
Innovative Reproduktions-
technologien für den
modernen Artenschutz

BioRescue Partner

BioRescue

GEFÖRDERT VOM

Bundesministerium
für Bildung
und Forschung

MDC MAX-DELBRÜCK-CENTRUM FÜR MOLEKULARE MEDIZIN IN DER HELMHOLTZ-GEMEINSCHAFT

大阪大学
OSAKA UNIVERSITY

AVANTEA

KYUSHU
UNIVERSITY

SAFARI PARK DVŮR KRÁLOVÉ

KENYA
WILDLIFE
SERVICE

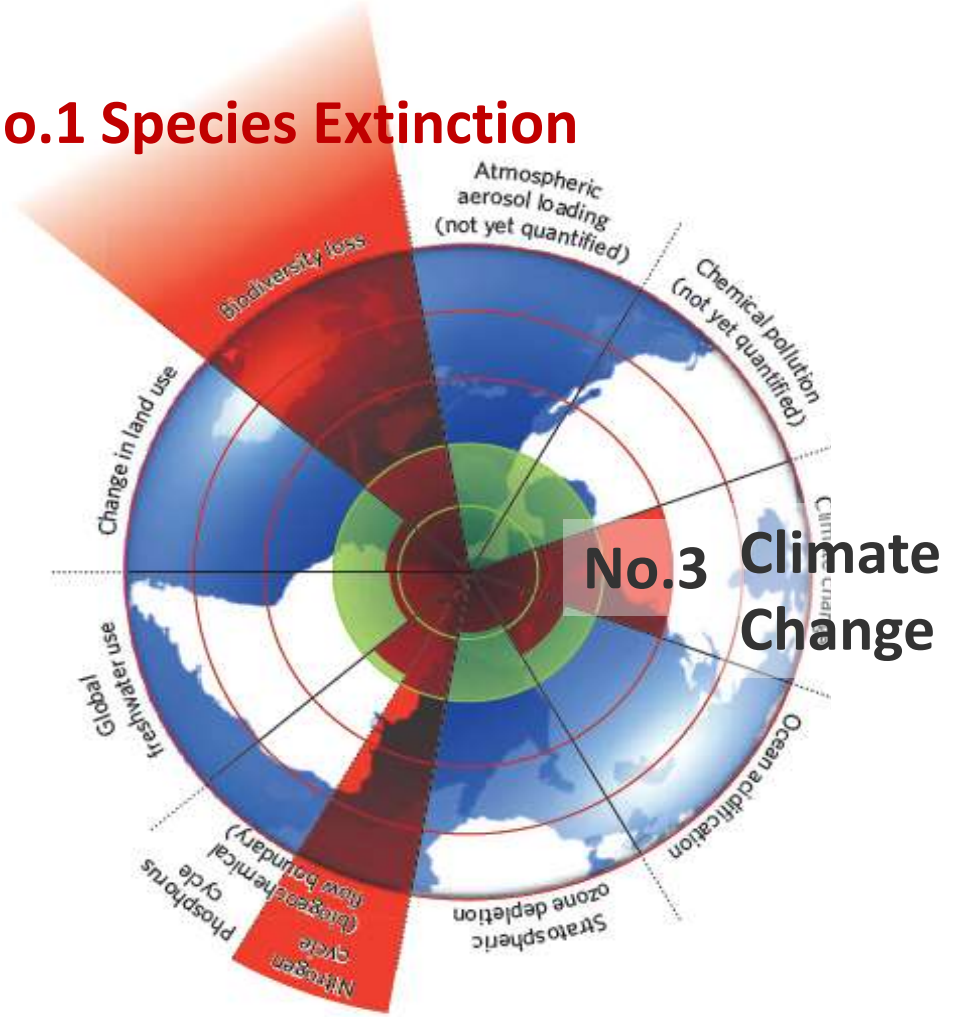
OL PEJETA
CONSERVANCY

Velvyslanectví České republiky v Nairobi
Embassy of the Czech Republic in Nairobi

Stabile and disbalanced Planetary Processes

At risk are (IUCN):

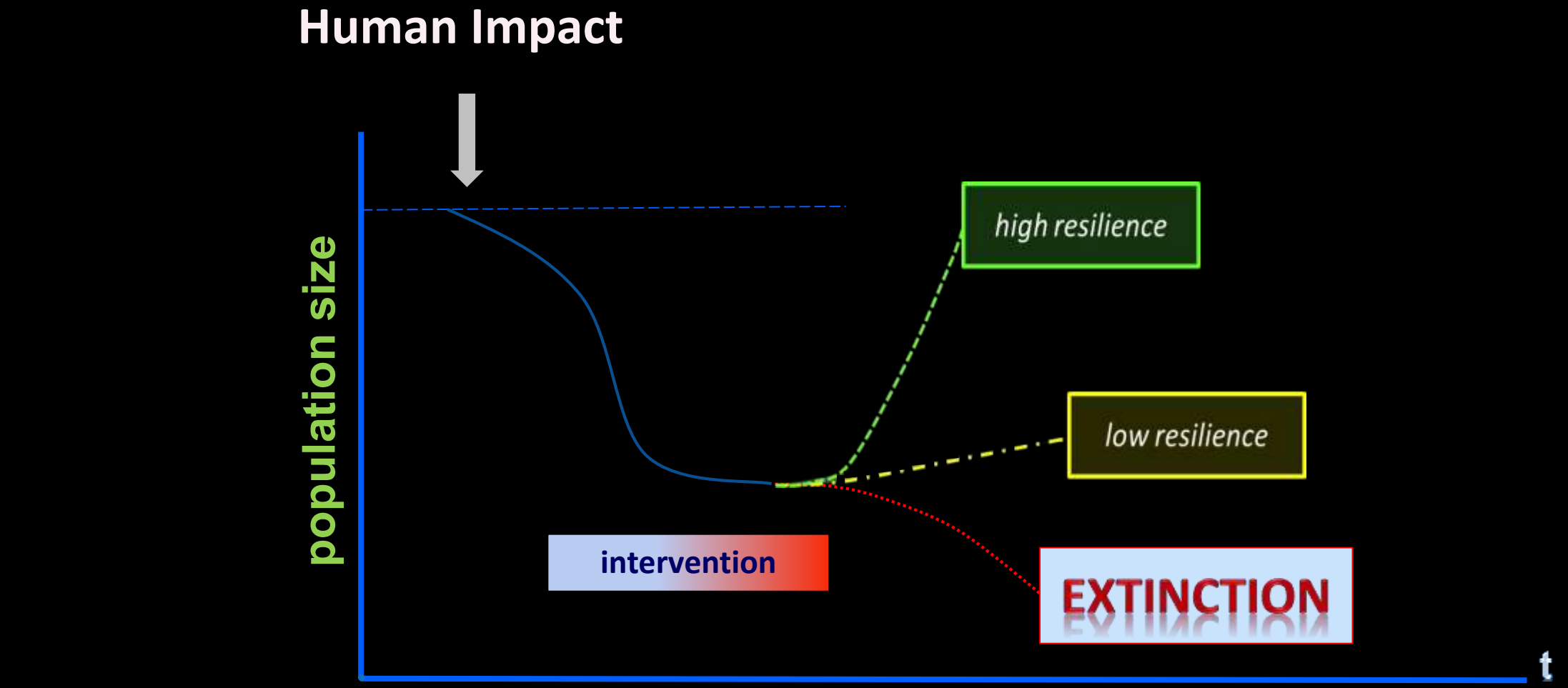
- 21% of all mammals
- 12% of all birds
- 28% of all reptiles
- 30% of all amphibians
- 32% of all fishes
- 27% of all insects
- 45% of all mollusks
- 35% of all crustaceans
- 56% of all arachnids
- ...



