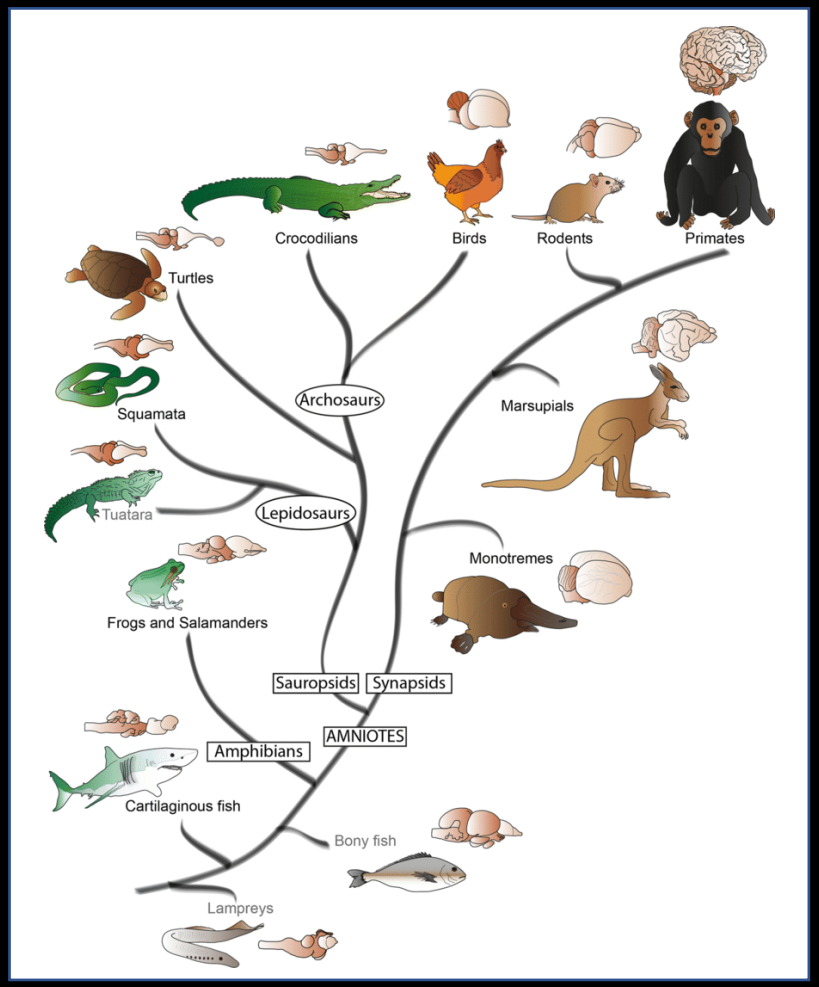
MUNI MED

Embryology III

autumn 2024

Interspecies differences in reproductive biology



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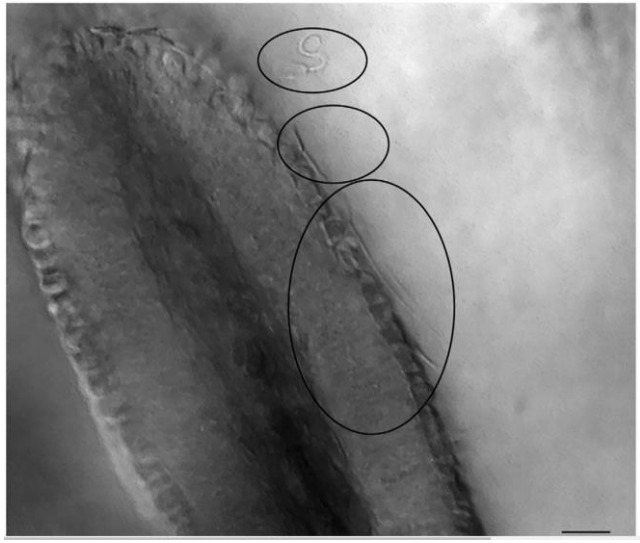
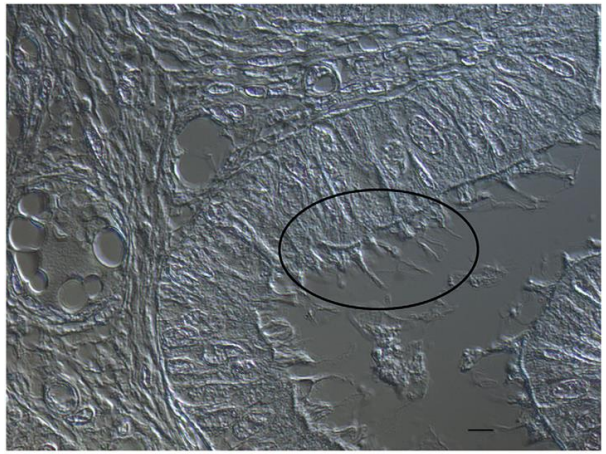
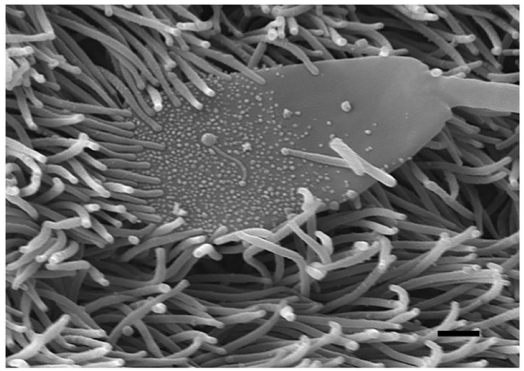
Sperm reservoirs

- sperm deposition in female tract
  for 3-5 days (most mammals), weeks (birds), moths (bats) , or even years (snakes)
- documented in mice, rats, hamsters, pigs, sheep, cows, horses
- anticipated to exist in humans, but no evidence *in vivo*

- binding of sperm head to the cilia of Fallopian tube epithelium (isthmus and ampula) while preserving their motility and fertilizing capacity
- sperm binding mediated by carbohydrate moieties on the cillia
- glycoproteins and mucopolysaccharides secreted by tubal cells maintain cell motility
- extracellular vesicles promote sperm survival, capacitation and hyperactivation

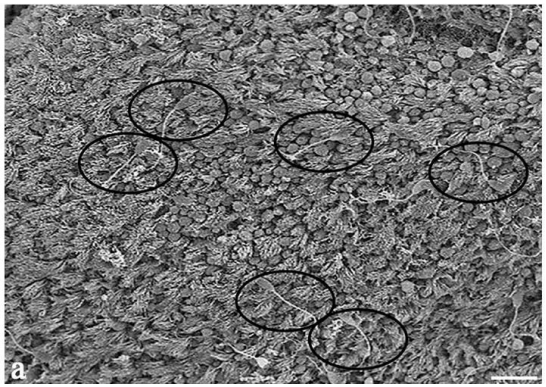






Kölle et al 2021

Sperm reservoirs



Sabine Kölle

- digital live cell imaging

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SCIENTIFIC REPORTS
nature research

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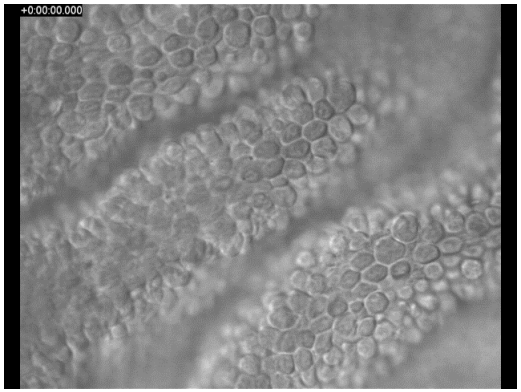
OPEN

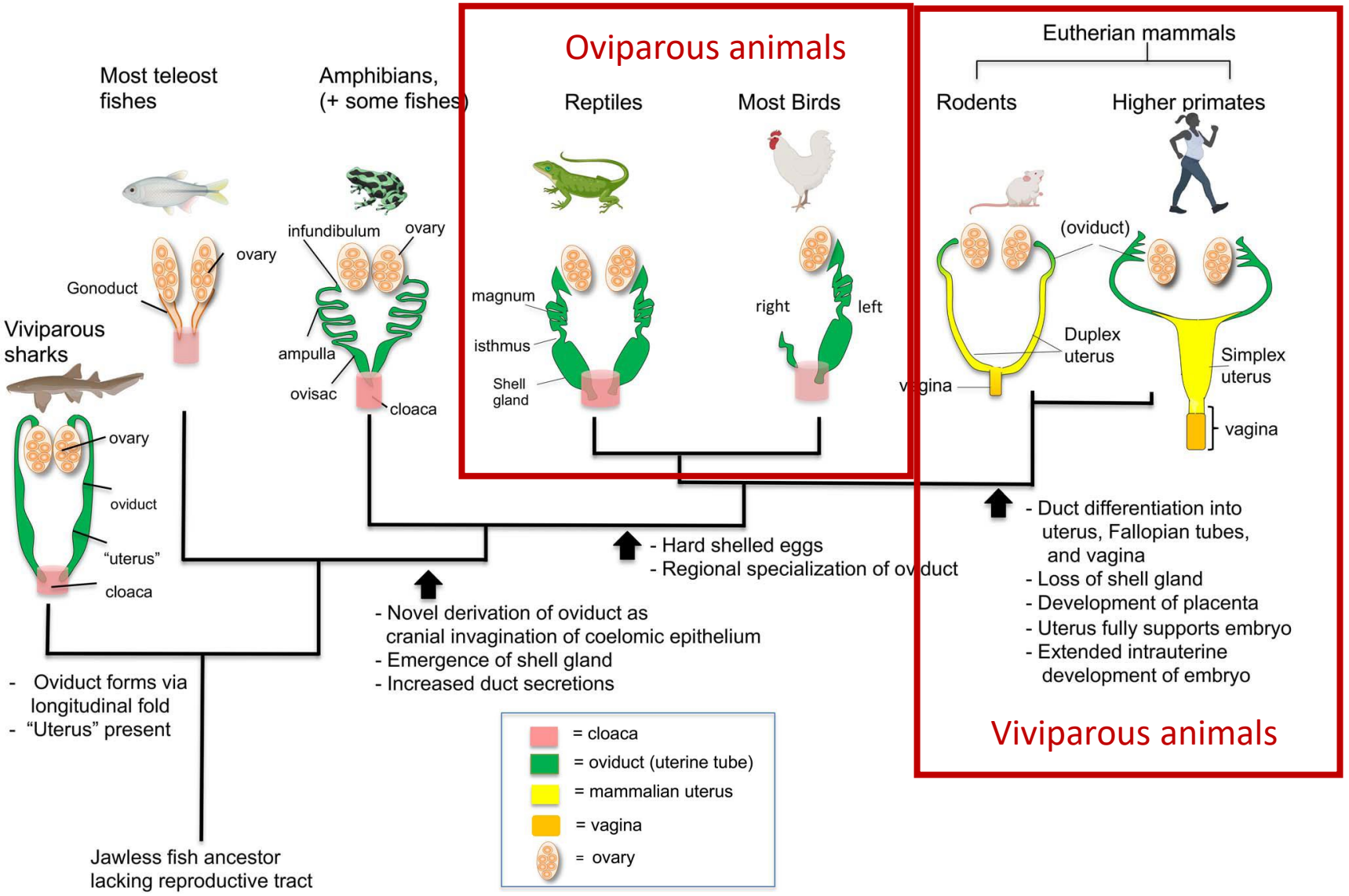
Bovine sperm-oviduct interactions are characterized by specific sperm behaviour, ultrastructure and tubal reactions which are impacted by sex sorting

Miguel Camara Pirez[1], Heather Steele[1], Sven Reese[2] & Sabine Kölle[1]✉



Camara Pirez et al 2020

Female reproductive tract development



Major et al, 2022

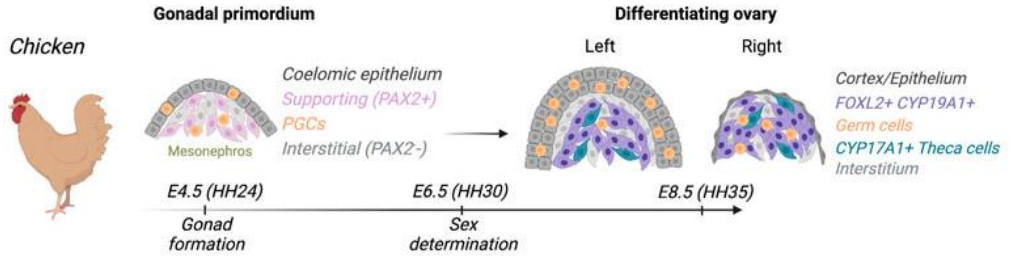
Reproductive tract development

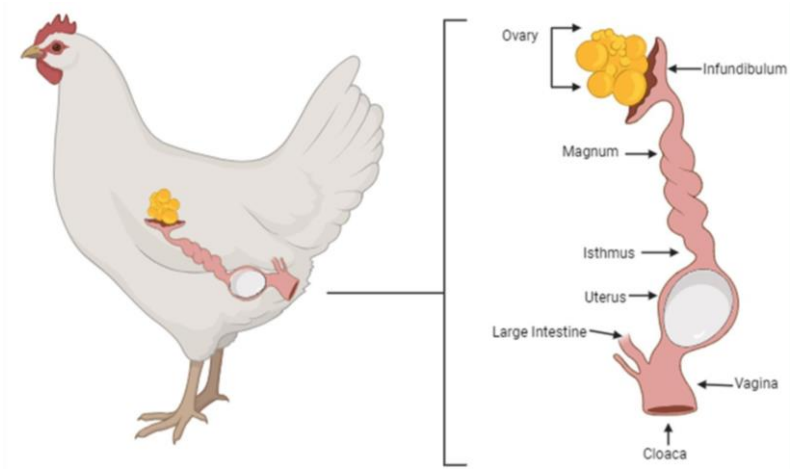
❖ Birds (avian species)

