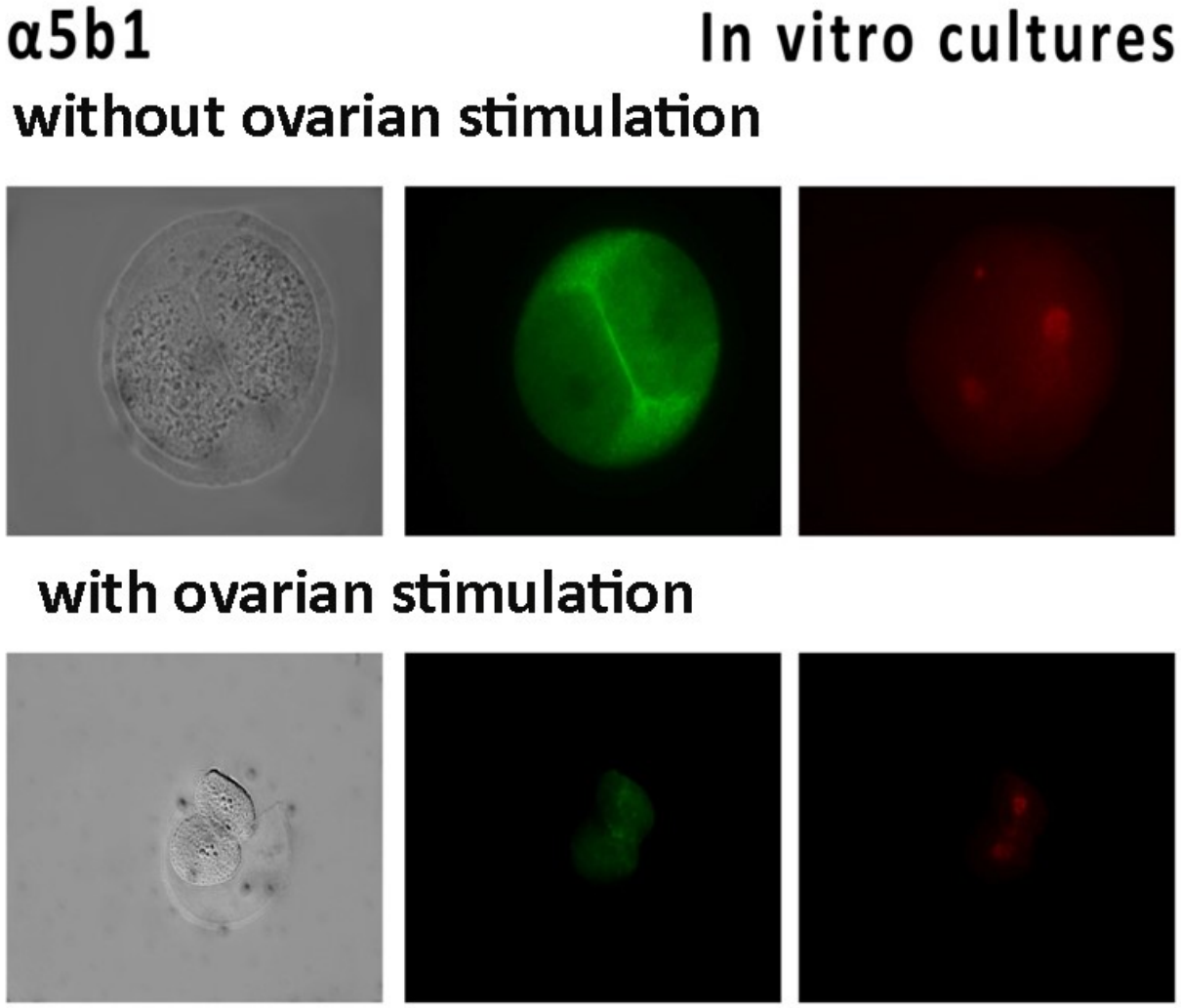
Example: PMSG on Day 1 at 1:00 p.m., hCG on Day 3 at 12:00 p.m. Cage each superovulated female with a fertile male 3-4 hours after the last injection (3-4:00 pm)