Rockström et al., Nature 461: 472-475 (2009)


courtesy of Dr. William Fowlds

Population Dynamics



The Washington Post
Democracy Dies in Darkness

Should we accept this position?

Outlook • Perspective

We don't need to endangered s s. Extinction is part of ion.

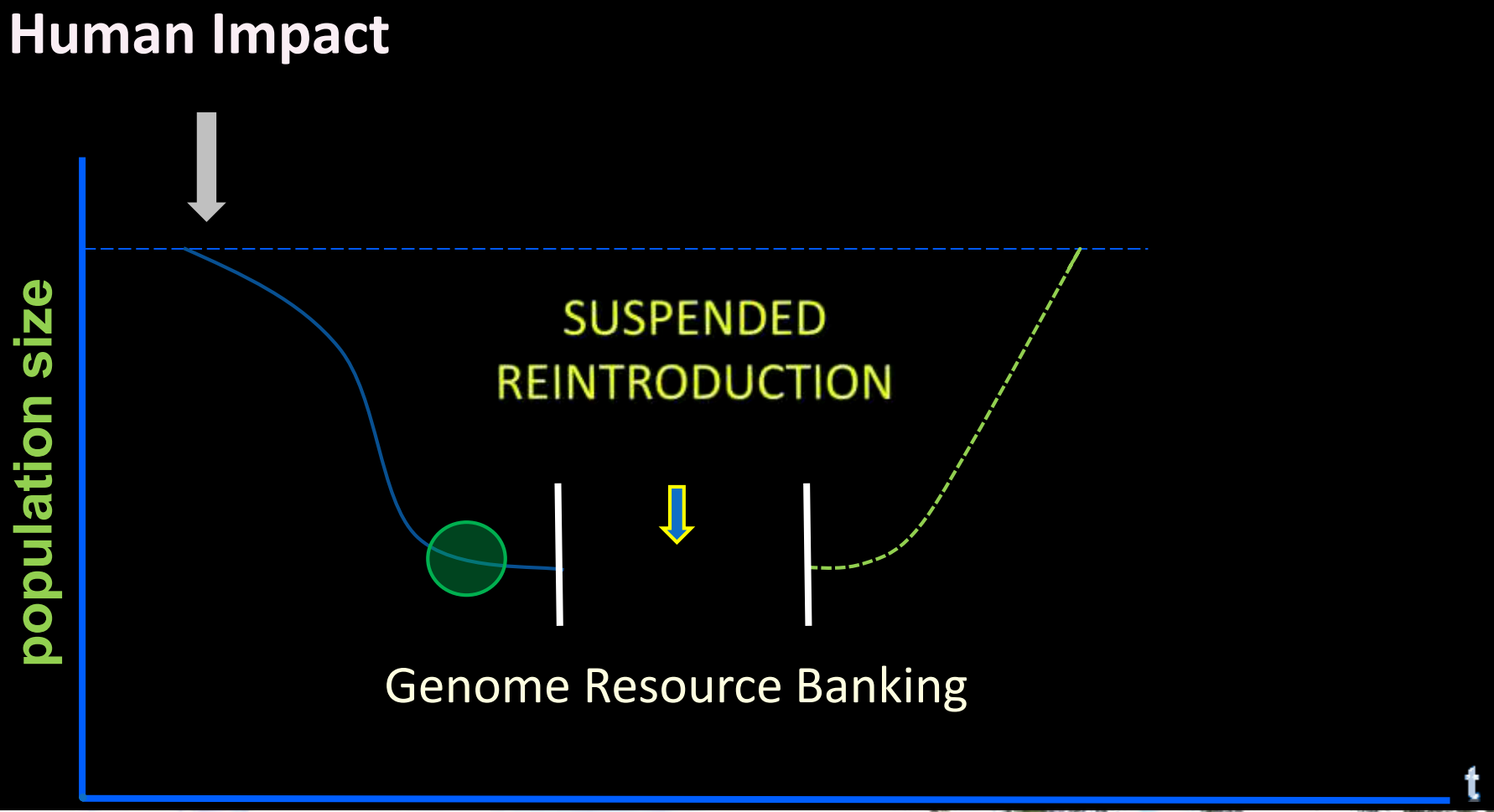
The c we should go out of our way to p omo sapiens.

. Alexander Pyron November 22

R. Alexander Pyron is the Robert F. Griggs Associate Professor of Biology at the George Washington University.

"Evolution loves death more than it loves you or me. This is easy to write,

Population Dynamics

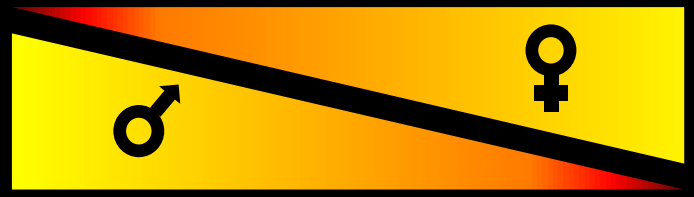



Cryobanking buys Time!
DEPT
4 5
DEPT
5


[w]