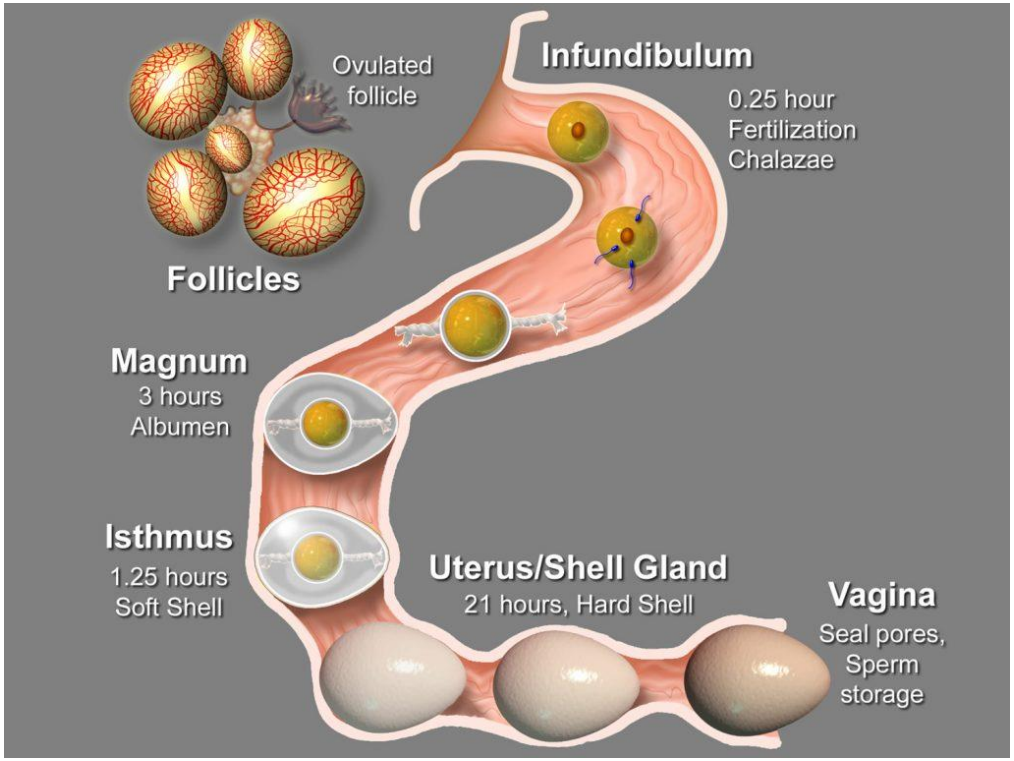
- right ovary does not form cortex and regresses

- only left oviduct developes

- ismus secrete egg shell membrane
- shell gland („uterus“) lays down calcificated shell



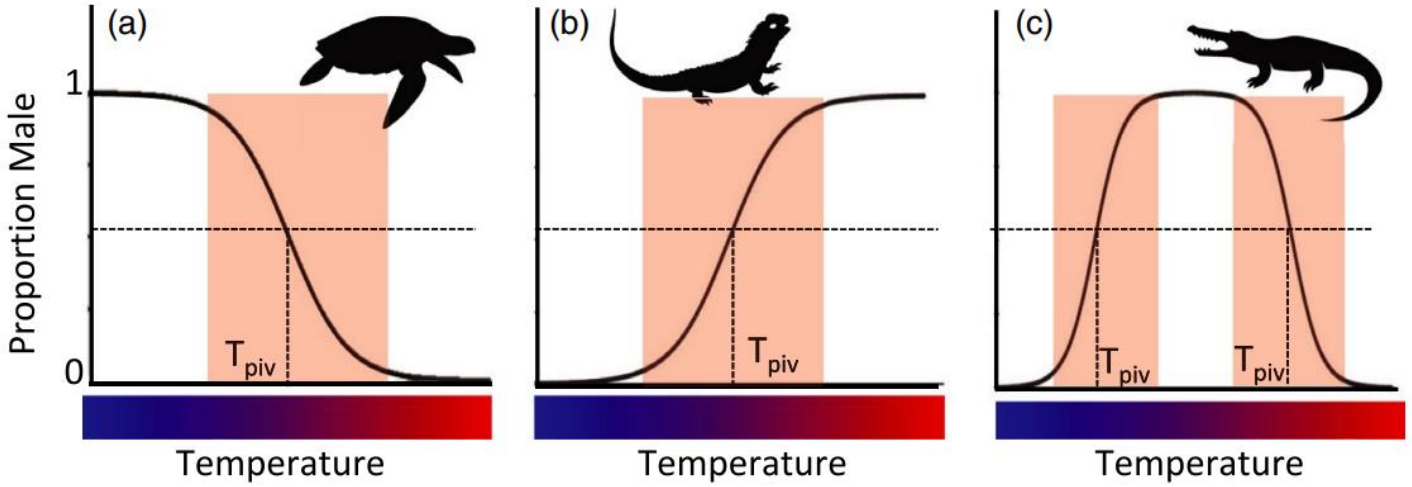




Sexual differentiation

❖ Temperature-dependent sex differentiation

- in reptile species
- gonadal differentiation and sex of the embryo is not determined by heterologous chromosomes but the incubation temperature during early and middle incubatinon period
- expression of sex-determining factors are subjected to temperature-dependent regulation (e.g. truncated gene products are dysfunctional)
- global warming can lead to overproduction of one sex

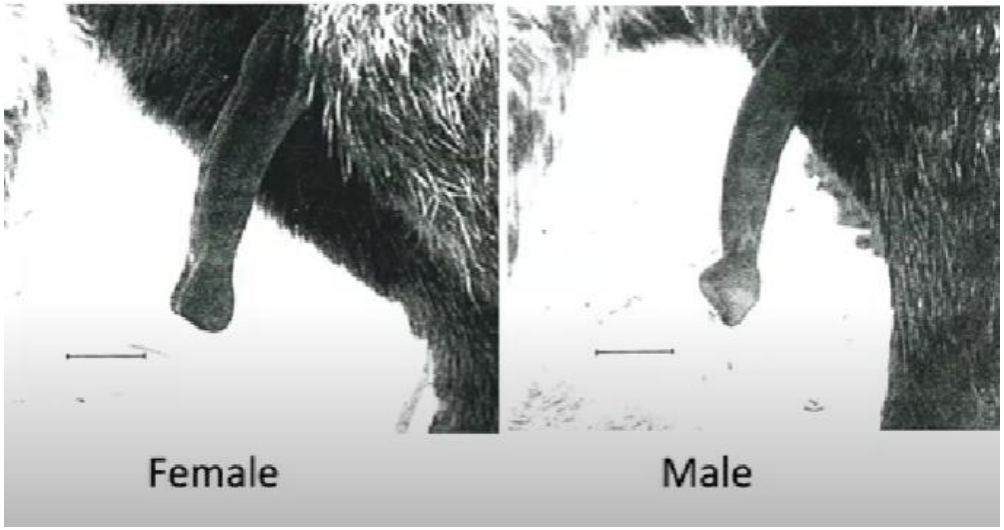


Lockey and Eizaguirre 2021

Sexual differentiation

❖ Spotted hyena

- gonadal androgen independent female masculinization
  → male-like external genitalia („pseudopenis“)
  → aggressive behaviour
- placenta converts androstendione from adrenal glands to testosterone before gonadal differentiation
- shorter gonadal androgen-free window during female prenatal development
- swolled phallus-like clitoris retracted during mating

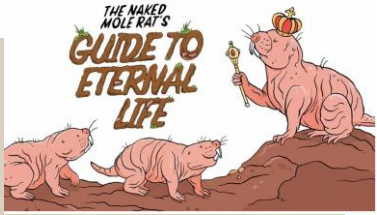


- difficult labour

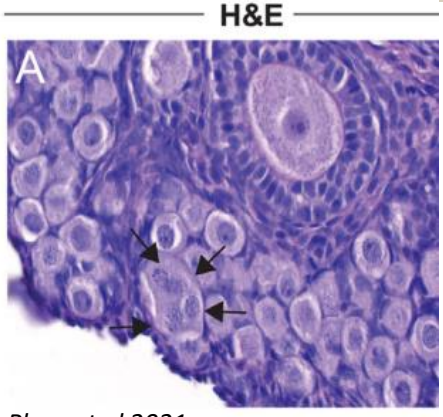
(60% of firstborn cubs are stillborn!)

Oogenesis

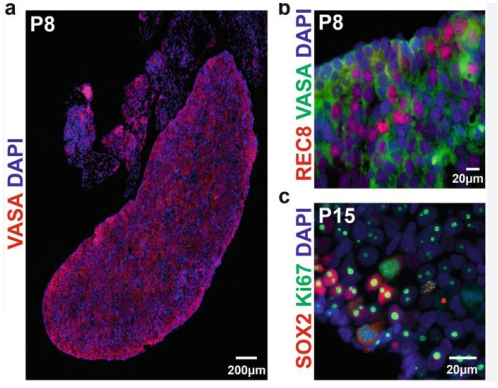
❖ Naked mole rat



- eusocial colony forming animals
- division to reproductive and working groups
- long life span, aging- and hypoxia resistance, rare cancer
- workers have the potential to become reproductively active if queen dies or new colony is formed
- large ovarian reserve, switch from anovulatory to ovulatory phenotype



Place et al 2021



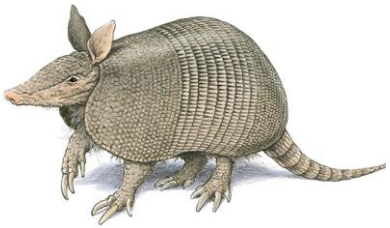
Brieño-Enríquez 2023

- model of postnatal oogenesis
- large germ cell nests in adult ovaries
- detection of putative germ line stem cells

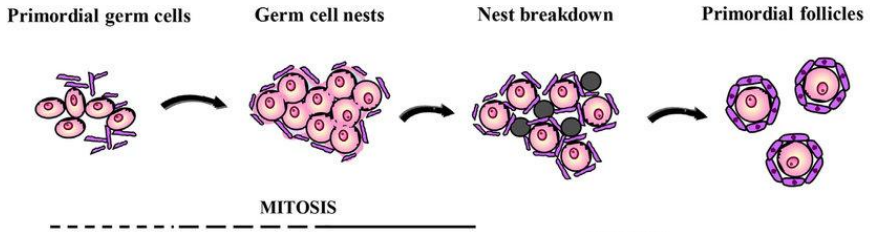
Oogenesis

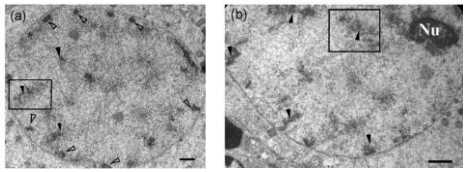
❖ Armadillo

(Chetopthractus villosus)



- germ cell nests (cysts) present in the adult ovary
- constitutive presence of female germ cells (leptotene and diplotene) connected by intercellular bridges
- model for investigating female meiosis and cyst break down process during folliculogenesis





Lepto-zygotene Pachytene

Received: 12 July 2019 | Accepted: 30 October 2019
DOI: 10.1002/mrd.23296
Molecular Reproduction & Development

RESEARCH ARTICLE

Germ cell cysts, a fetal feature in mammals, are constitutively present in the adult armadillo