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



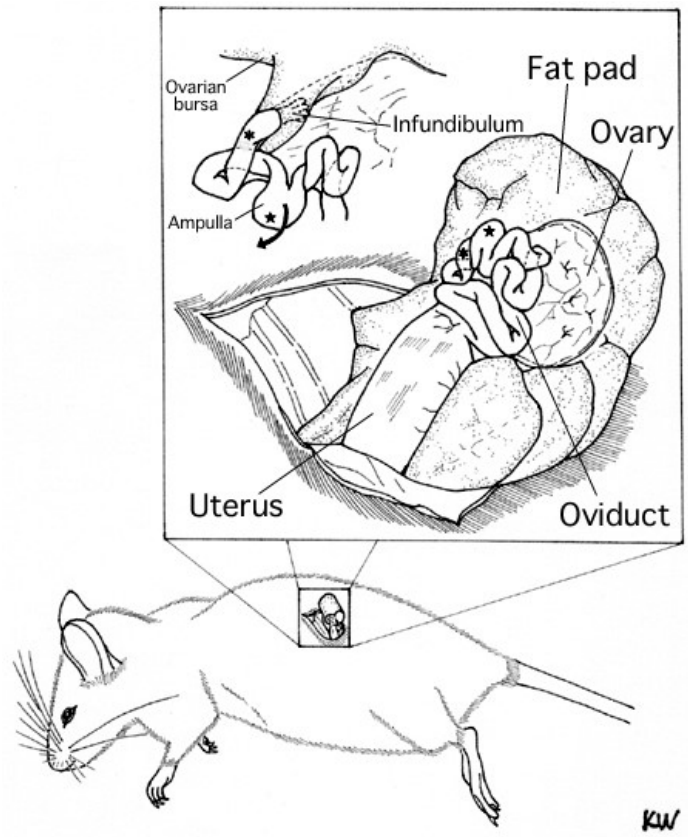
- Pronuclei are formed 6-7 h post- fertilization
- Within 2-3 h the second polar body is formed
- First cleavage occurs about 24 h post-fertilization
- The 8-cell embryos undergo compaction to form a solid ball of cells
- Blastocysts consist of the Inner Cell Mass (ICM), the trophoectoderm and the blastocoel

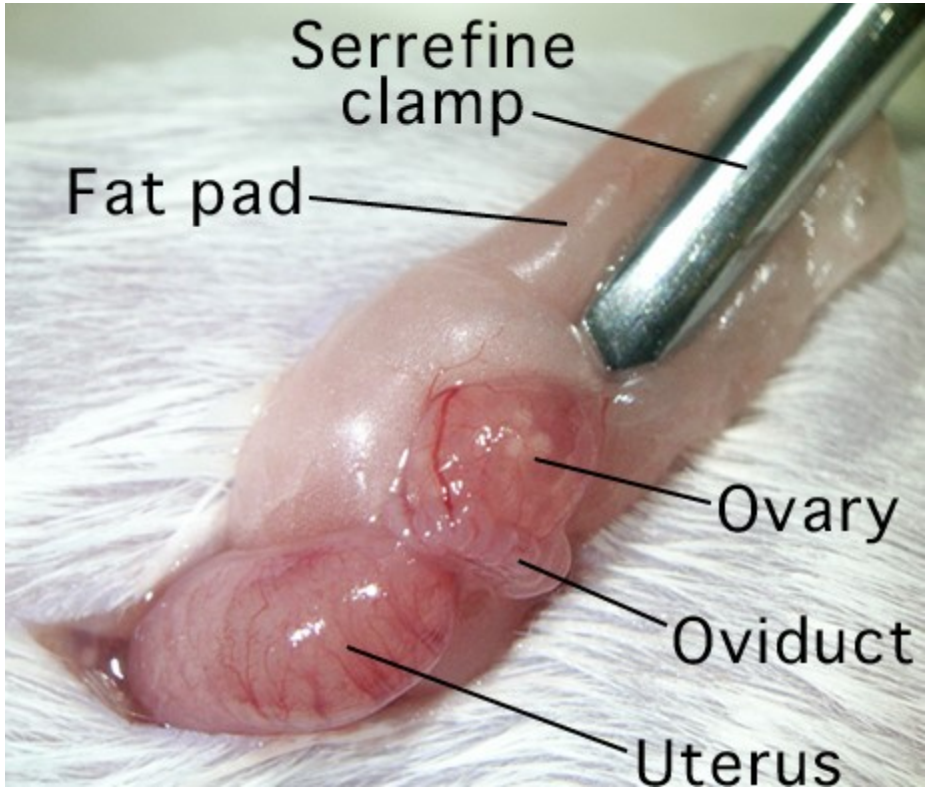
However,
ovarian stimulation shows different pattern of integrin expression in 2-cell and 4-cell embryos.