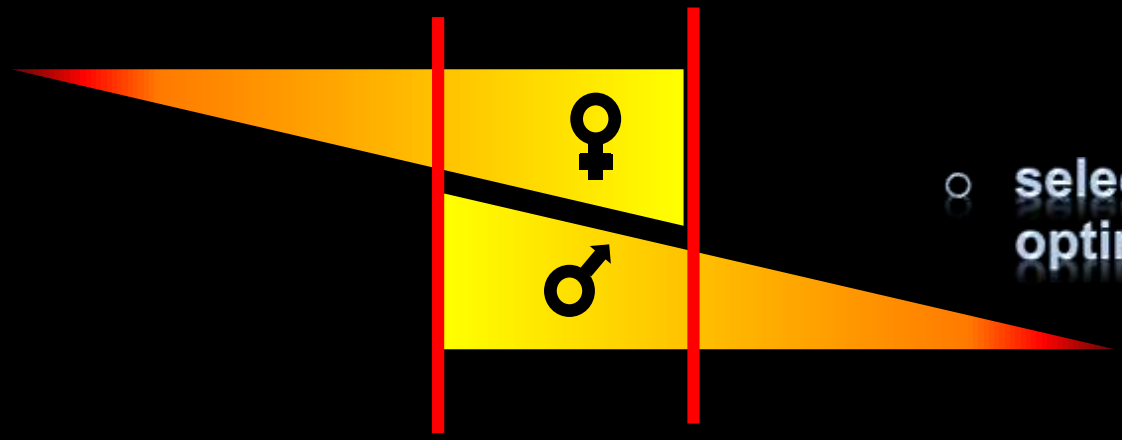
DISTRIBUTION OF FERTILITY PARAMETER

Human



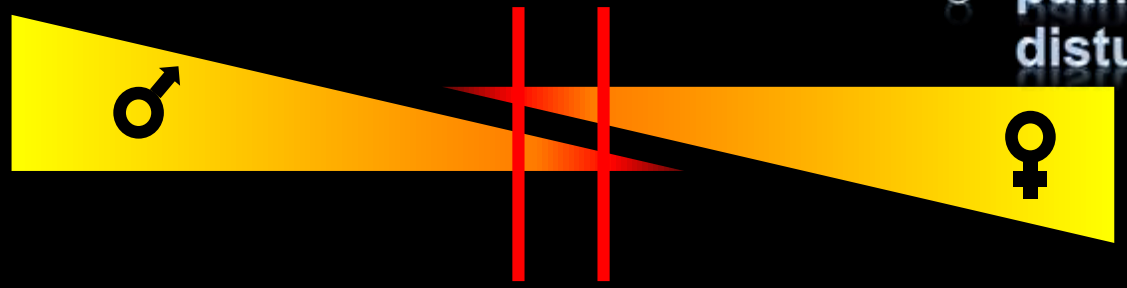
- randomized distributed

Livestock



- selected to optimum

Endangered species



- pathological disturbed

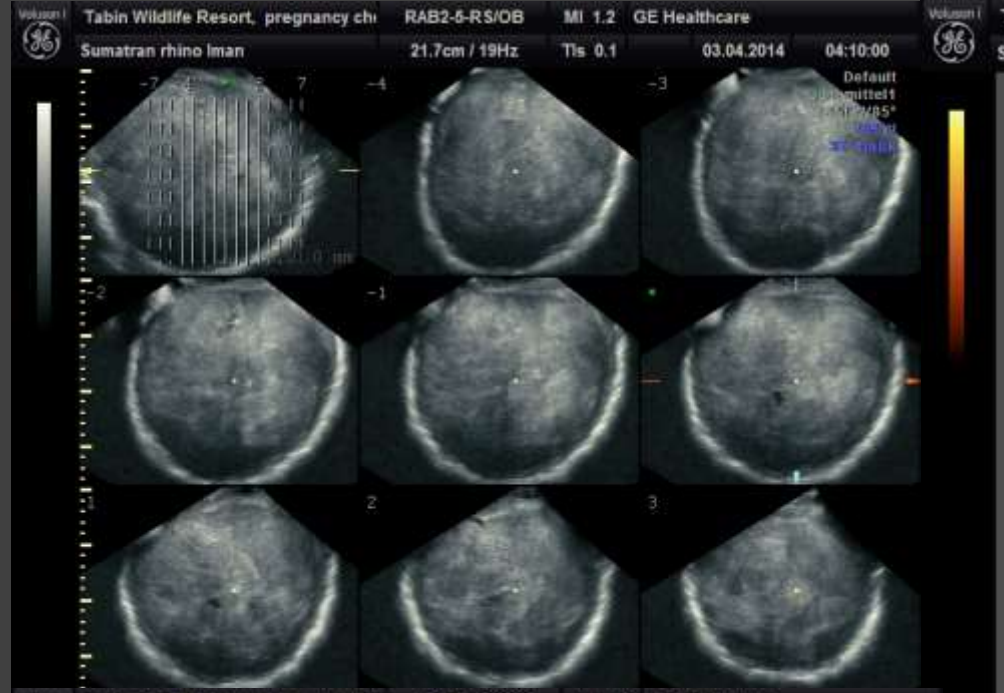
Capture of the last Sumatran rhino in Sabah (18. Dec. 2011)

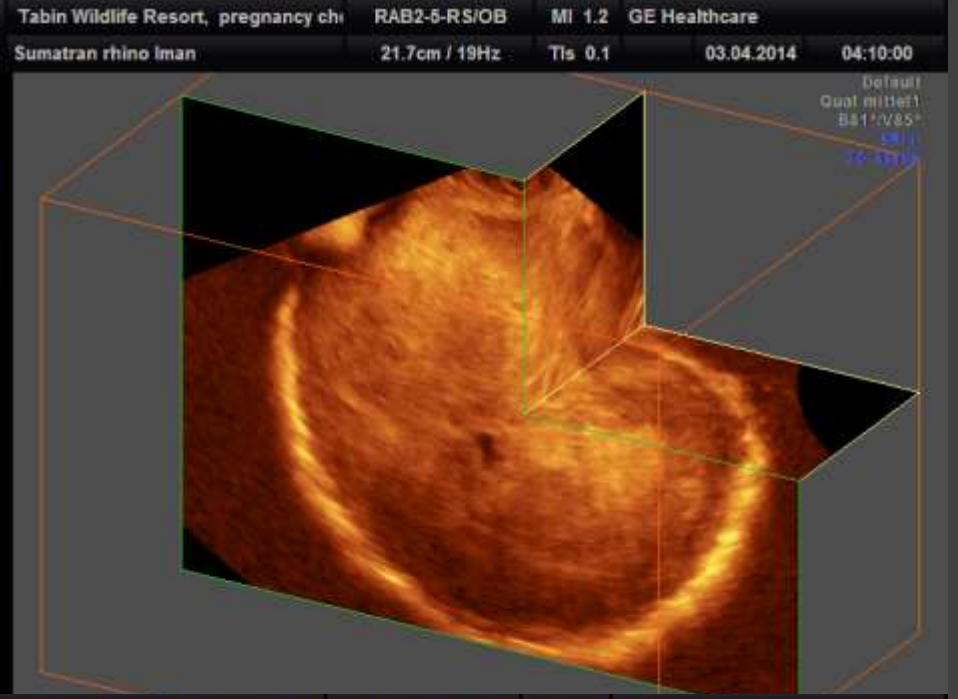




Two weeks later





Tabin Wildlife Resort, pregnancy ch
RAB2-5-RS/OB
MI 1.2
GE Healthcare
Sumatran rhino Iman
21.7cm / 19Hz
TIs 0.1
03.04.2014
04:10:00


Tabin Wildlife Resort, pregnancy ch
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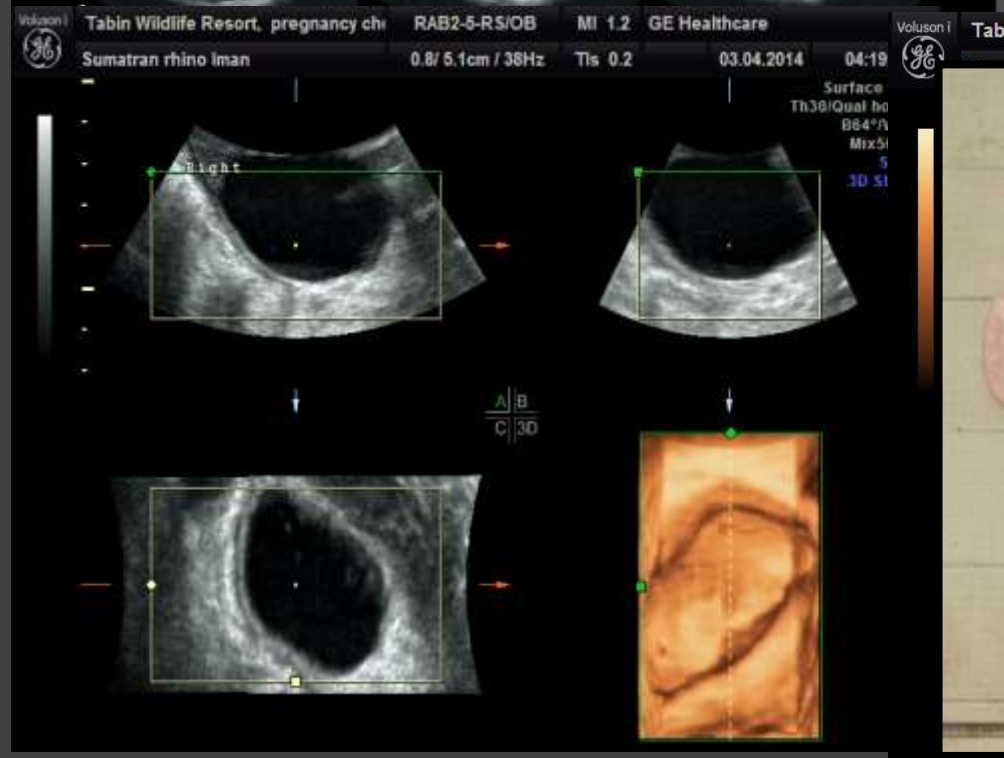
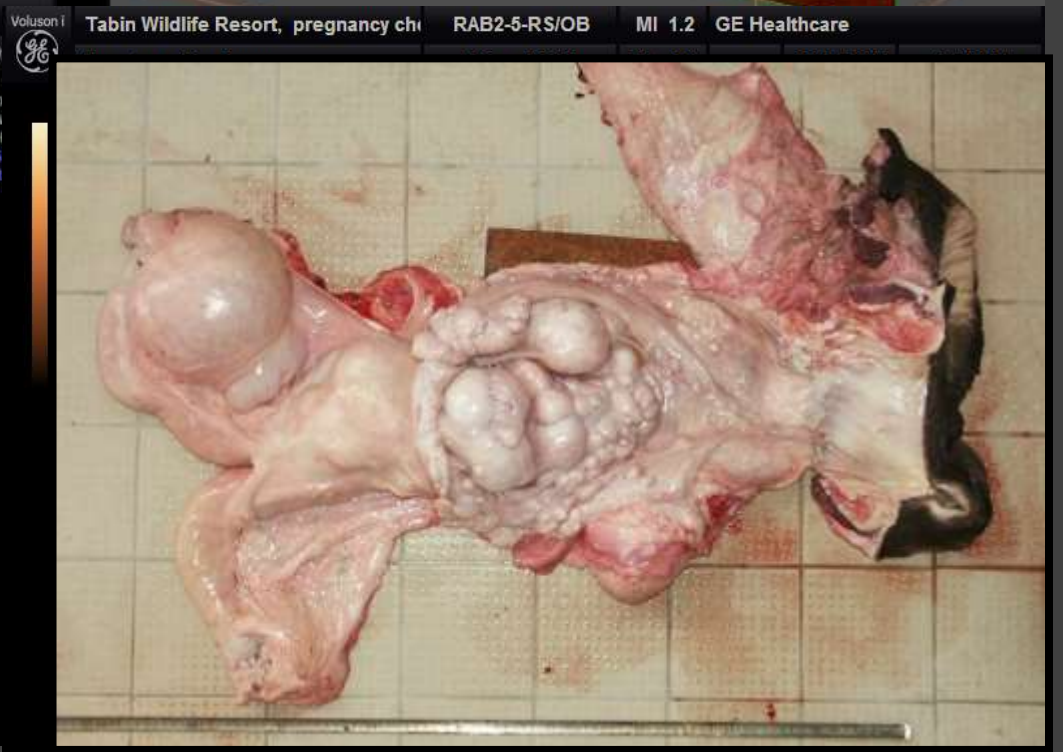