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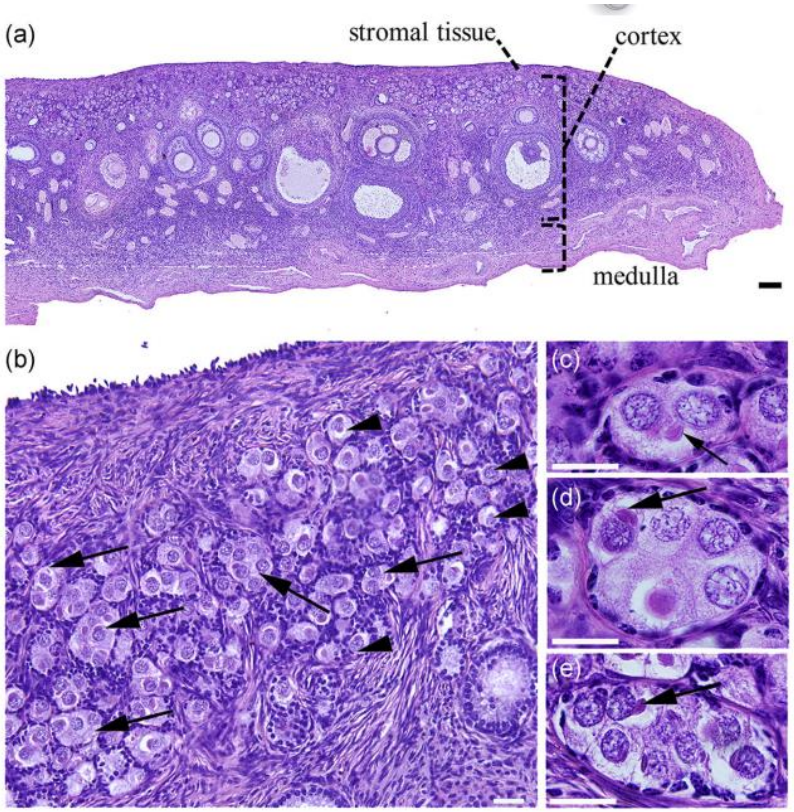
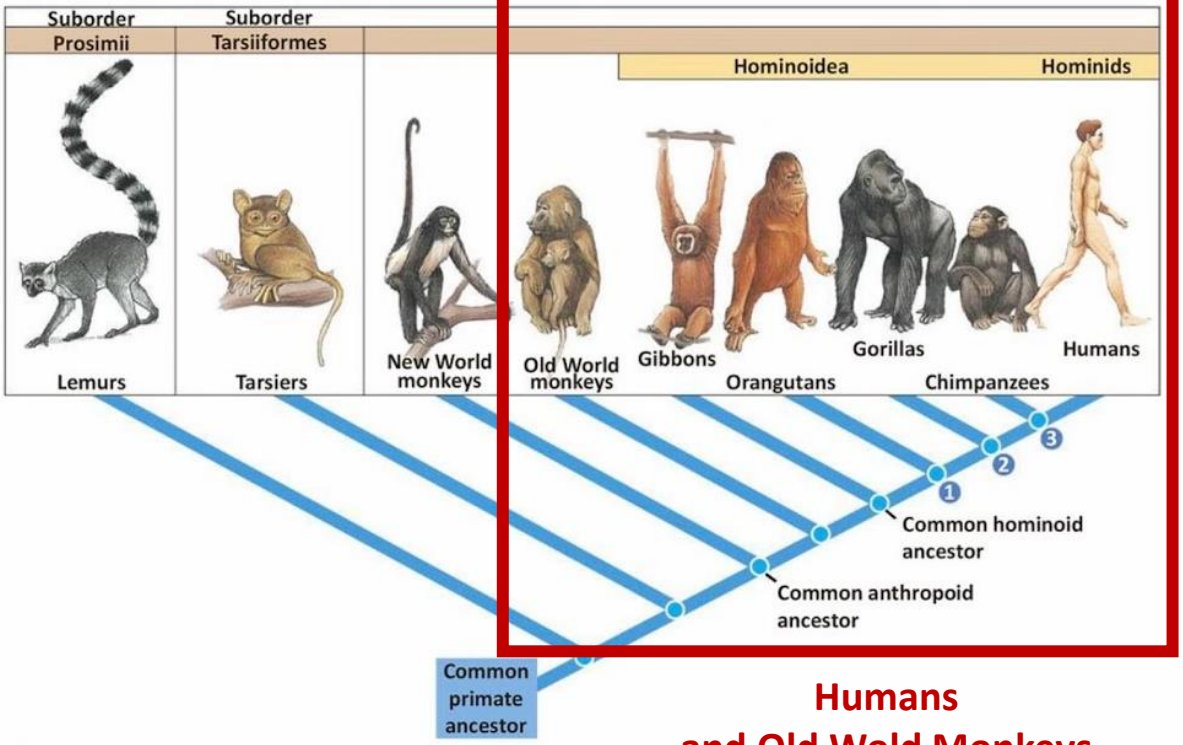


Reproductive cyclicity

❖ Menstruating species

Bats
(Chiroptera)



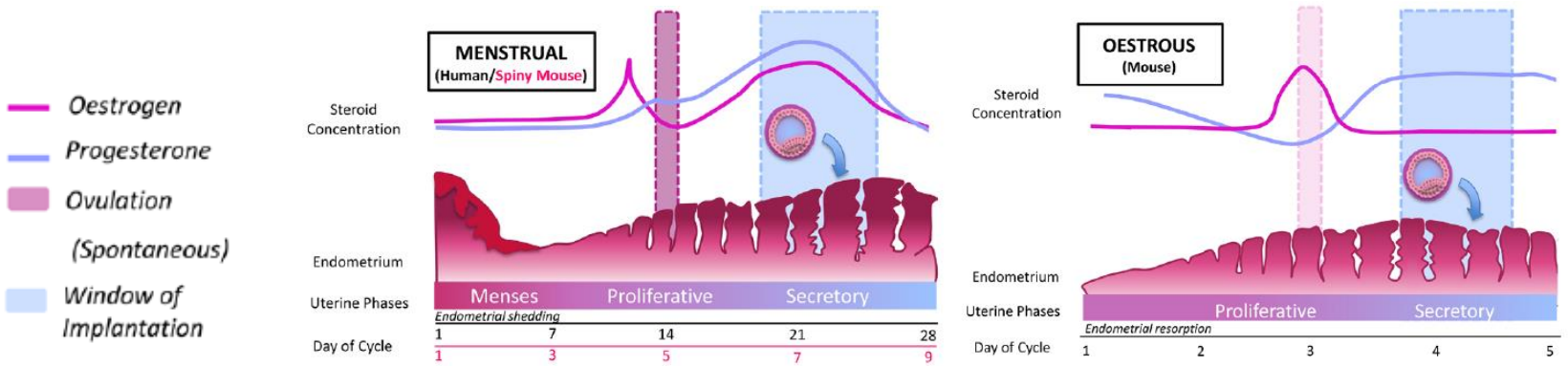
Humans and Old Wold Monkeys

Elephant shew
(*Rhynchocyon petersi*)
Insectivorous mammal

Spiny mouse
(*Acomys cahirinus*)
the only known rodent exhibiting menstruation!

Reproductive cyclicity

	Menstruation cycle	Oestrous (Estrous) cycle
Endometrium fate in non-conception cycles	shedded	reabsorbed
Vaginal bleeding	yes	no/very few
Sexual receptivity	uniform	strong sexual urge
Duration	fixed (~28 days)	variable (7-21 days)
Follicular phase	Long (50%)	Short (20%)
Ovulation	Middle of cycle	Beginning/end of cycle
Luteal phase	50%	80%
Luteolysis	Ovarian PGF2α	Uterine PGF2α
Fertile period	up to 6 days	24 hours
P4 effect on sexual receptivity	P4 → ↓GnRH inhibits sexual receptivity	P4 → ↓GnRH does not inhibit sexual receptivity
Menopause	occurs	not described



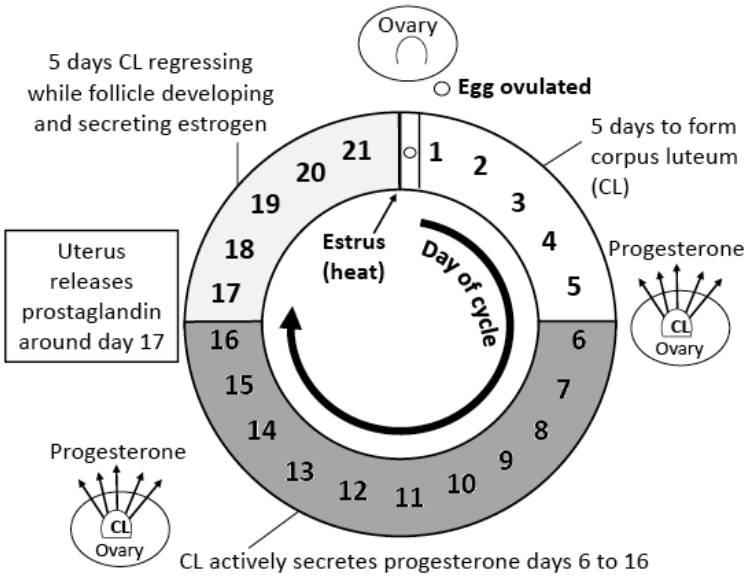
Bellofiore et al 2018

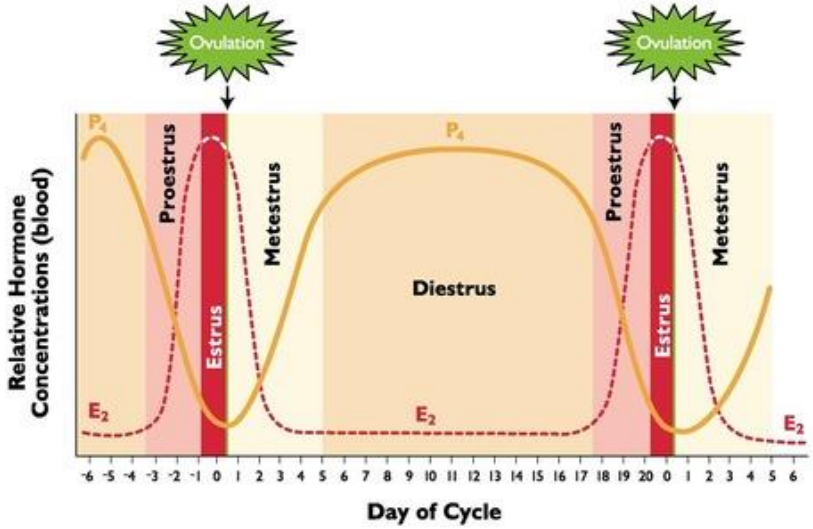
Reproductive cyclicity

❖ Estrous cycle

Follicular phase

- **Proestrus** (before desire)
  - ↑E2
  - preparation for mating
- **Estrus** (desire)
  - ↑↑ E2
  - ovulation, sexual receptivity



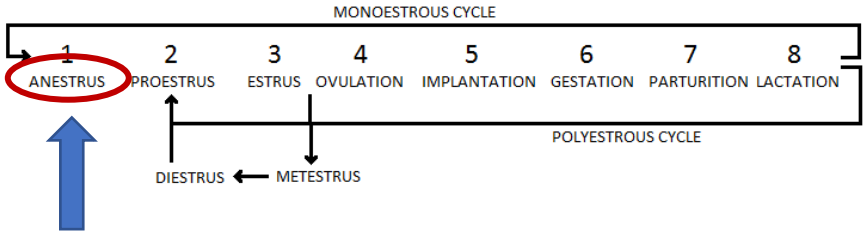


Luteal phase

- **Metestrus** (after desire)
  - ↓ E2, P4↑
  - preparation for pregnancy
- **Diestrus** (between desire)
  - P4↑↑
  - quiescent stage before P4 regresses and next cycle starts

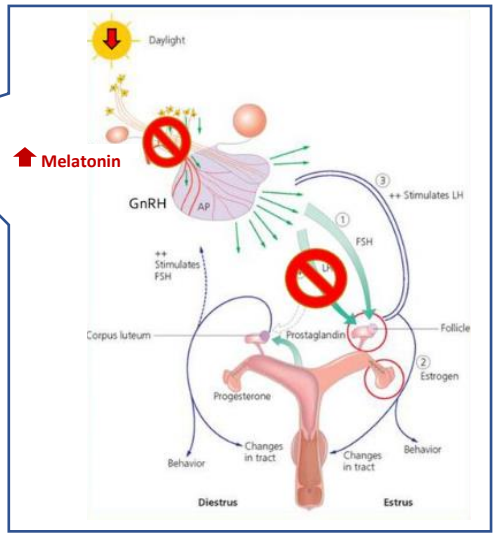
Reproductive cyclicity

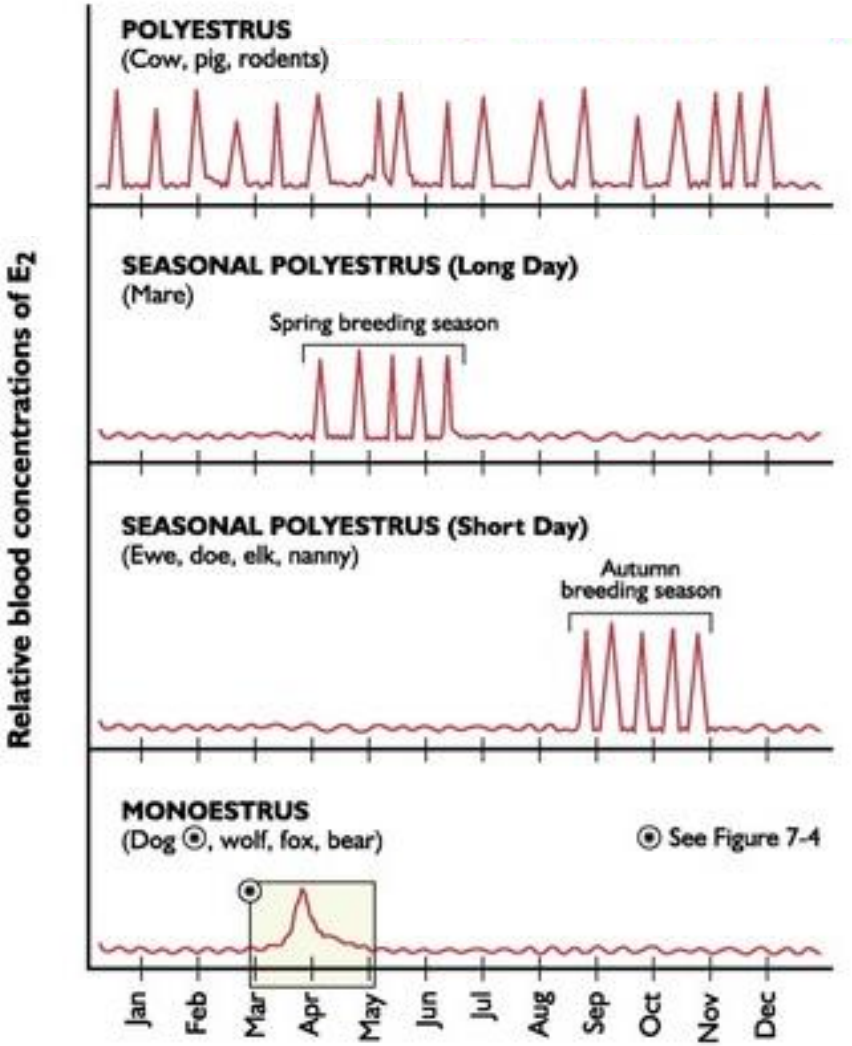
❖ Estrous cycle



ANESTRUS = acyclicity

- Prolonged period of sexual rest
  - ← pregnancy
  - ← lactation
  - ← presence of offspring
  - ← stress
  - ← pathology
  - ← season