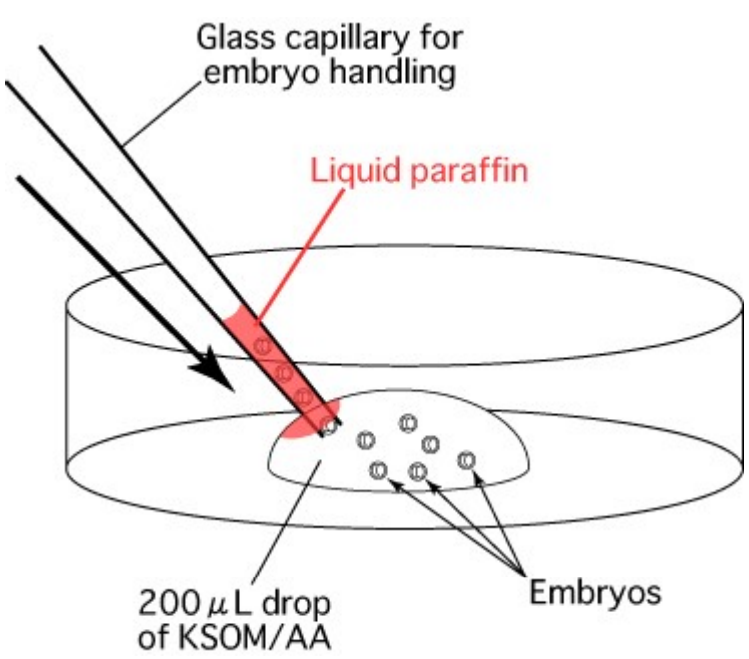


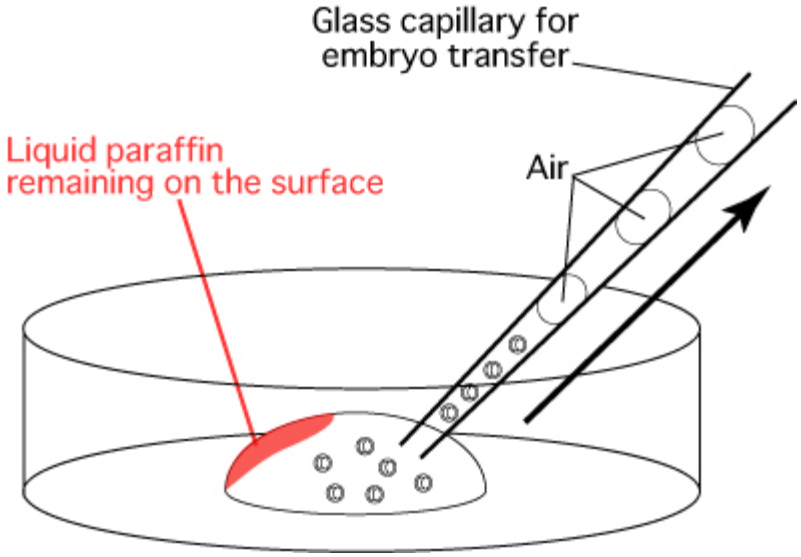
Embryo transfer

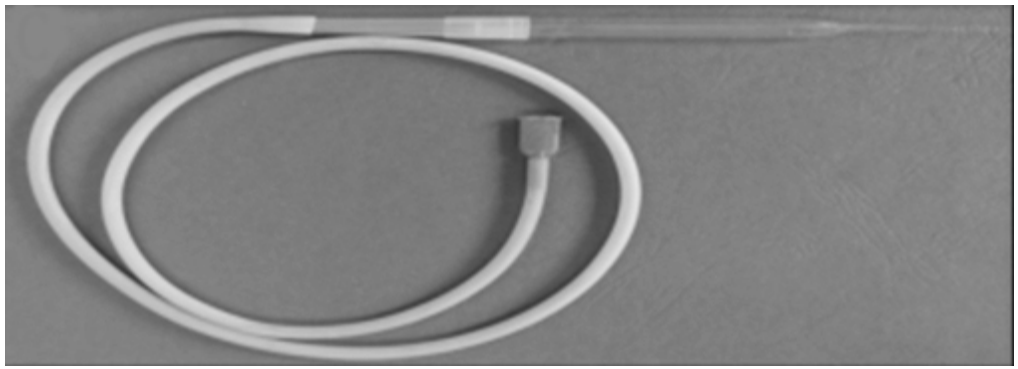
Most commonly day 3 embryos (day 0 corresponds to plug day) are transferred to the uterus of day 2 of pseudopregnant females (females mated with sterile males)