Tabin Wildlife Resort, pregnancy ch
RAB2-5-RS/OB
MI 1.2
GE Healthcare
Sumatran rhino Iman
0.8/ 5.1cm / 38Hz
TIs 0.2
03.04.2014

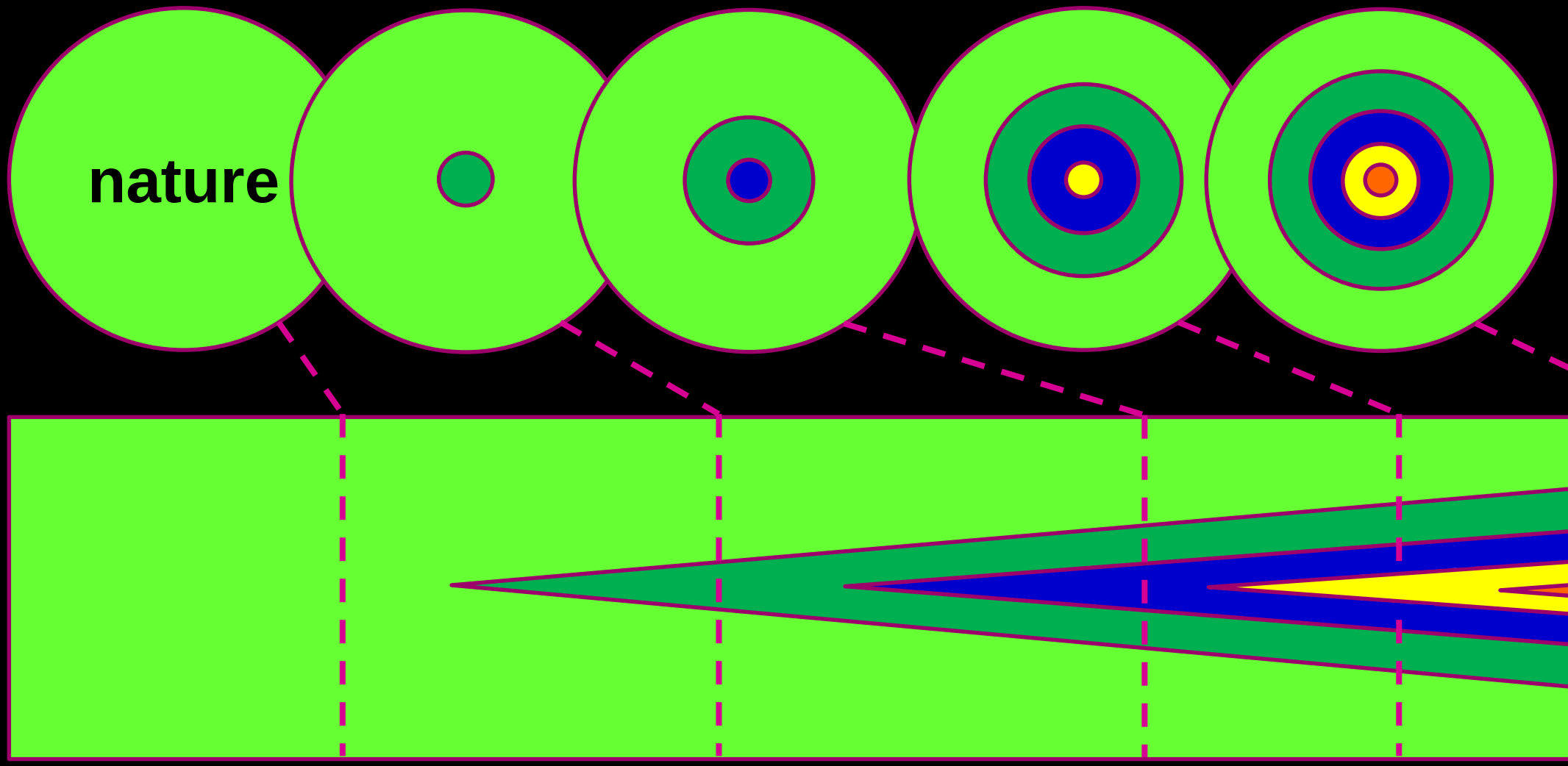

Tabin Wildlife Resort, pregnancy ch
RAB2-5-RS/OB
MI 1.2
GE Healthcare

Inbreeding effects


29-11-2020 10:33:13
camera_1

Current and future Conservation Measures

Anthropozean



Richard Lydekker (1849 – 1915)

Head of Northern White Rhinoceros.