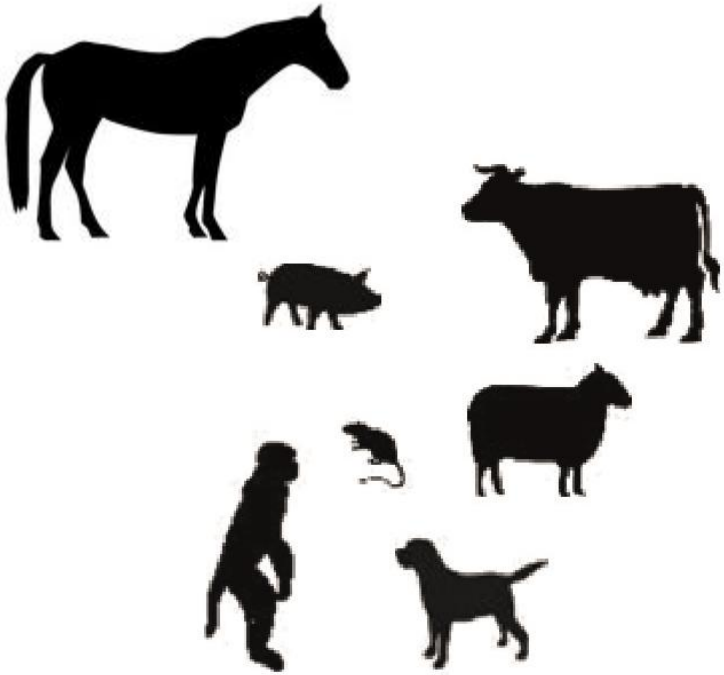




Reproductive cyclicity

❖ Spontaneous ovulation

- endogenous ovulation trigger



Mare (*Equus*)
Sow (*Sus scrofa*), Cow (*Bob taurus*),
Ewe (*Ovis aries*), Mouse (*Mus musculus*),
Macaque (*Macaca mulata*),
Dog (*Canis vulgaris*)

❖ Induced ovulation

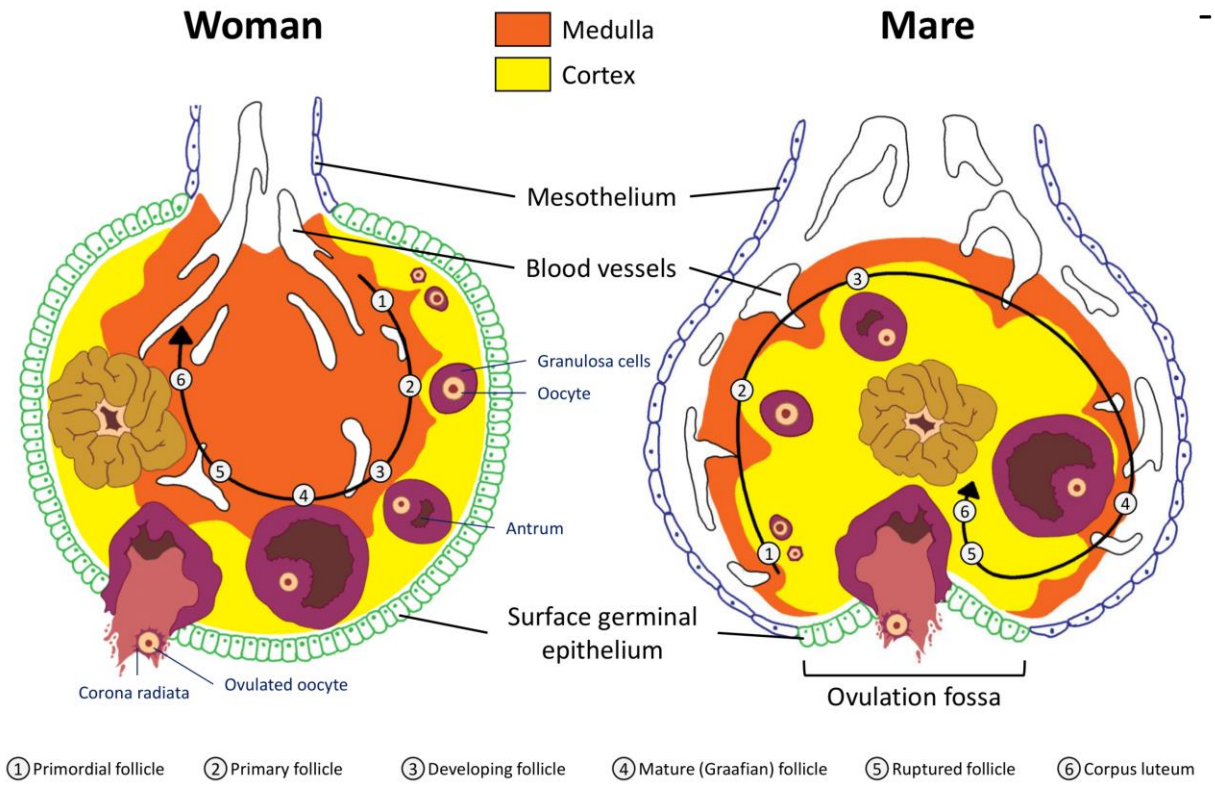
- ovulation occurs only after stimulus (e.g. presence of male, mating behaviour, copulation)

Dromadery (*Camelus dormaderius*),
Rabbit (*Oryctolagus cuniculus*),
Alpaga (*Vicugna pacos*), Shrew (*Suncus murinus*),
Rhinoceros (*Diceros bicornis*)
Cat *(Felis catus)*

Ovarian tissue organisation and embryo motility

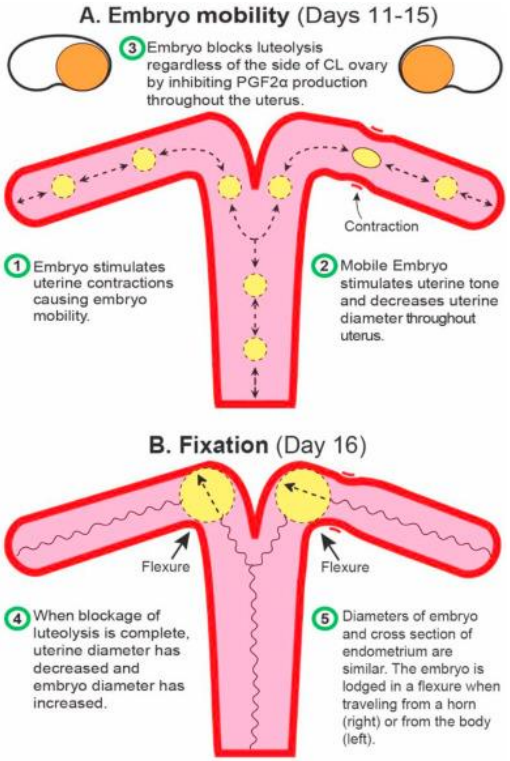
„inside-out“ ovaries

- germinal epithelium is in the inside of the ovary
- ovulated oocytes must pass through the ovulation fossa



Benammar at al 2021

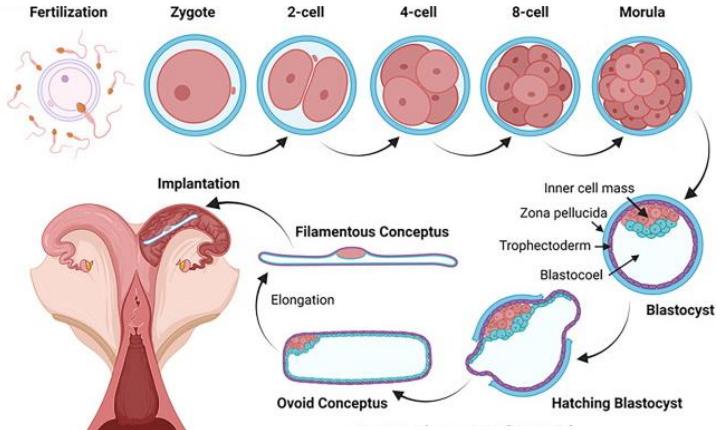
- embryo motility and delayed implantation



Ginther 2021

Preimplantation conceptus elongation

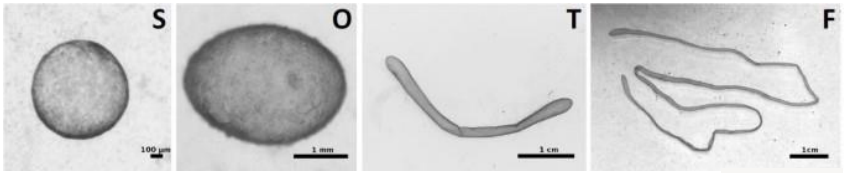
- occurs in post-hatching embryos in *Ungulates*



Davenport et al 2023

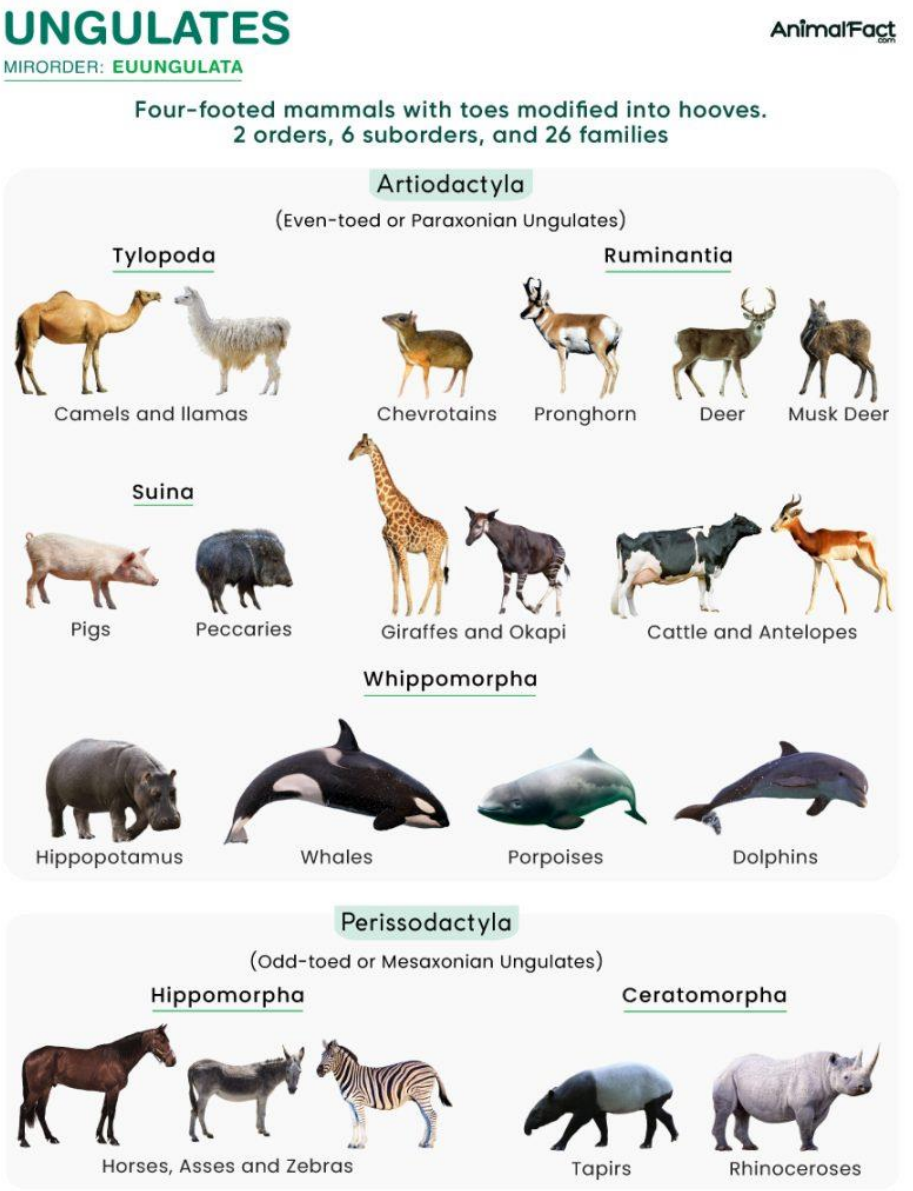
- transformation form spherical to filamentous shape