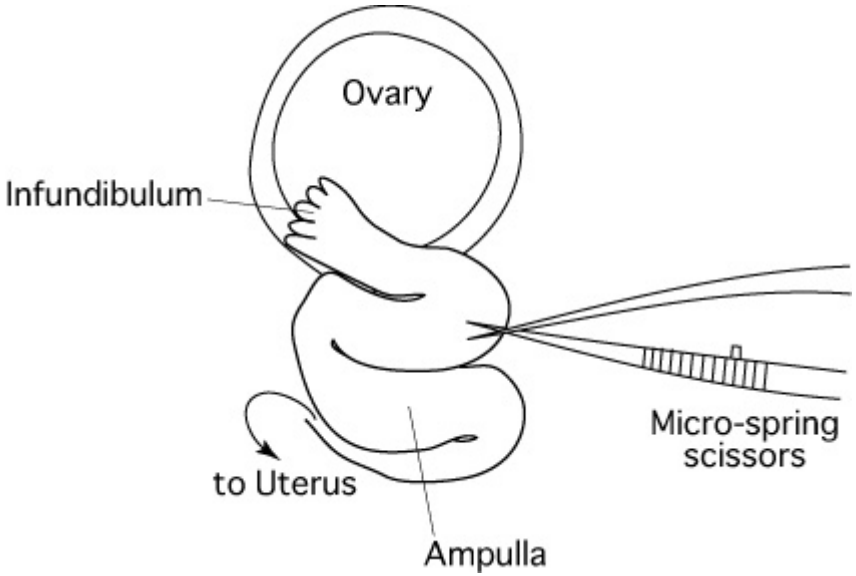


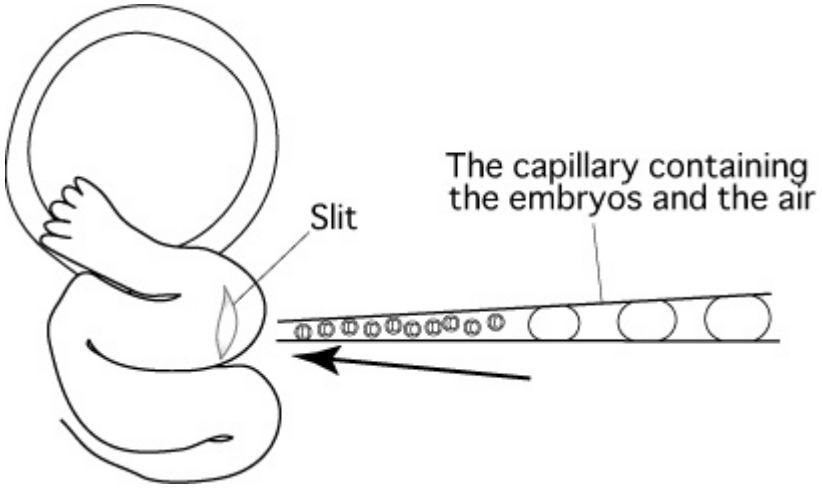


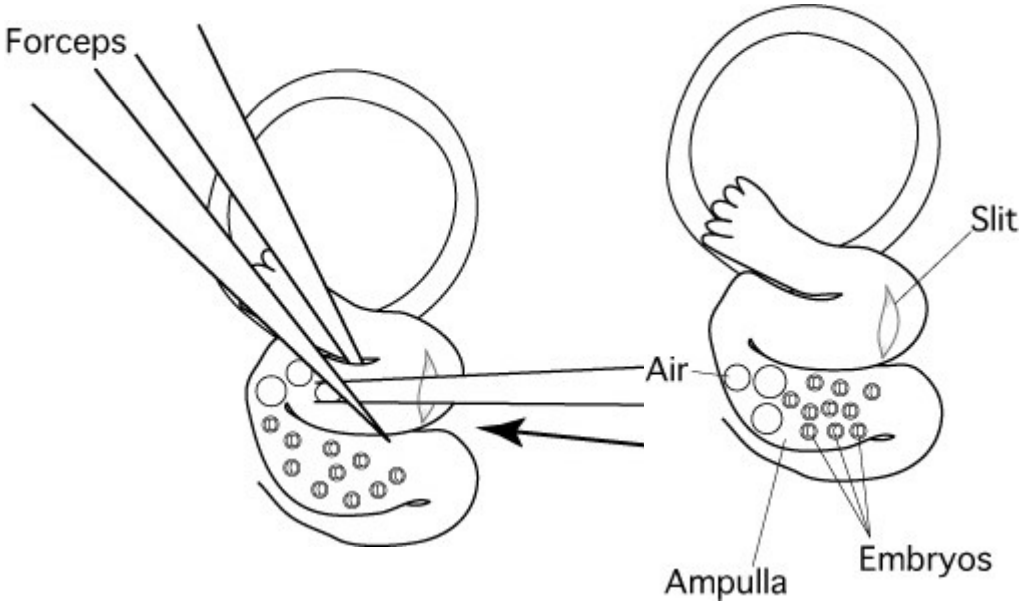

Glass capillary for
embryo handling
Liquid paraffin
200 μL drop
of KSOM/AA
Embryos