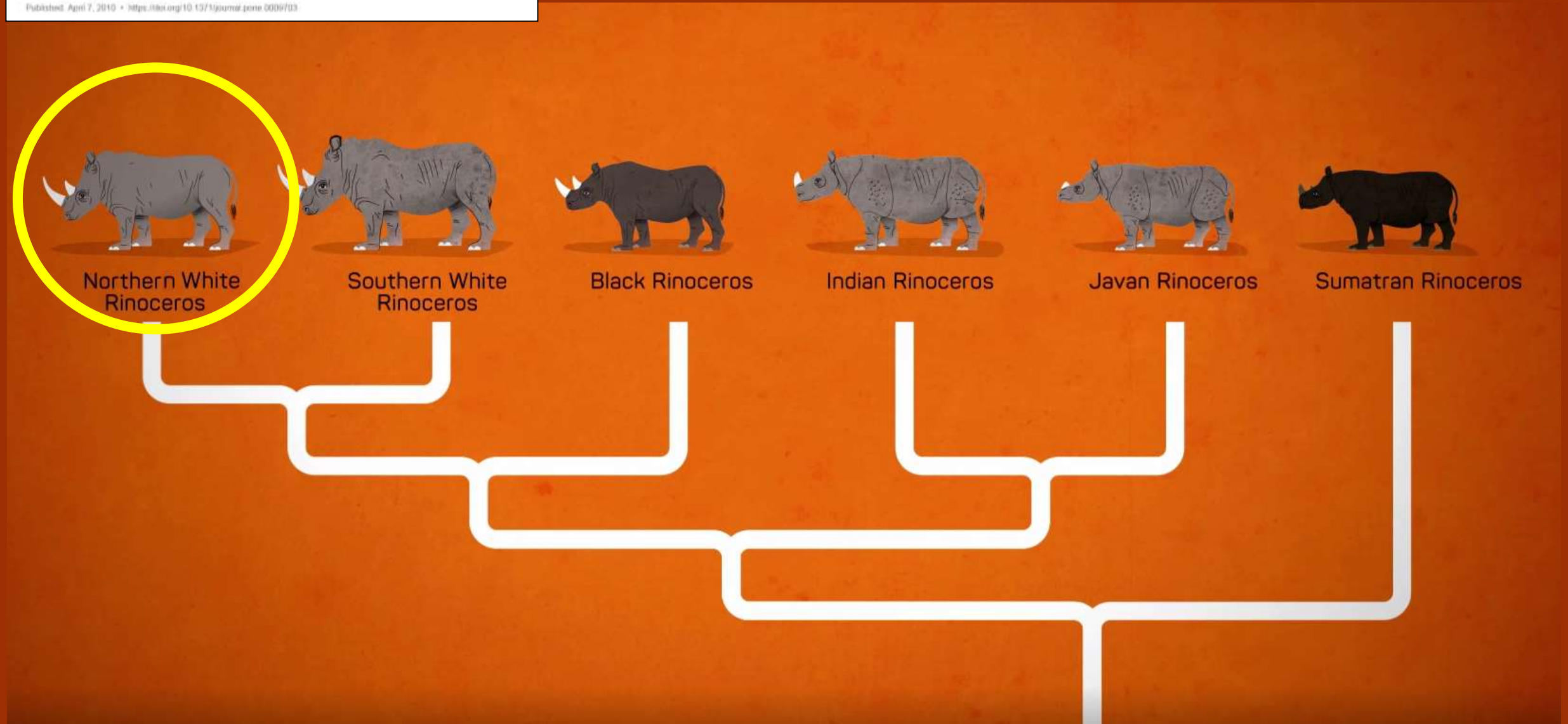
Skull and horns of White Rhinoceros from Lado in the possession of Sir Edmund G. Loder, Bart.

110 years later practically extinct

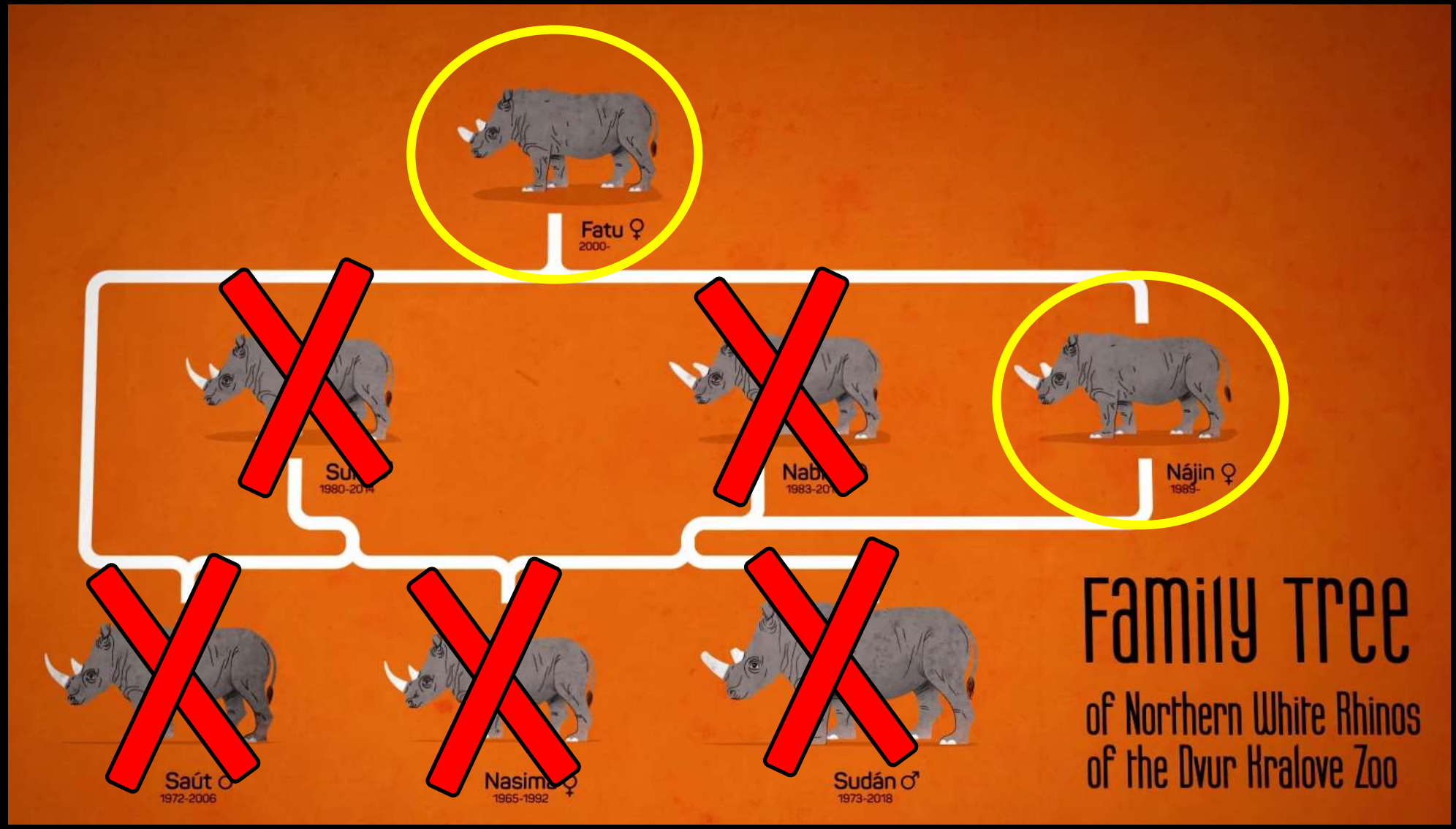
White Rhinoceros of Lado, Records of Big Game, 1914.