150 μm → 30 cm-100 cm



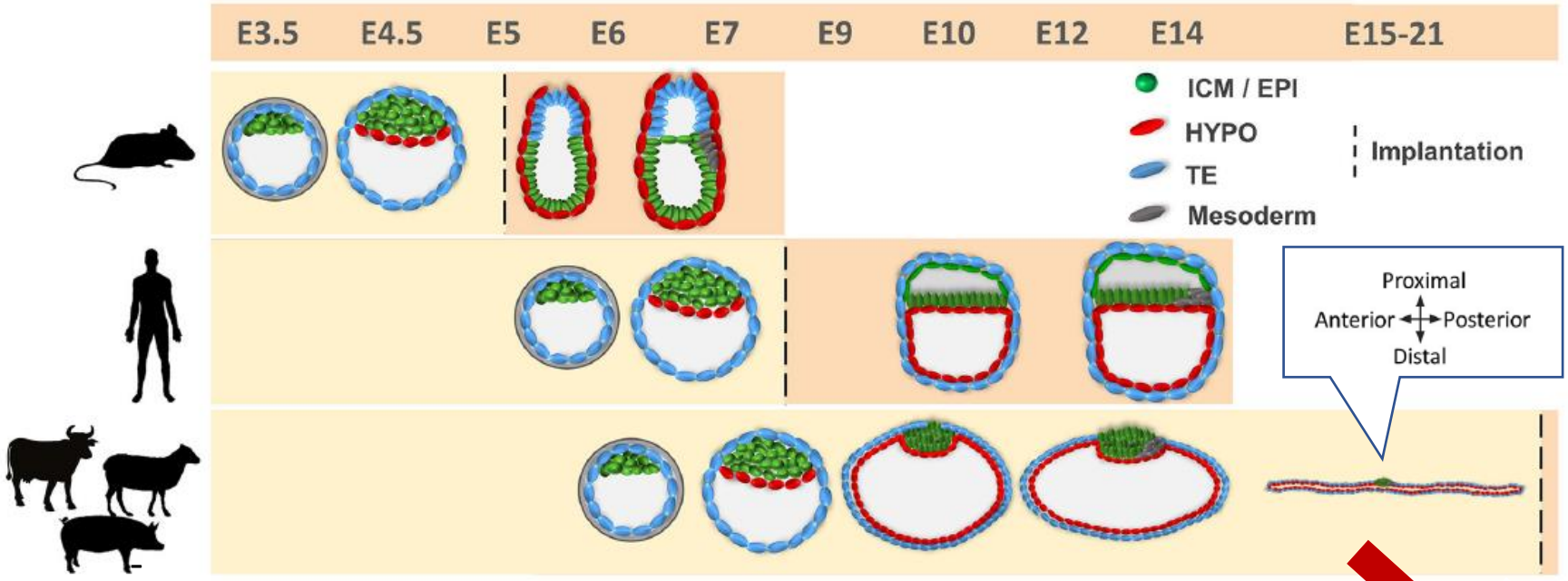
Turenne et al 2012

- failure to elongate (~30%) contribute to high high embryonic loss



Preimplantation conceptus elongation

- massive proliferation of extraembryonic tissues (TE + hypoblast)
- in contrast to mouse and humans, epiblast in ungulates does not cavitate but form flat embryonic disc and anterio-posterior axis in established

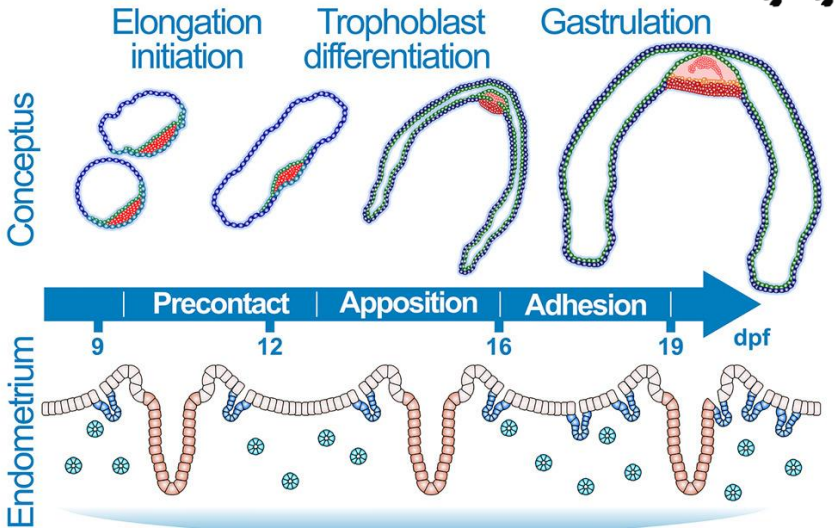


polar TE removed and epiblast exposed prior attachment

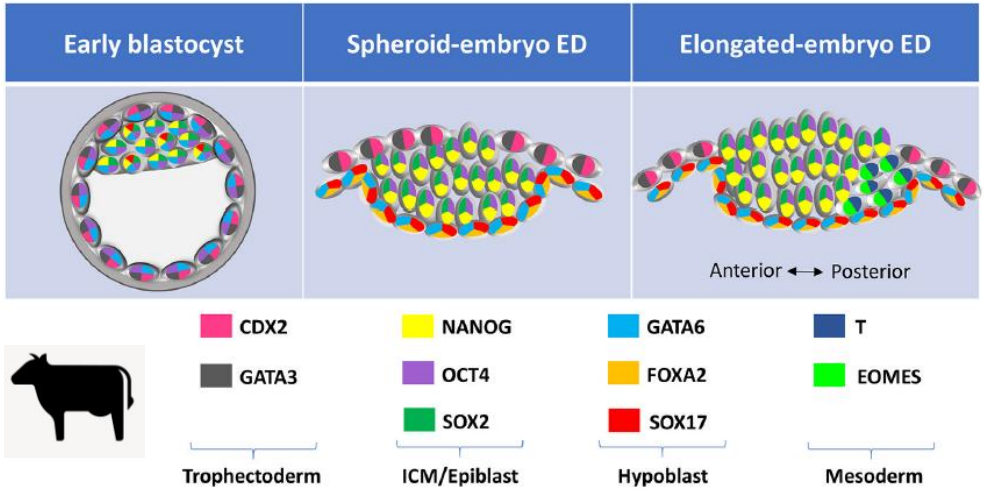
Pérez-Gómez et al. 2021

Preimplantation conceptus elongation

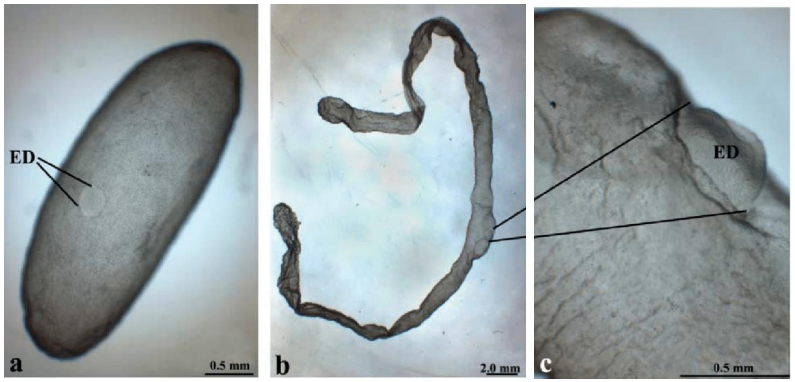
- 3 germ layers and germ line are specified **before implantation**
- development of epiblast is more vulnarable process than development of TE and hypoblast
- different expression of lineage specific markers than in mouse



Jia et al. 2023



Pérez-Gómez et al. 2021



ED = embryonic disc

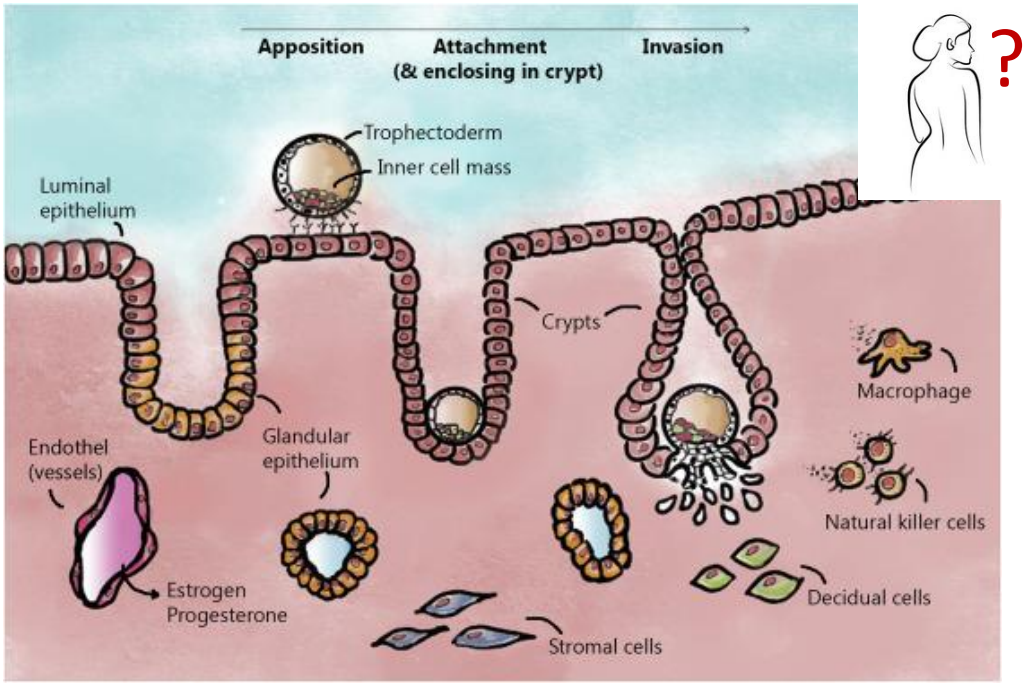
Tveden-Nyborg et al 2005

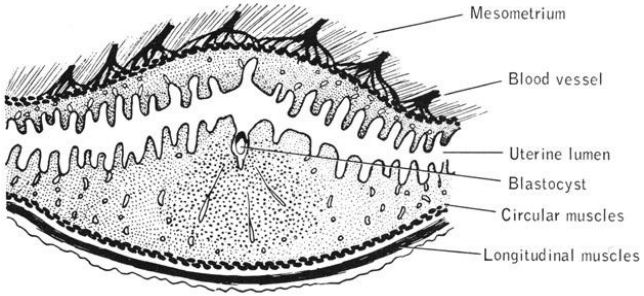
Embryo implantation

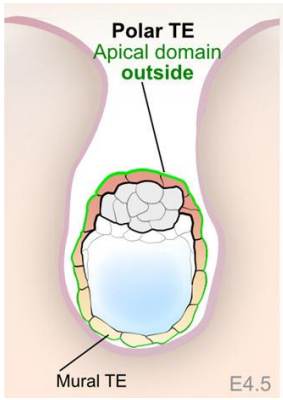
❖ Architecture of luminal epithelium

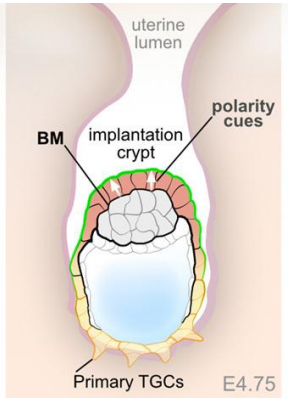
= „luminal folds“„implantation chambers“, „uterine crypts“

- invaginations of endometrial luminal epithelium
- enable engulfing of the embryo after apposition and facilitate proper embryo orientation
- disorganisation of luminal foldings can compromise implantation efficiency