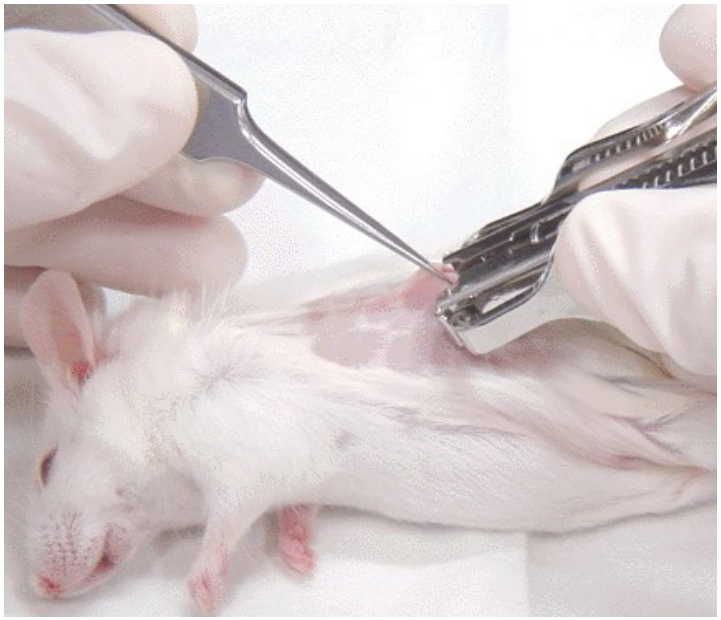

Glass capillary for
embryo transfer
Liquid paraffin
remaining on the surface
Air





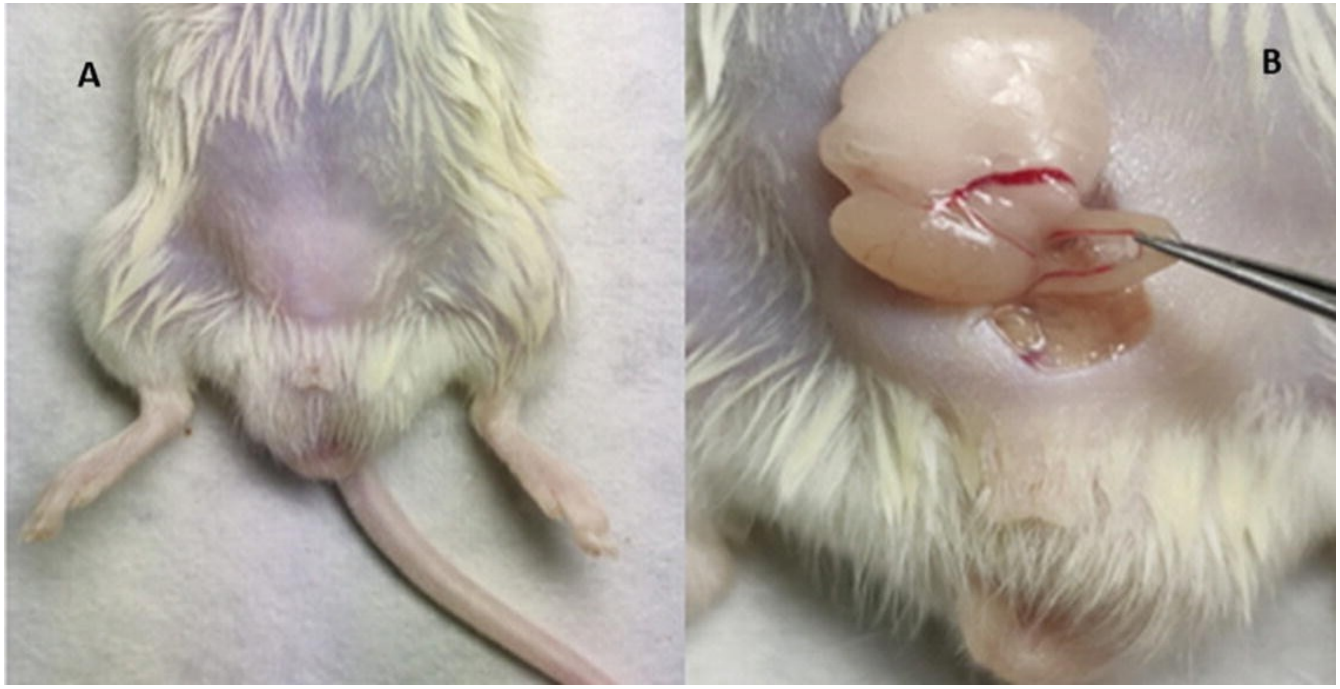


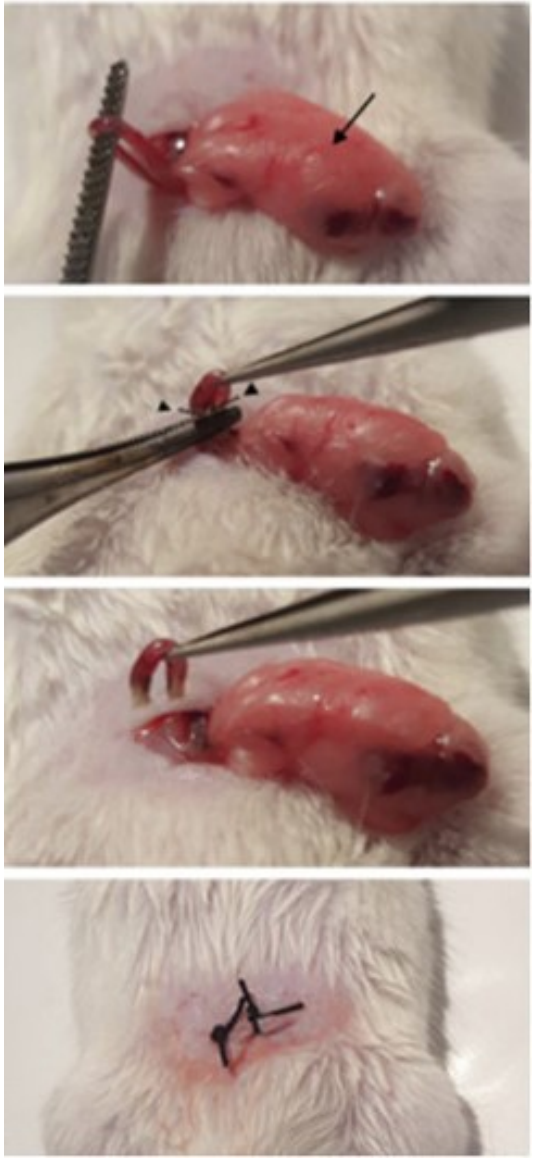




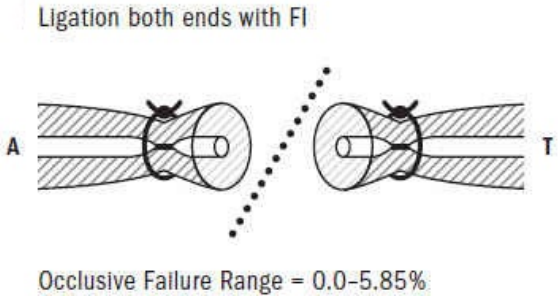
Push the ovary, oviduct and uterine horn back into the abdomen and close the wound using wound clips