Mr. Lydekker announced the discovery in a notice of The Field - February 22, No. 2878: 319, 1908:

Phylogeny of the Rhinocerotidea



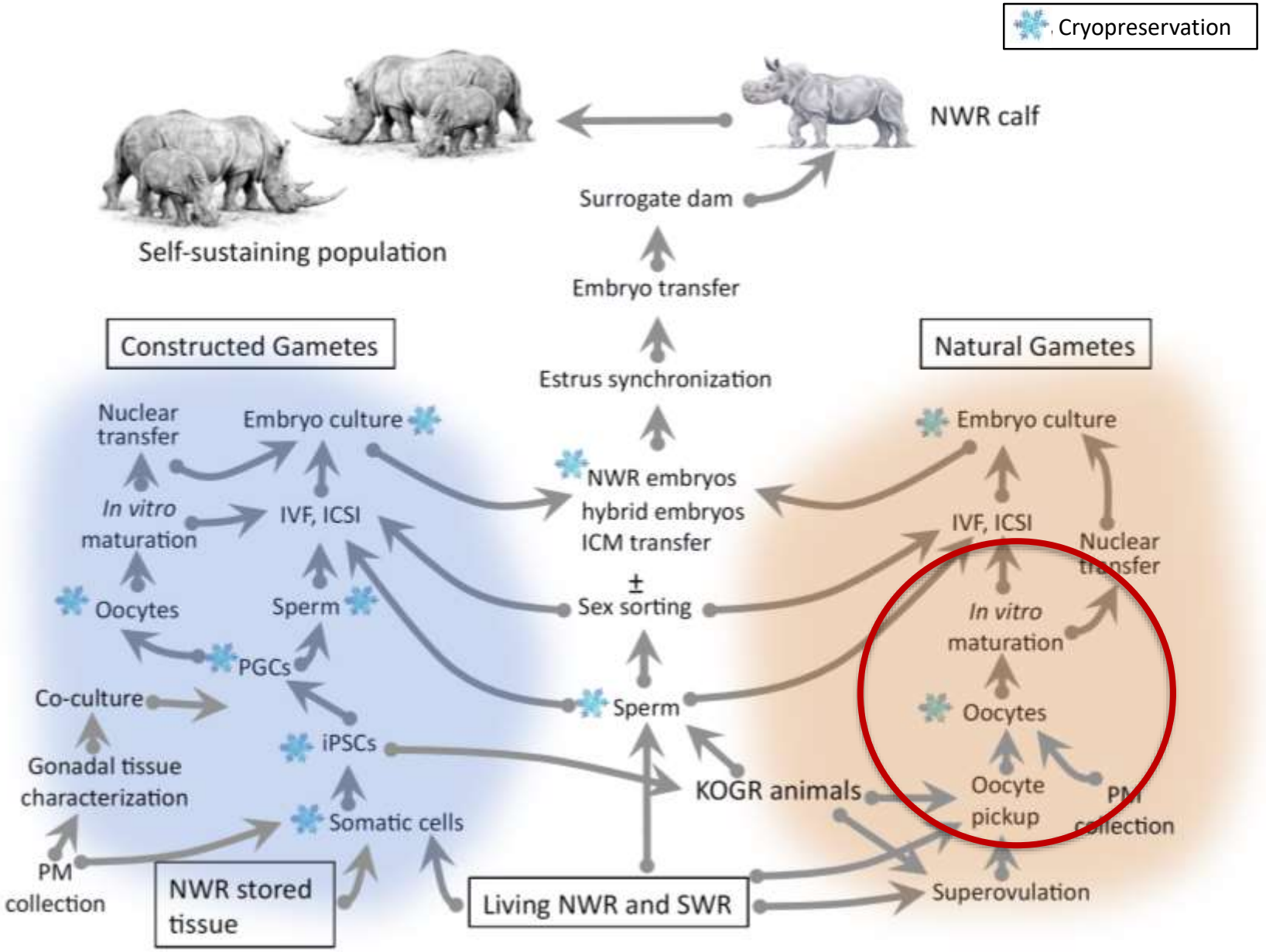
Last of their Kind




Is the northern white rhinoceros doomed?

Twenty-four / seven

BioRescue


Cryopreservation
NWR calf
Surrogate dam
Self-sustaining population
Embryo transfer
Constructed Gametes
Natural Gametes
Estrus synchronization
Nuclear transfer
Embryo culture
NWR embryos
hybrid embryos
ICM transfer
In vitro maturation
IVF, ICSI
Oocytes
Sperm
±
Sex sorting
PGCs
Co-culture
Sperm
iPSCs
Gonadal tissue characterization
KOGR animals
Somatic cells
PM collection
NWR stored tissue
Living NWR and SWR
Oocyte pickup
Superovulation

Northern white Rhino
Ol Pejeta, Kenya

Anaesthesia in wild patients

QUESTIONNAIRE on Medical History of Patients

Age:___yrs Height:___cm Weight___kg

Do you smoke?

Do you drink alcohol regularly?

Do you take any kind of drugs?

Do you have diabetes?

Do you have high blood pressure?

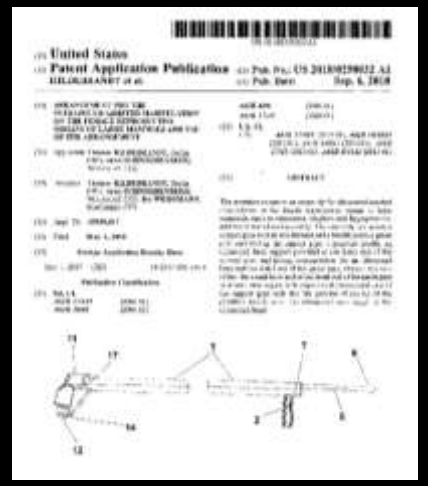
Do you have any known intolerances?

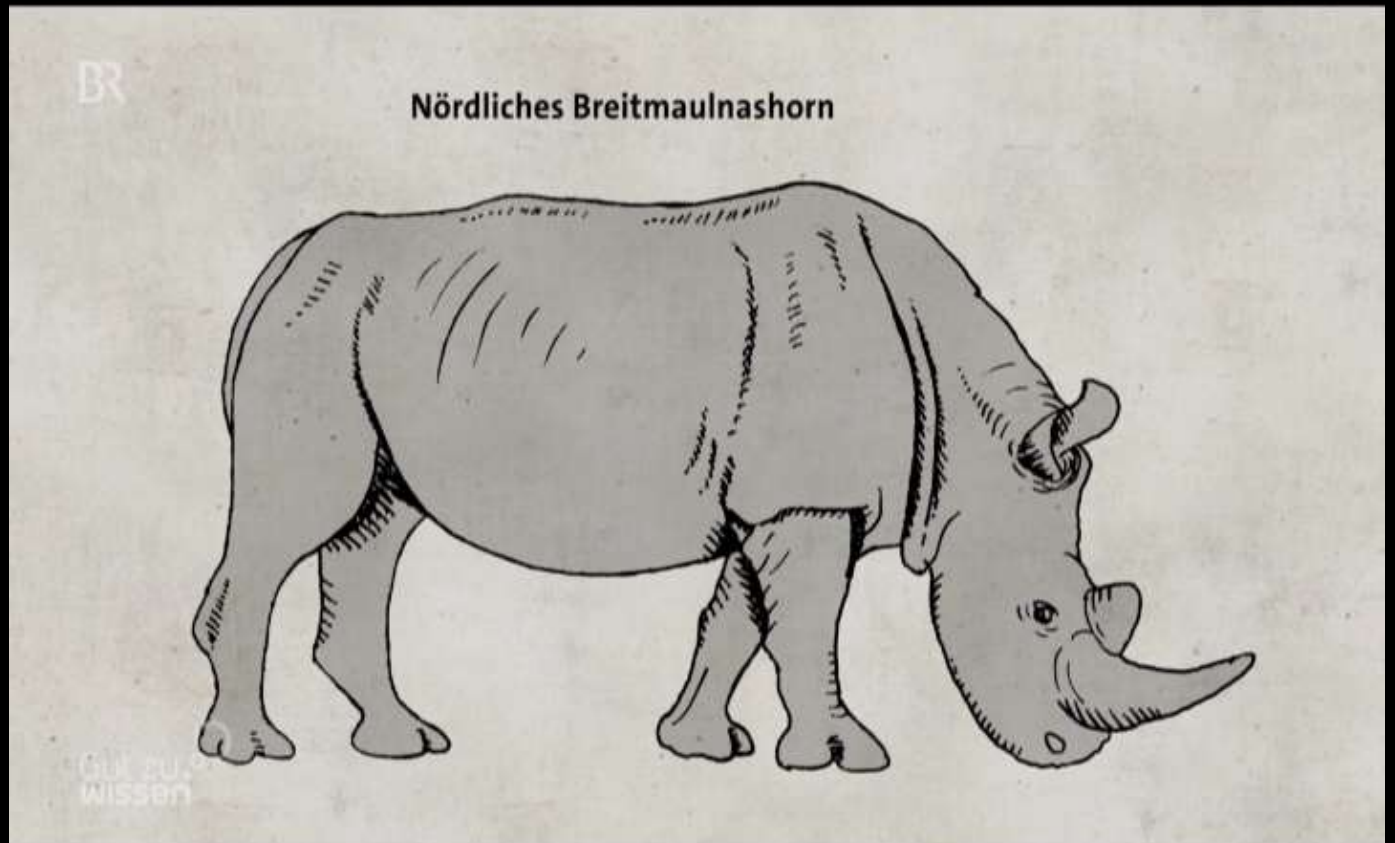
Anaesthesia incident

Anaesthesia incident

Transrectal oocyte collection in white rhinos using a patented system

(by U.S. Patent No. 10,779,859; European Patent EP 3 369 397; ARIPO Patent AP/P/2018/010558; Republic of South Africa Patent No. 2018/01416)