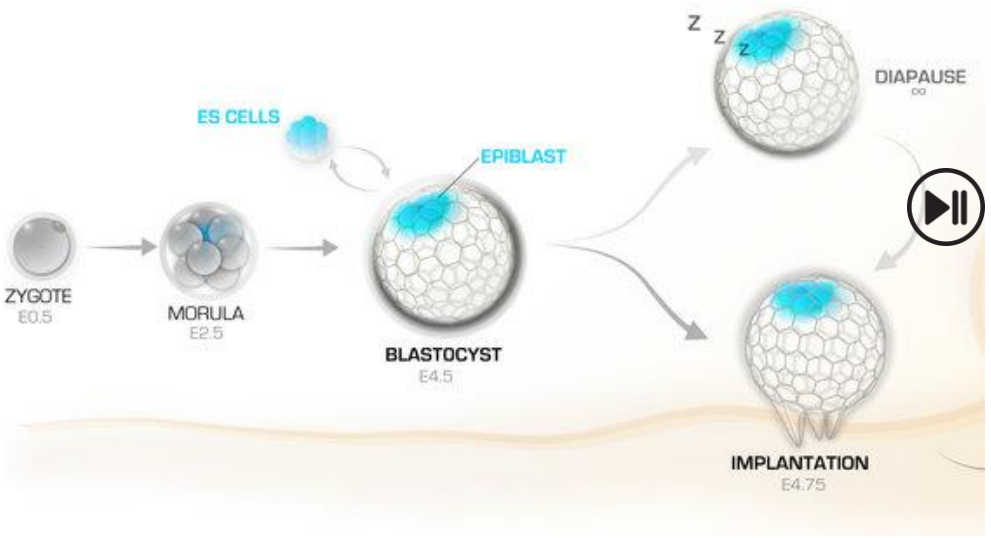






Embryonic diapause

- **reversible pause of early embryonic development**
- switching to (near) dormant state
- uncoupling mating and birth time
- common in non-mammalian species and documented in > 130 mammalian species
- can last from days to months
- obligate/facultative
- lactational/seasonal (photoperiod-dependent)
- the delivery is timed to favourable conditions → survival advantage to the offspring and mother



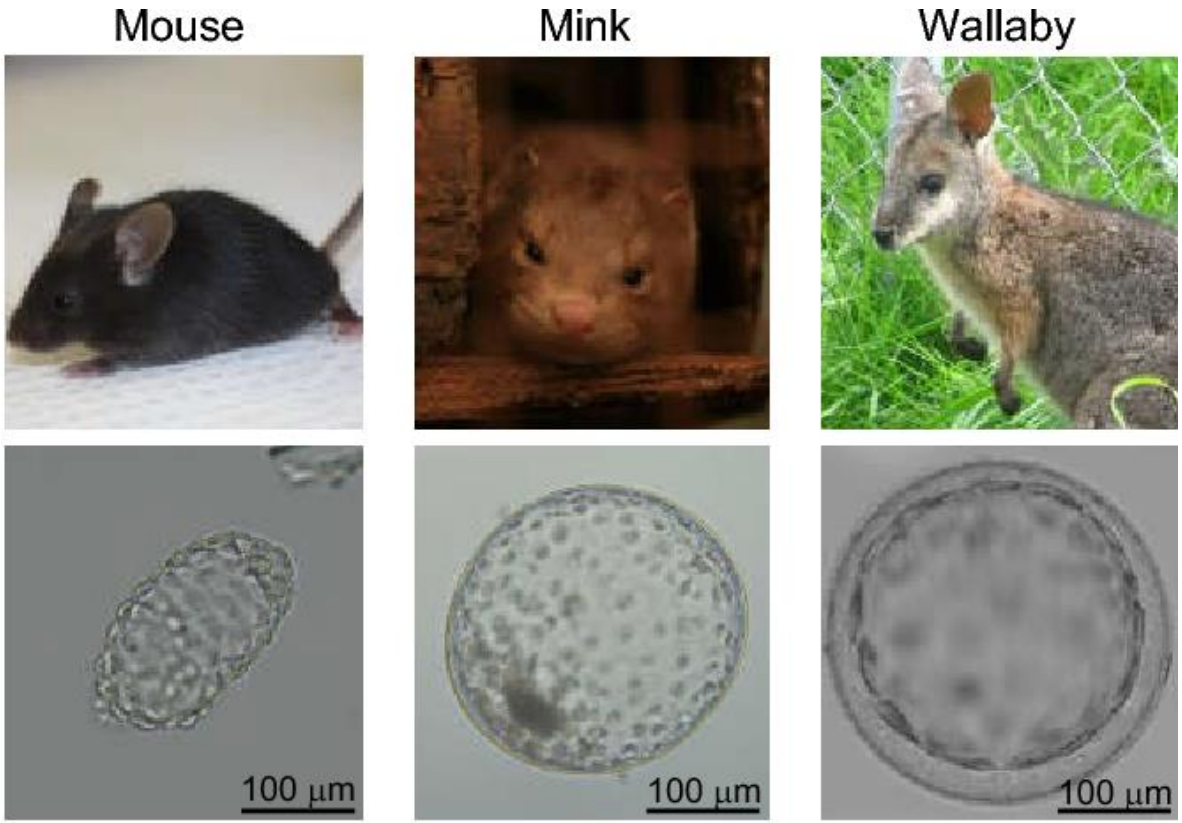
Mammalian diapause

	Eutherian		Marsupial
	Mouse	Roe deer	Mink	Wallaby
# of embryos	6-12	1-4	2-12	1
occurence	facultative	obligate	obligate	facultative or obligate
duration	up to ~40 days	4-5 months	2 weeks	11 months
post-implantation gestation length	16 days	4-5 months	31 days	9 days
growth during diapause	first 5 days	continuously	no proliferation	no proliferation
embryo cell number (diapause start-end)	140-200 cells	100-20k cells	250-500 cells	80 cells
embryo morphology	hatched blastocyst elongated	hatched blastocyst lemon shaped	blastocyst in zona pellucida	blastocyst in zona pellucida, mucin layer and shell coat

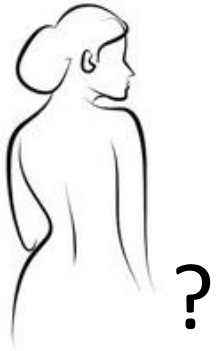
van der Weijden and Aydan Bulut-Karslioglu 2021

Embryonic diapause

- mammalian embryo development arrests in blastocyst stage („paused pluripotency“)
- prior/after hatching



Renfree et al 2017

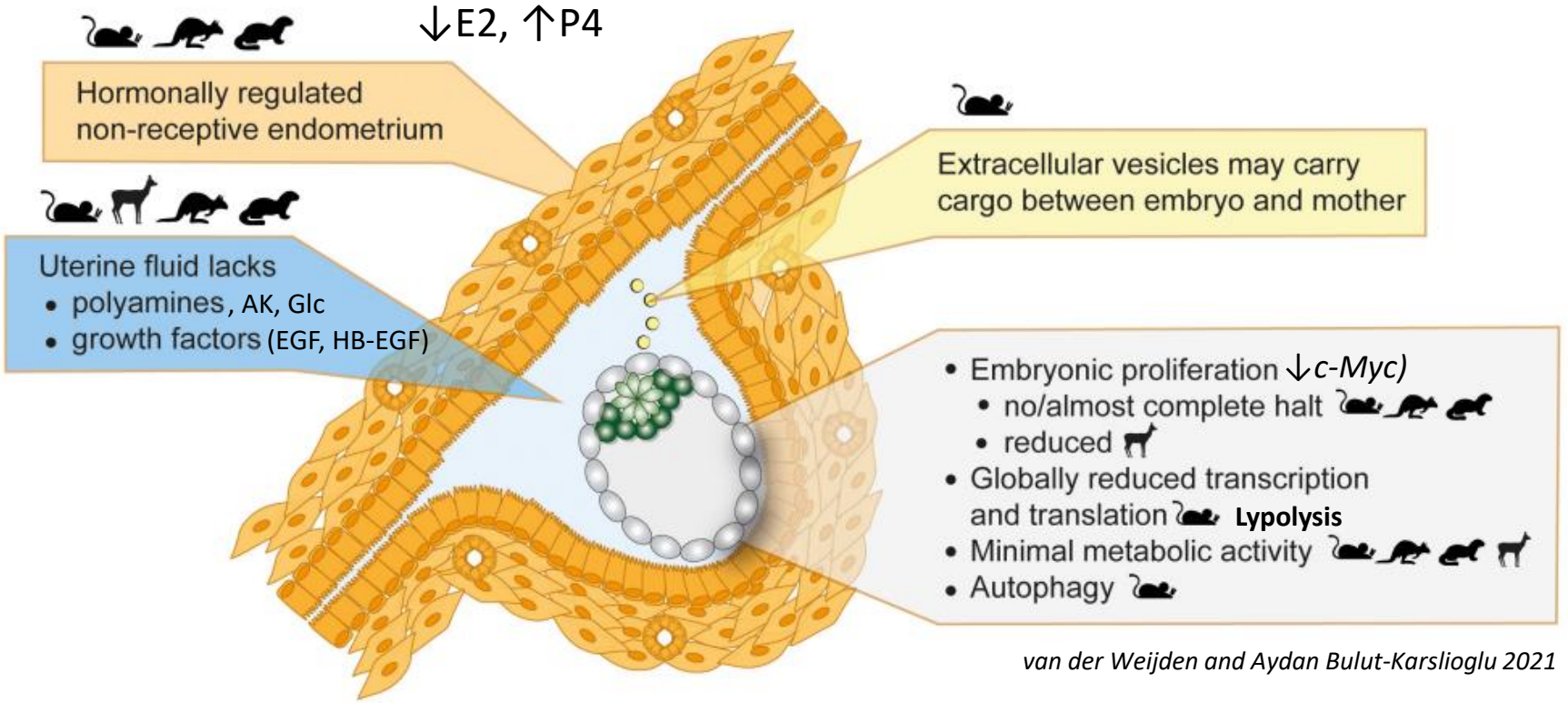


occasional reports of implantation delay in humans

BUT embryonic diapause is not a common reproductive strategy

Embryonic diapause

- under maternal control
  (non-receptive endometrium, endometrium signalling, uterine milieu)
- dormant state characterized by reduction of mitotic activity *(*and metabolism
- quiescence (exist of cell cycle G1 → G0 in ~17% cells)



van der Weijden and Aydan Bulut-Karslioglu 2021

- inducible in non-diapause species
  - sheep embryos can enter diapause under diapause conditions *(Ptak et al 2012)*

Embryonic diapause

van der Weijden et al 2021

Amino acids activate mTORC1 to release roe deer embryos from decelerated proliferation during diapause

Vera A. van der Weijden[a], Jochen T. Bick[a], Stefan Bauersachs[b], Anna B. Rüegg[a], Thomas B. Hildebrandt[c], Frank Goeritz[c], Katarina Jewgenow[d], Pieter Giesbertz[e], Hannelore Daniel[e], Emilie Derisoud[f,g], Pascale Chavatte-Palmer[f,g], Rupert M. Bruckmaier[h], Barbara Drews[a], and Susanne E. Ulbrich[a,1]

PNAS 2021 Vol. 118 No. 35 e2100500118

- endometrial tissue-derived extracellular vesicles contain microRNA let-7 that suppress mTORC1 and thus inhibit proliferation

Liu et al 2020

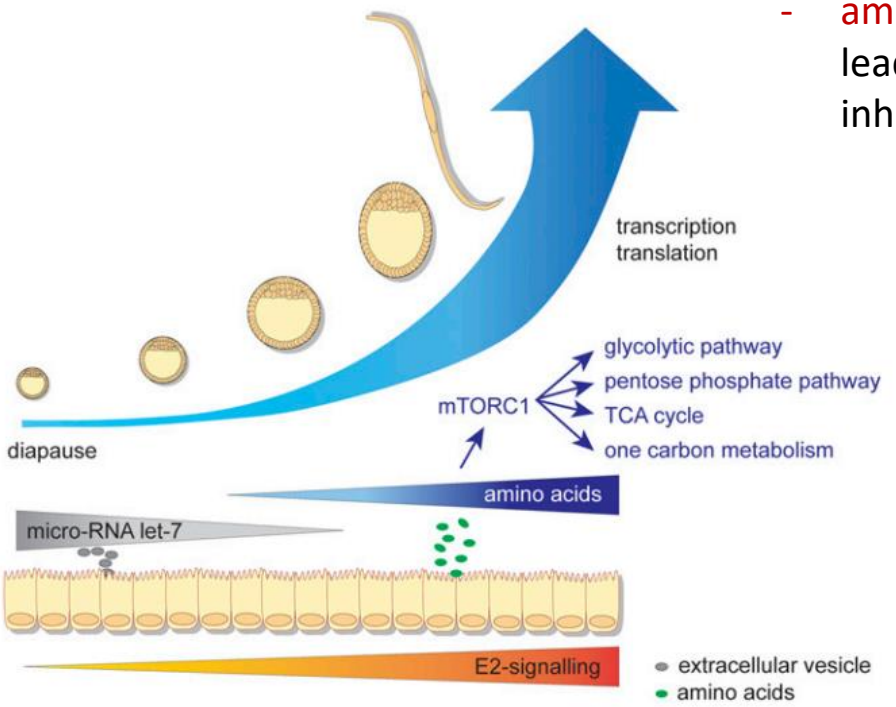
SCIENCE ADVANCES | RESEARCH ARTICLE

DEVELOPMENTAL BIOLOGY

Let-7 derived from endometrial extracellular vesicles is an important inducer of embryonic diapause in mice