Vasectomy

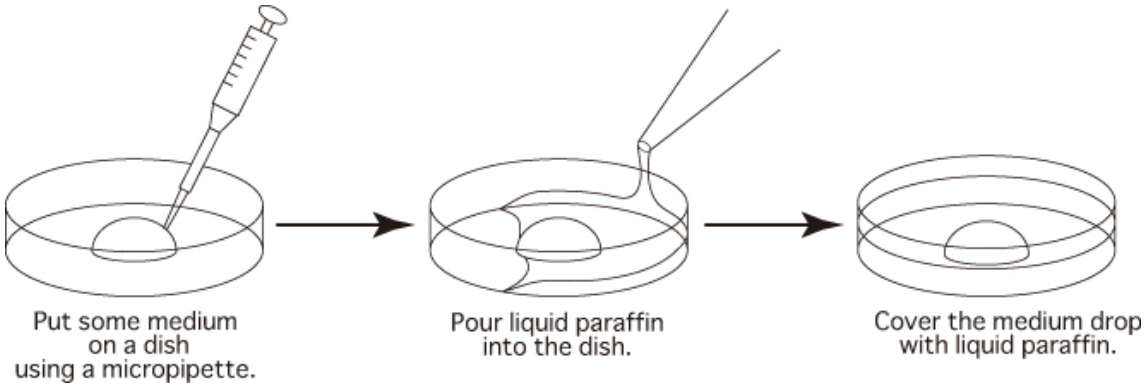


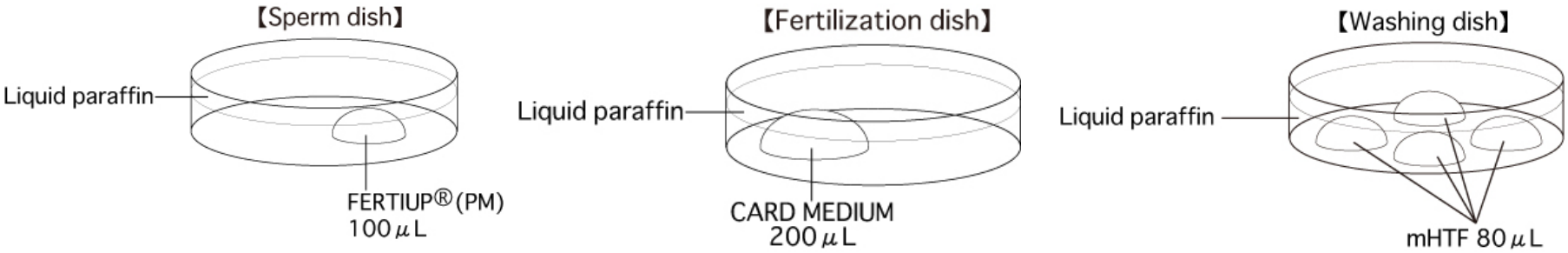


(**a**) Incision site for vasectomy. (**b**) A tubular structured vas deferens



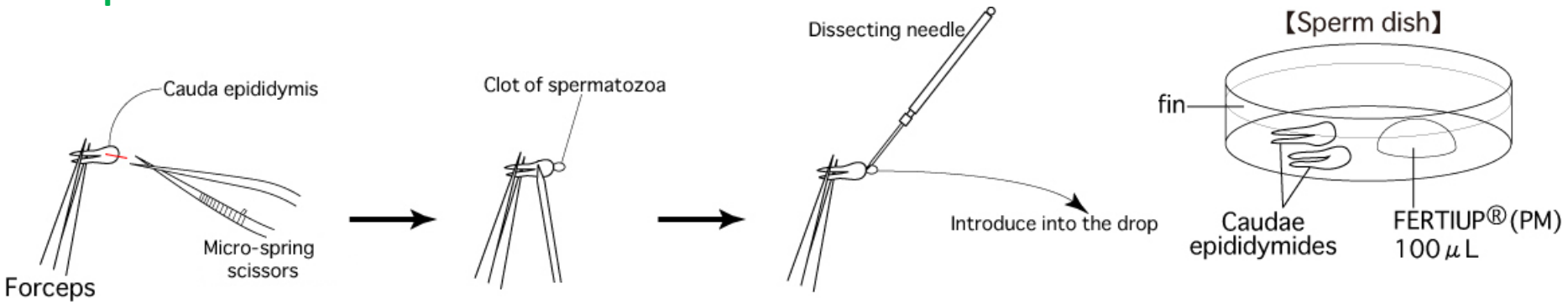
IVF: preheated-equilibrated media- standard CO2