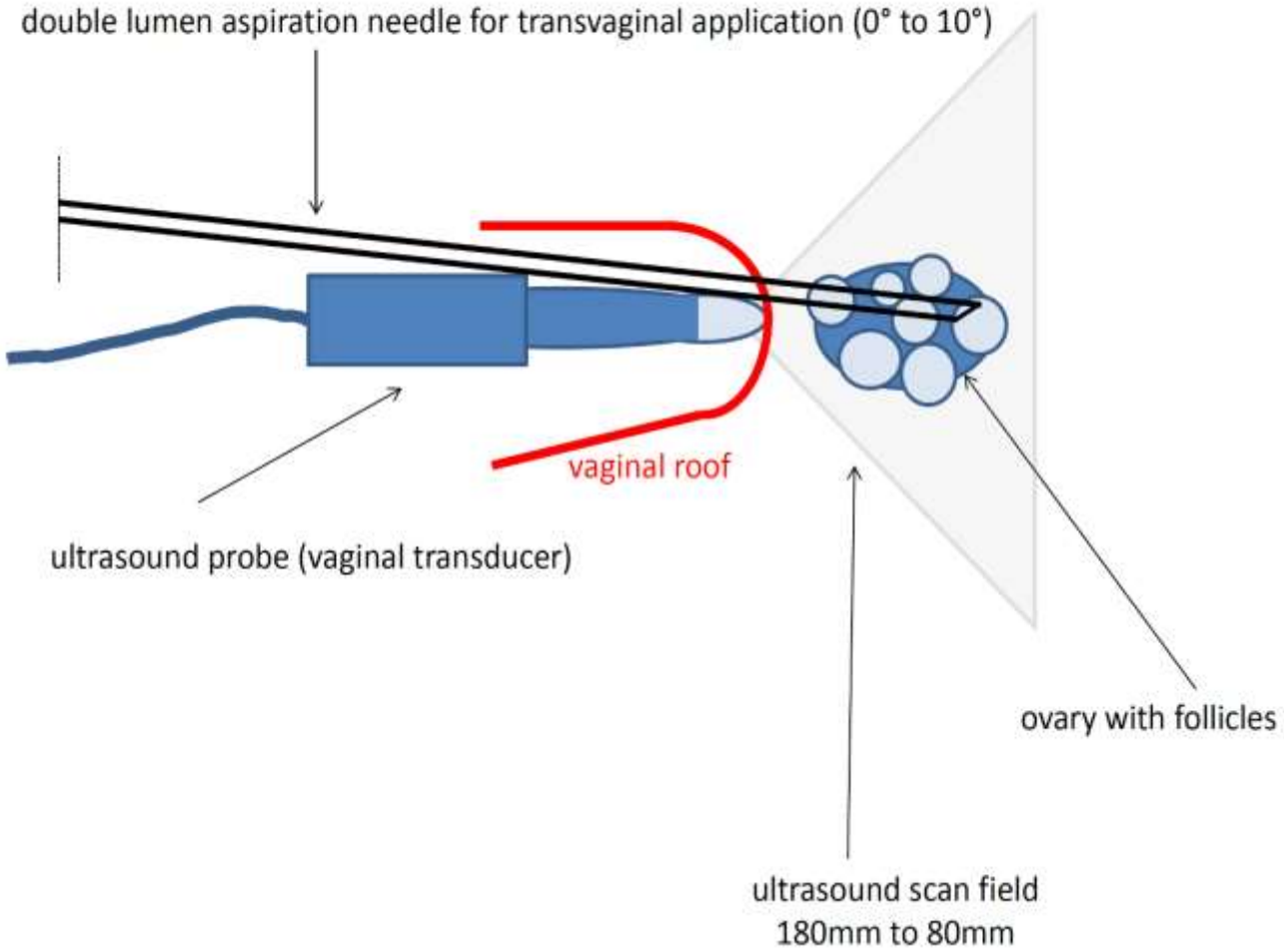


General Anesthesia

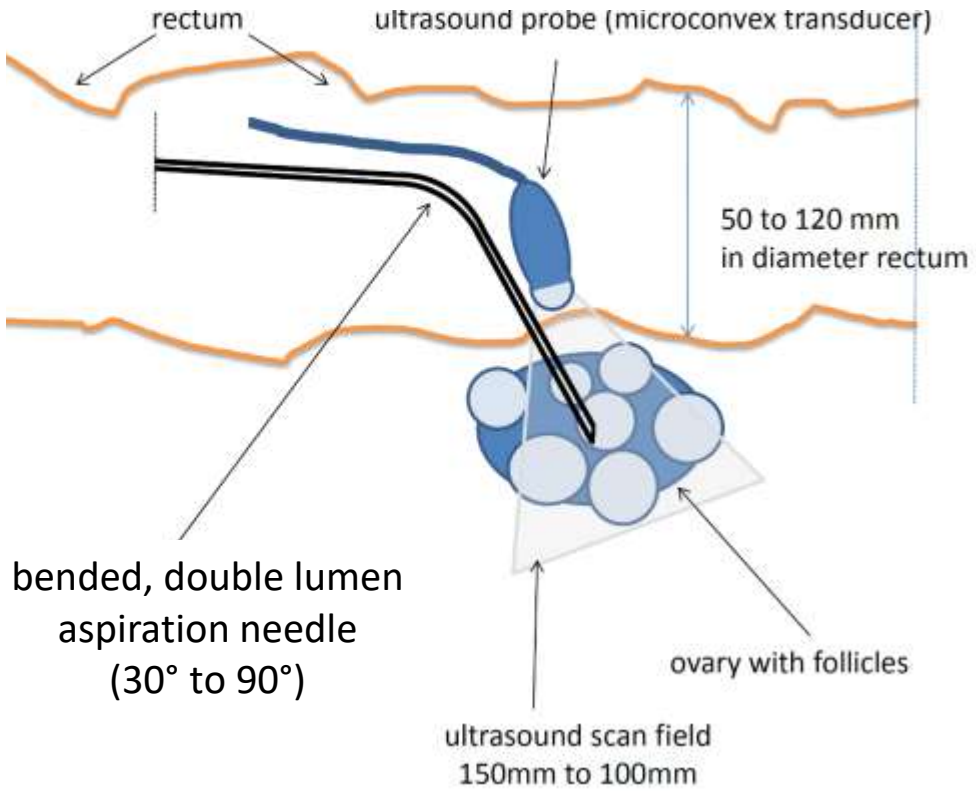
New oocyte aspiration technique

to 90°

classic (5 - 10 cm)



new (150 – 200 cm)