W. M. Liu[1,2*], R. R. Cheng[2*], Z. R. Niu[2,3*], A. C. Chen[2], M. Y. Ma[4], T. Li[5], P. C. Chiu[1,2], R. T. Pang[1,2], Y. L. Lee[1,2], J. P. Ou[5], Y. Q. Yao[4], W. S. B. Yeung[1,2,6†]

- overexpression of let-7 delayed mouse embryo development and slightly prologed survival of human blastocysts *in vitro*



Ye et al 2024

Development
For advances in developmental biology and stem cells

RESEARCH ARTICLE | 11 APRIL 2024

Nutrient deprivation induces mouse embryonic diapause mediated by Gator1 and Tsc2

In collection: Reproductive biology

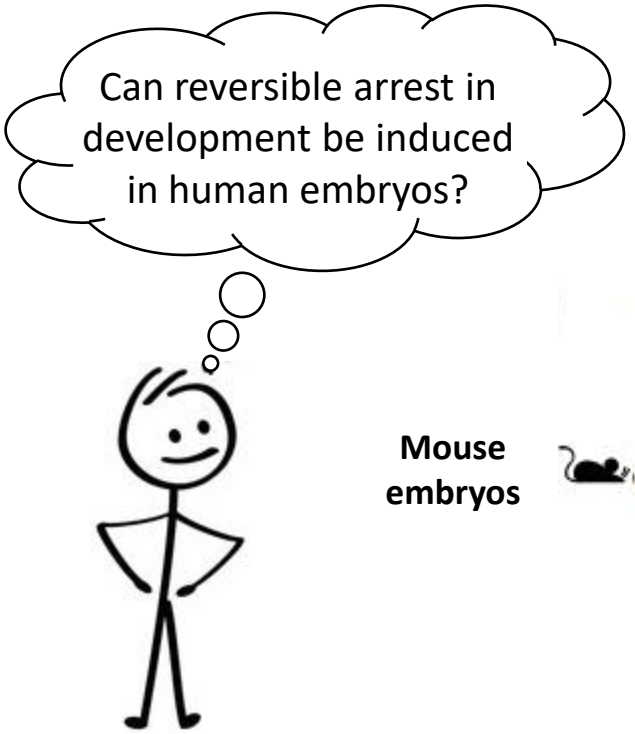
Jiajia Ye, Yuting Xu, Qi Ren, Lu Liu, Qiang Sun

- aminoacid starvation leads to mTORC1 inhibition

diapause

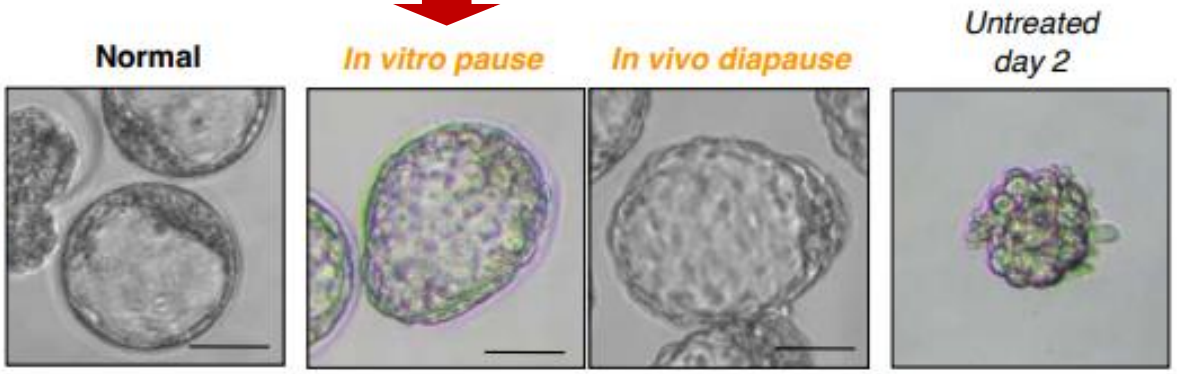
van der Weijden et al 2021

Embryonic diapause



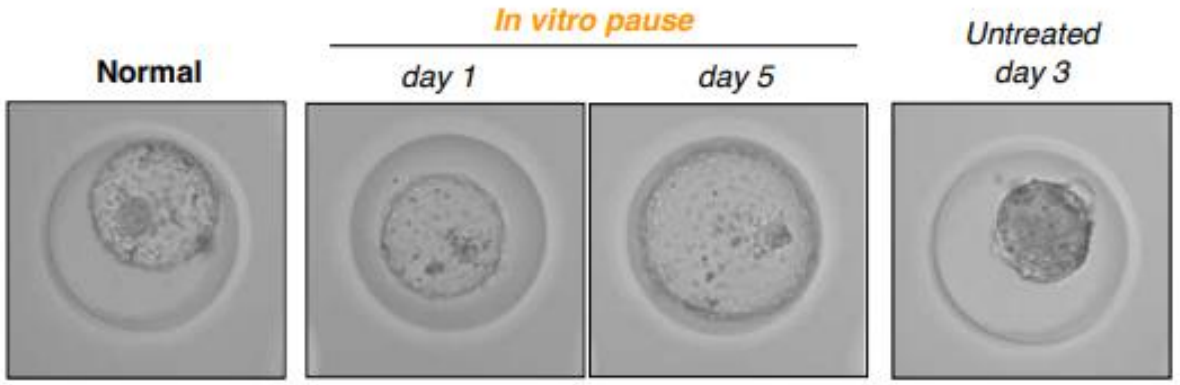
Mouse embryos

mTOR inhibitor



Human blastoids

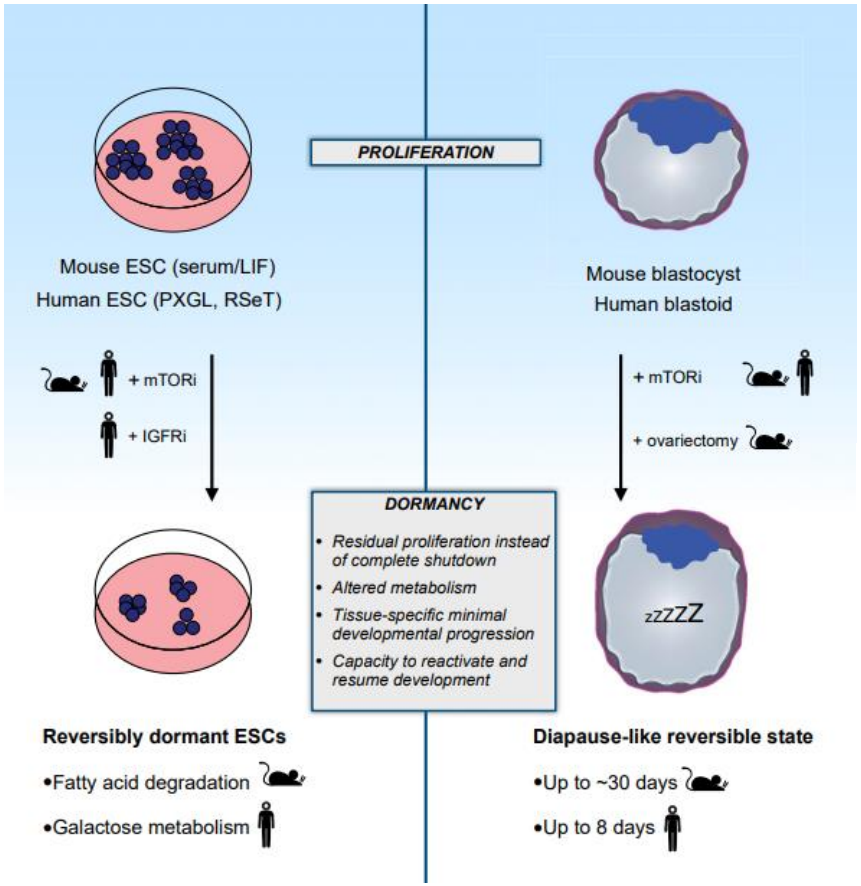
mTOR inhibitor

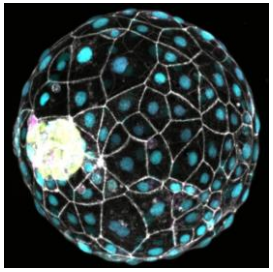


Embryonic diapause

Scientists discover ‘Pause Button’ in human development

26 September 2024





Nicolas Rivron

Cell

Article

mTOR activity paces human blastocyst stage developmental progression

Dhanur P. Iyer,[1,2,10] Heidar Heidari Khoei,[3,10] Vera A. van der Weijden,[1] Harunobu Kagawa,[3] Saurabh J. Pradhan,[3] Maria Novatchkova,[4] Afshan McCarthy,[5] Teresa Rayon,[6] Claire S. Simon,[5] Ilona Dunkel,[7] Sissy E. Wamaitha,[5] Kay Elder,[8] Phil Snell,[8] Leila Christie,[8] Edda G. Schulz,[7] Kathy K. Niakan,[5,9] Nicolas Rivron,[3,11,*] and Aydan Bulut-Karslioğlu[1,11,12,*]
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[10]These authors contributed equally
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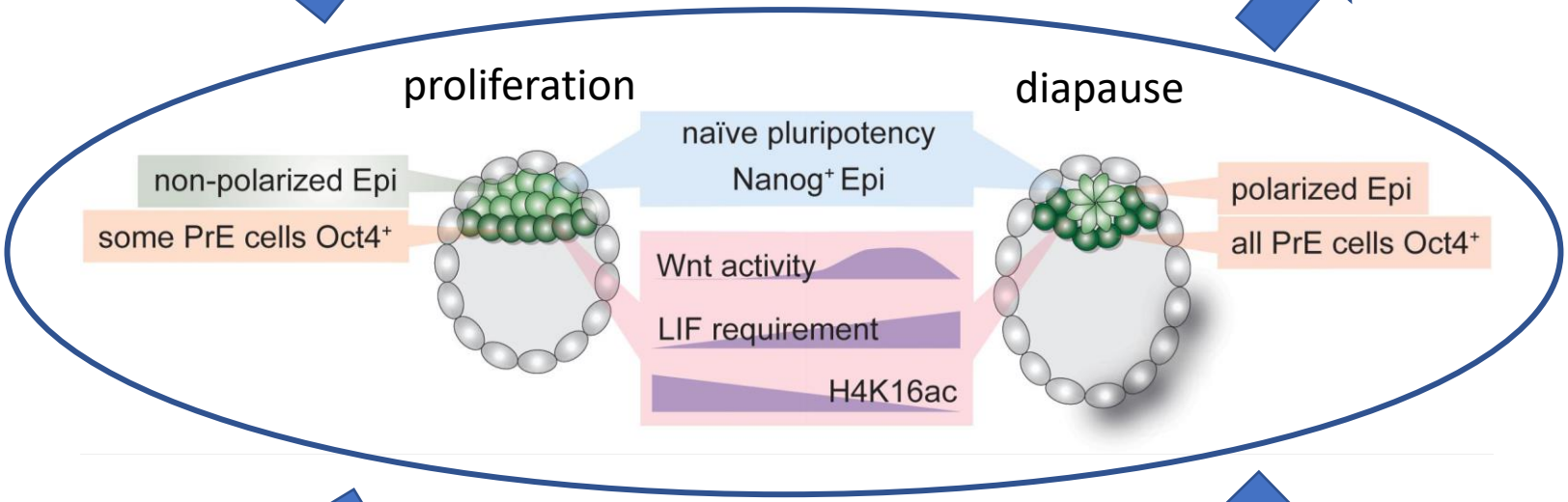
*Correspondence: nicolas.rivron@imba.oeaw.ac.at (N.R.), aydan.karslioglu@molgen.mpg.de (A.B.-K.)
https://doi.org/10.1016/j.cell.2024.08.048

- Human pluripotent cells are capable of entering a reversible dormant state
- Human blastoids under mTOR inhibition show a diapause-like response
- Dormant human blastoids show altered developmental progression and attachment
- Species-specific metabolic profiles of mouse and human cells can be seen in dormancy

Embryonic diapause

Improve efficiency of ES lines derivation

Promoting survival of IVF embryos
(extending time for, cell specification, DNA repair and/or autophagy)



Cancer research
(dormancy/reactivation clues)

A time window for PGT ?

van der Weijden and Aydan Bulut-Karslioglu 2021

Embryonic diapause

❖ Superfetation

- pregnancy with embryos from more than one ovulation
- pregnancy with the offspring fathered by more than one male