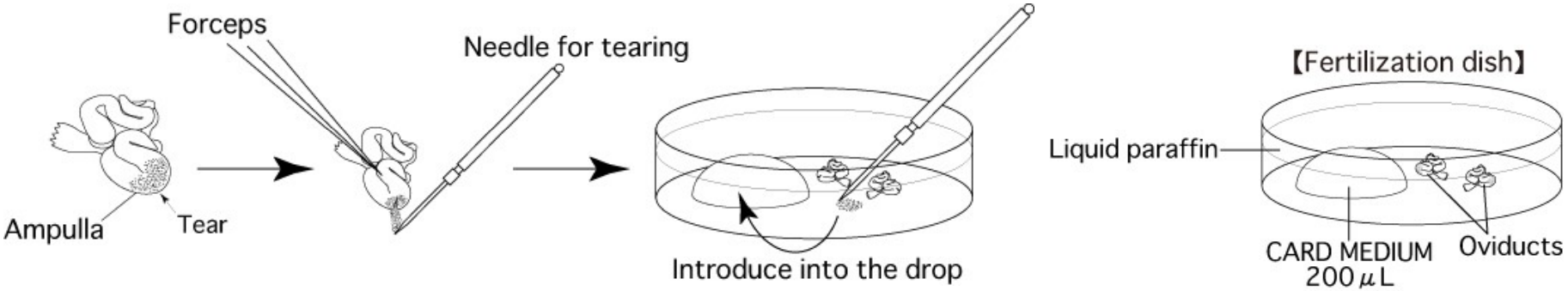


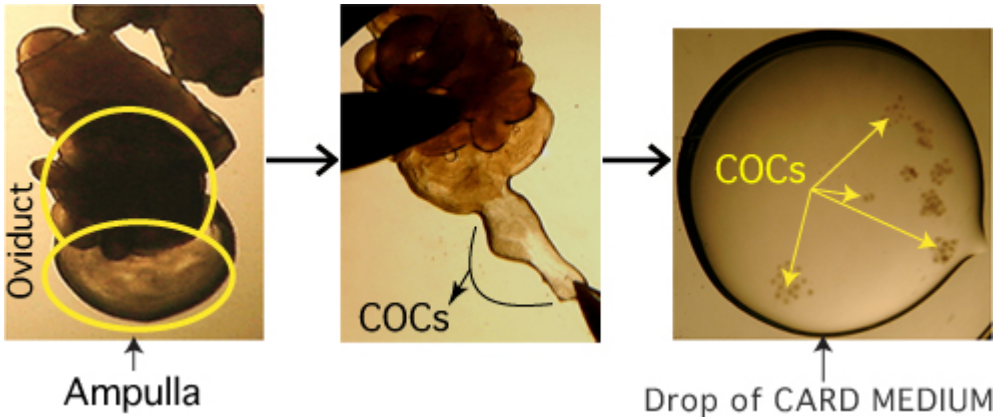
http://card.medic.kumamoto-u.ac.jp/card/english/sigen/manual/mouseivf.html

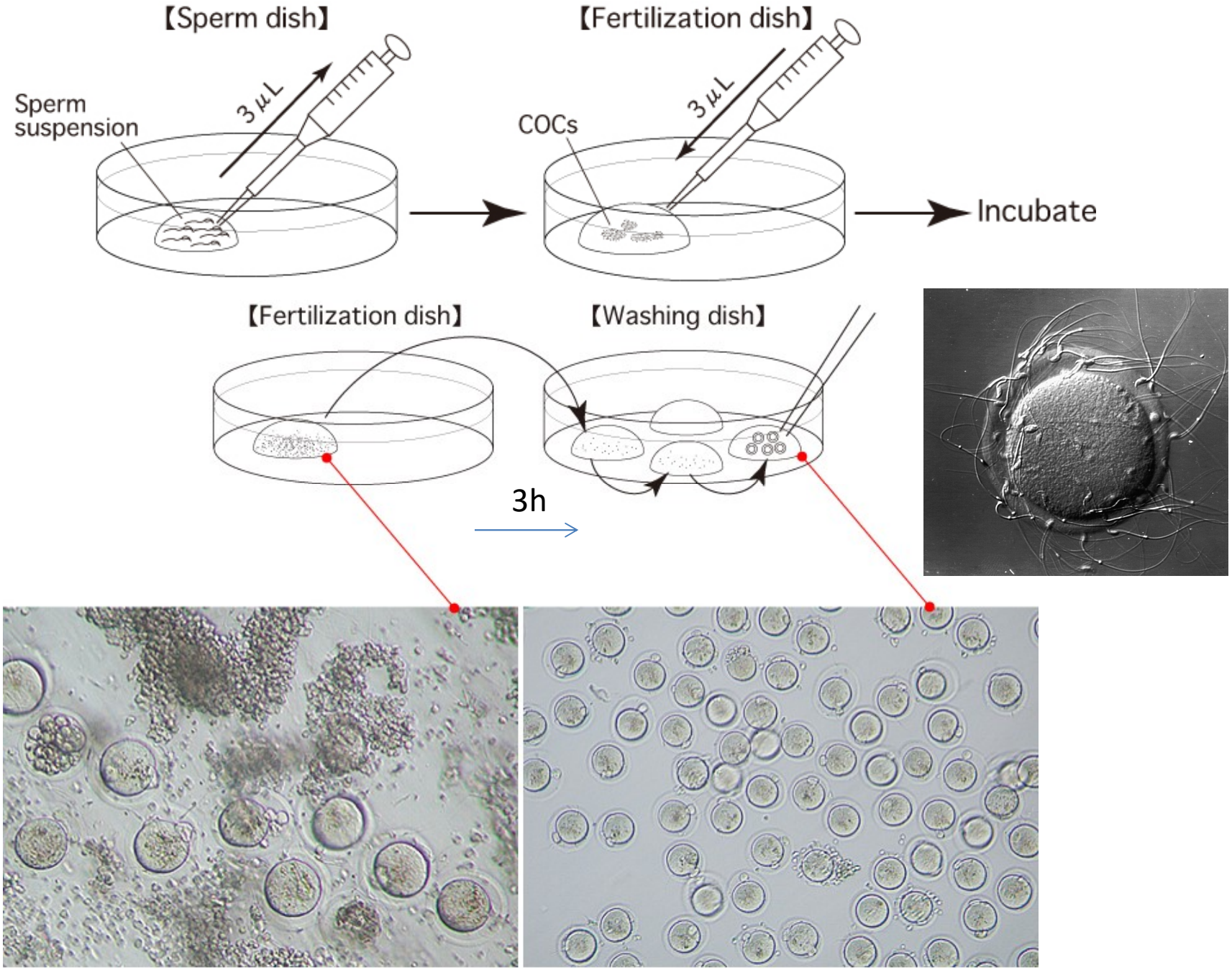
sperm



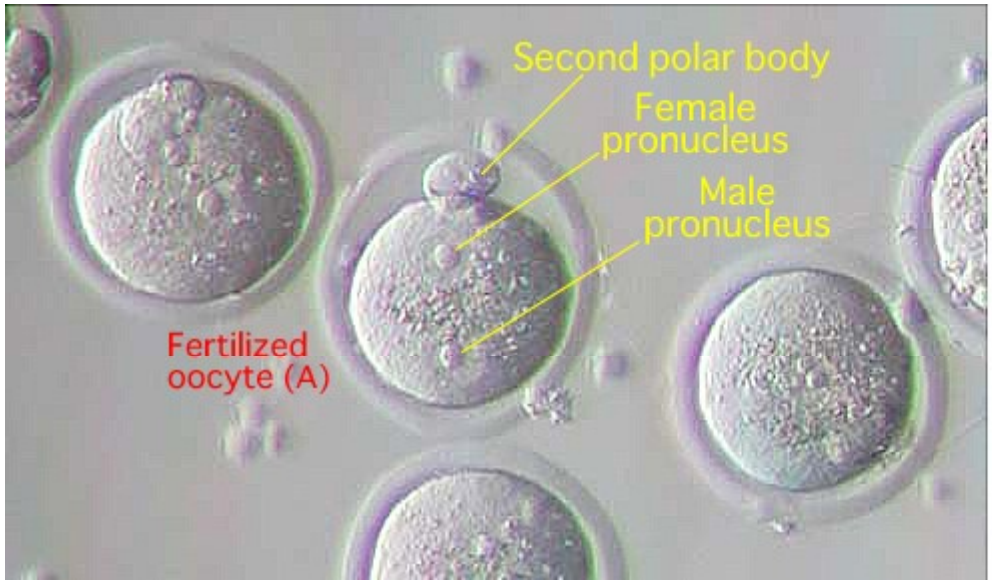
Oocytes-COCs

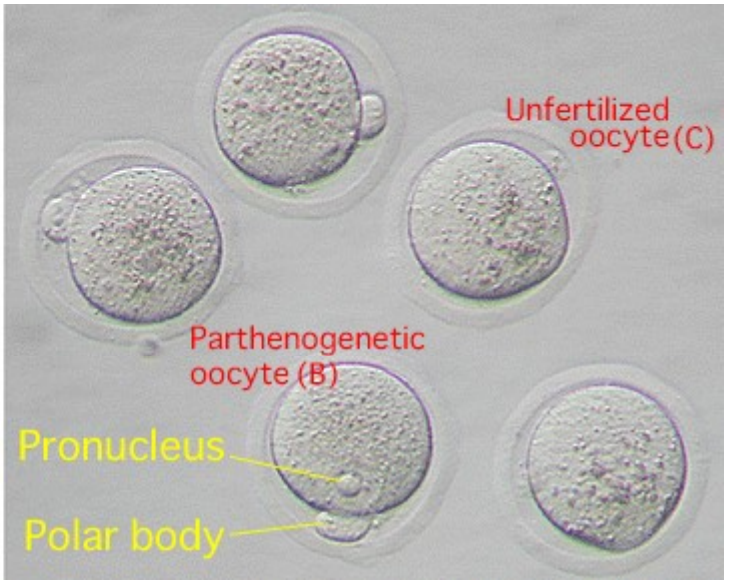





【Sperm dish】
Sperm suspension
3μL
【Fertilization dish】
COCs
3μL
Incubate
【Fertilization dish】
【Washing dish】
3h

6h later


Second polar body
Female pronucleus
Male pronucleus
Fertilized oocyte (A)


Unfertilized oocyte (C)
Parthenogenetic oocyte (B)
Pronucleus
Polar body

However,
different pattern of integrin expression in *in vivo* and *in vitro* growing embryos.