Estrus and Mating To male 1 → Ovulation 1 → Embryos in diapause

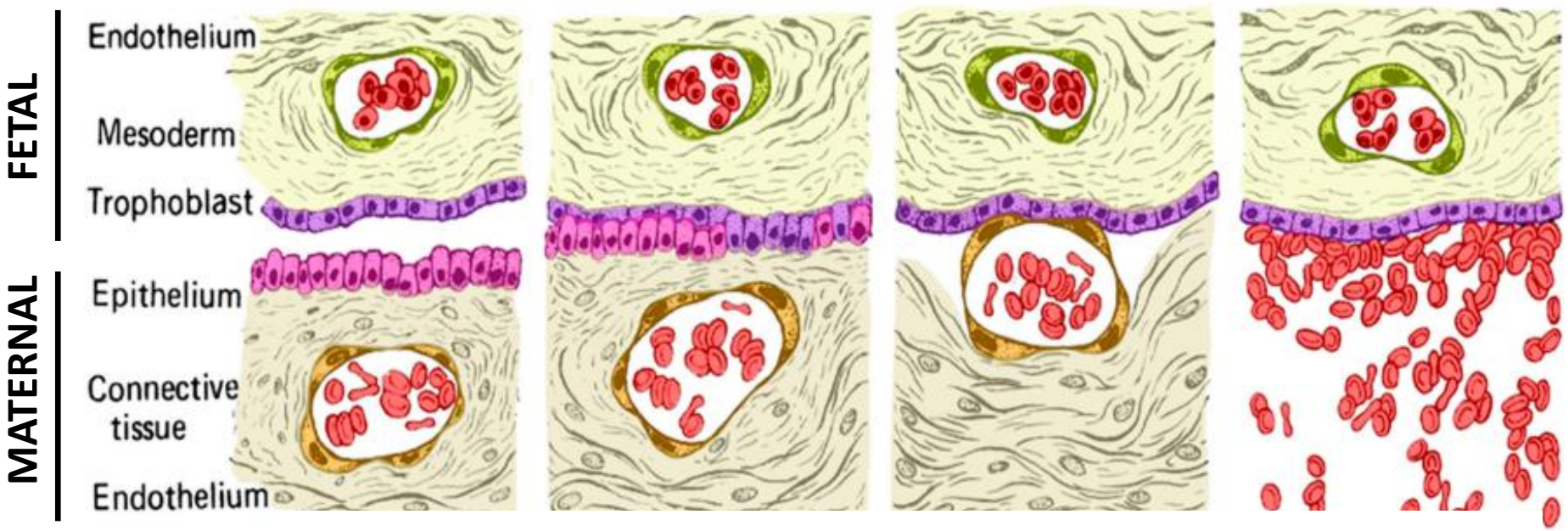
Embryos implant In the uterus

Estrus and Mating To male 2 → Ovulation 2 → Embryos in diapause

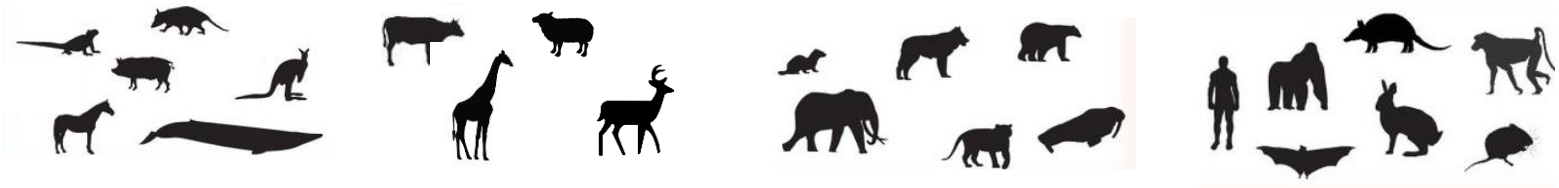
Multiple parentage for the litter

Placentation

MATERNAL-FETAL INTERFACE

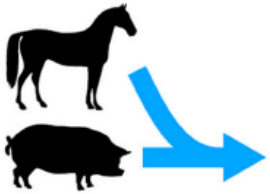


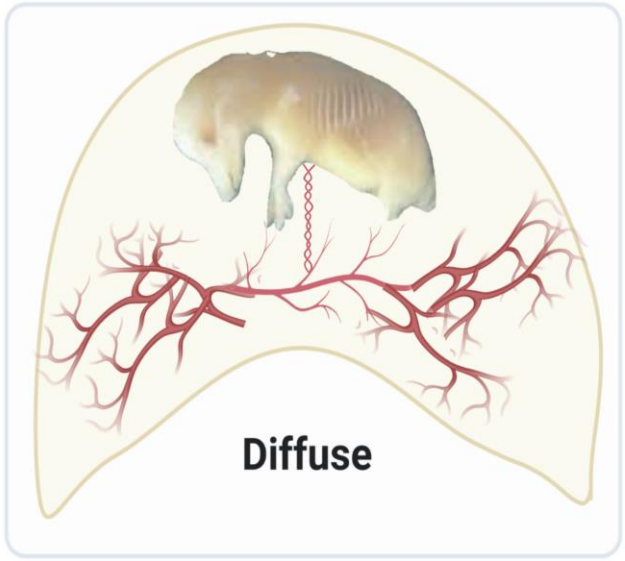
EPITHELIOCHORIAL **SYNDESMOCHORIAL** **ENDOTHELIOCHORIAL** **HEMOCHORIAL**

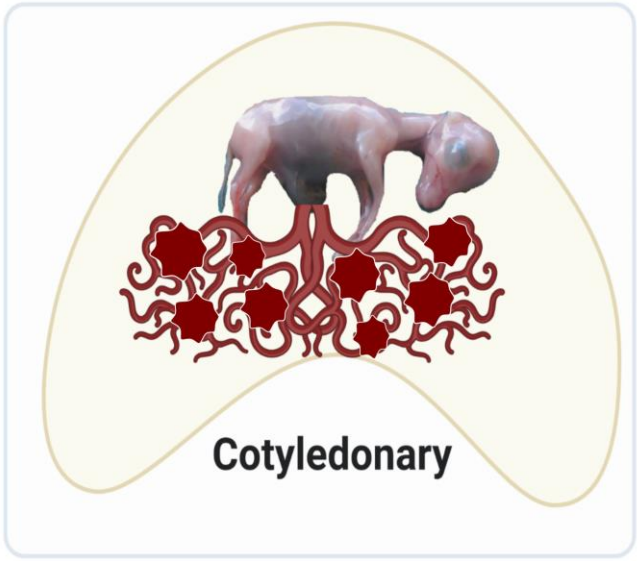


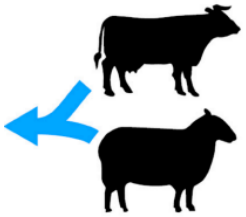
Placentation

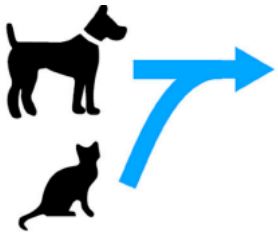
DISTRUBUTION OF CHORIONIC VILLI

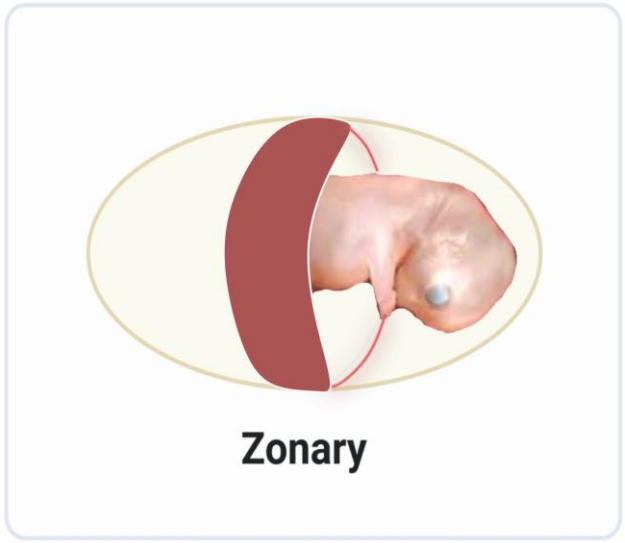


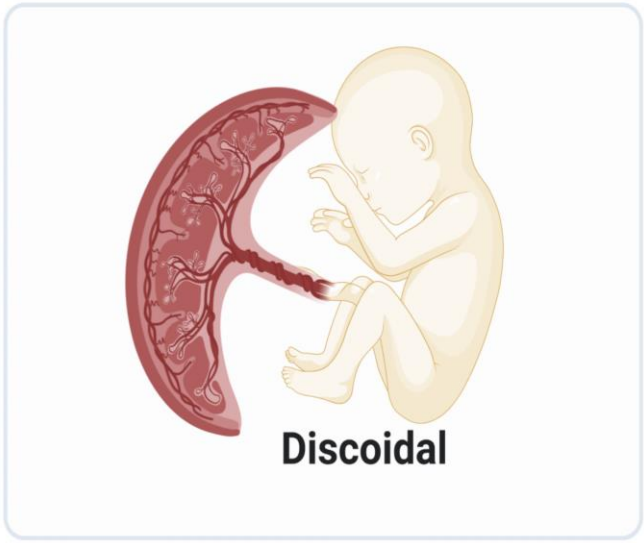


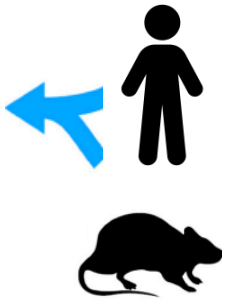






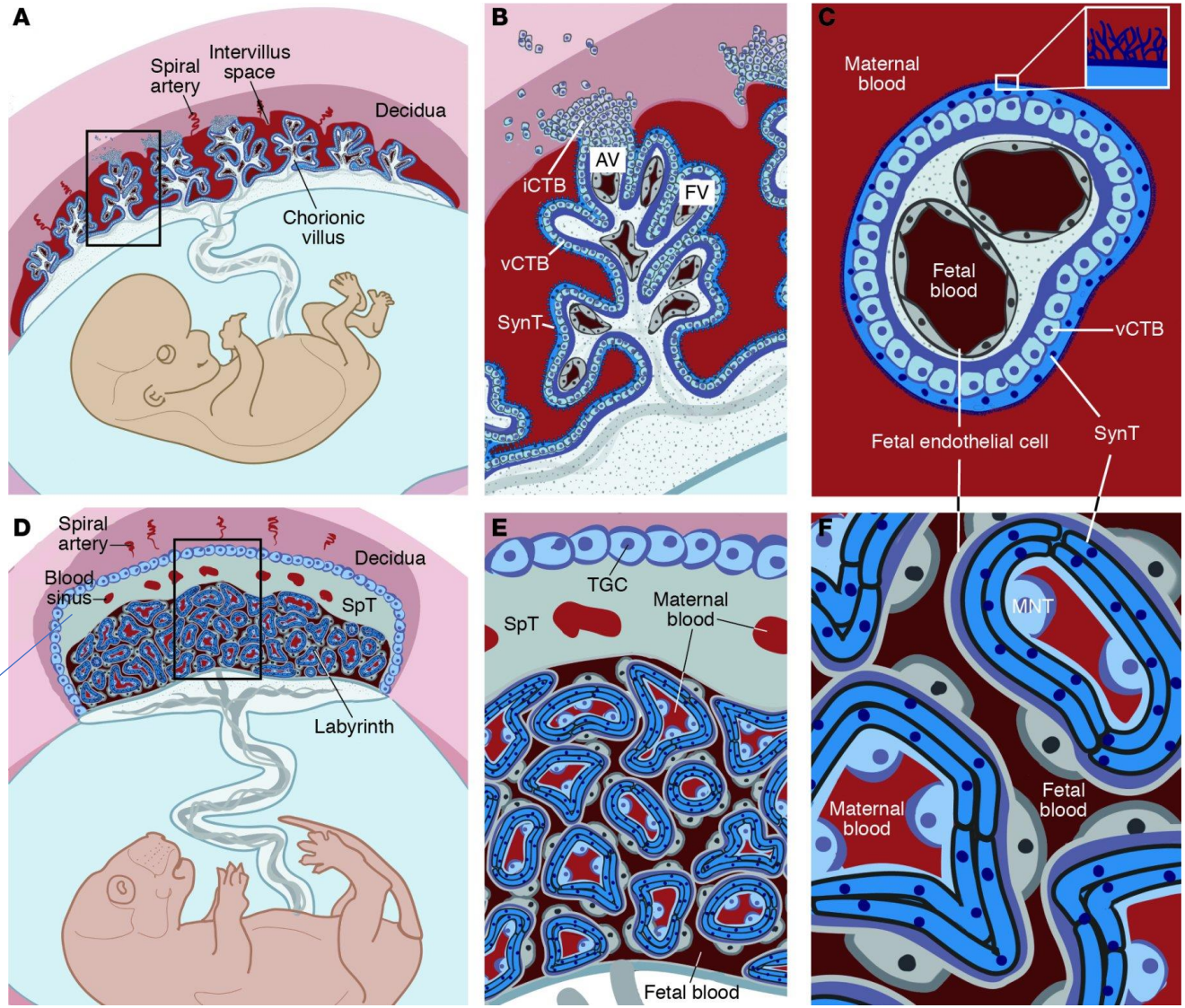






Placentation

junctional zone



Maltepe et al 2010

Conserving endangered species

‘Rewinding human mistakes’: can IVF save the world’s most threatened species?

Technology, viewed as last-ditch insurance policy, this week led to first successful white rhino embryo transfer

https://ivfmeeting.com/products/copy-of-session-26-assisted-reproductive-technology-in-endangered-species-conservation

https://safaripark.cz/en/conservation/northern-white-rhinos/colossal-biosciences-joins-biorescue-on-its-mission-to-save-the-northern-white-rhino-from-extinction