U.S.-PAT: 10.779.859

European PAT: EP 3 369 397

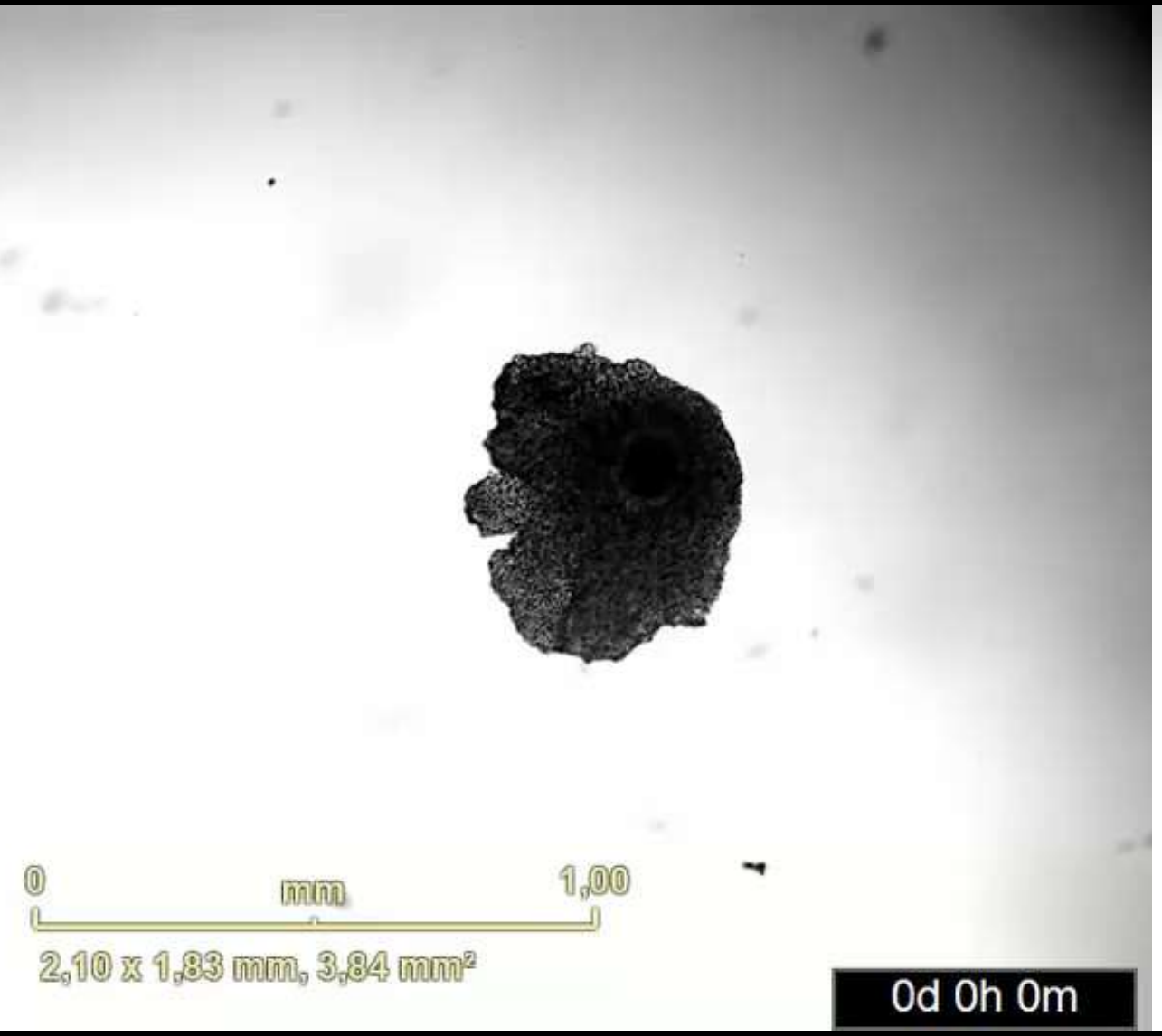
3D-ultrasound of a stimulated rhino ovary

Before IVM

After IVM

AVANTEA

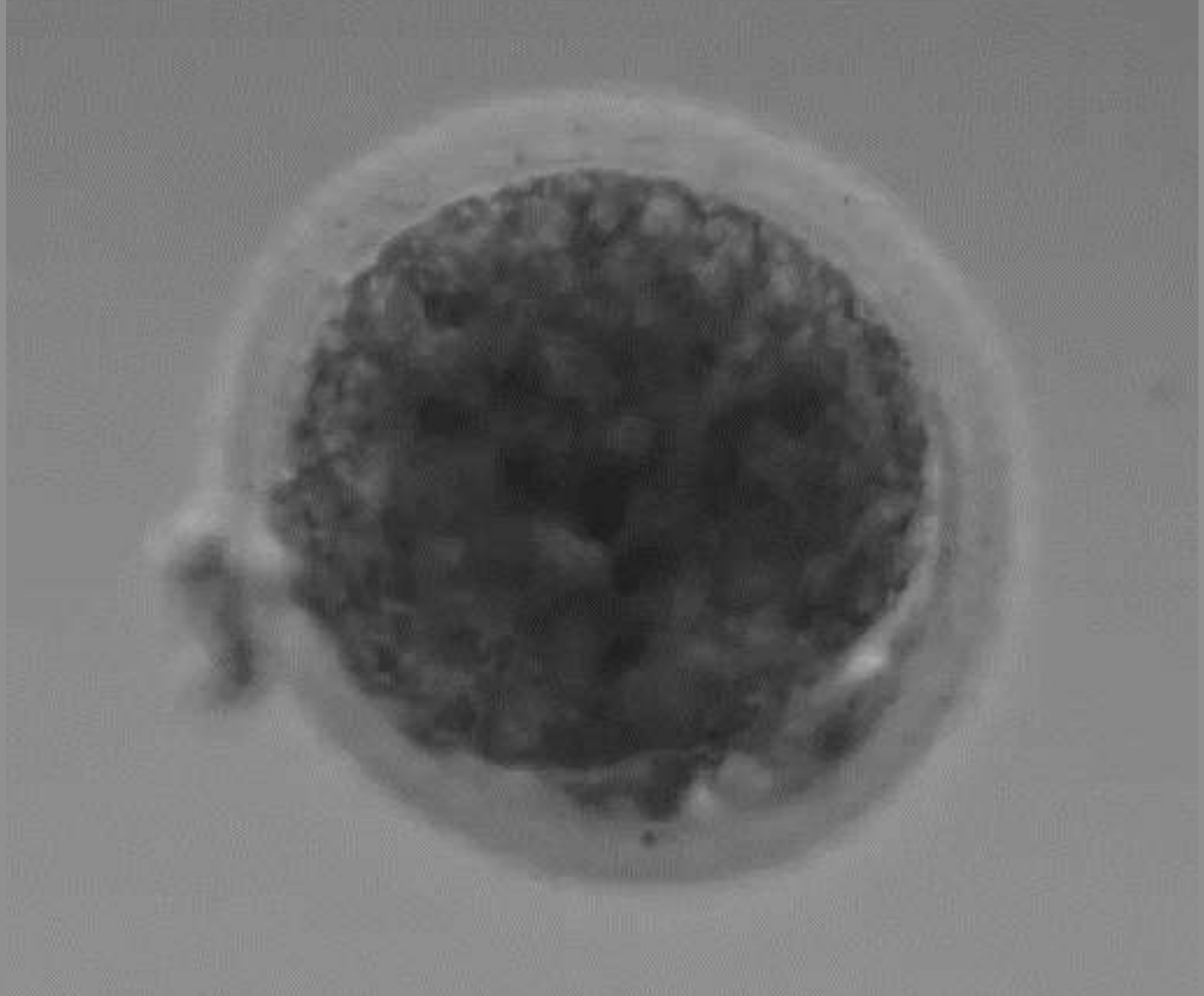
In Vitro Maturation of rhinoceros inmature rhinoceros oocytes (36 hours)



ICSI

Intra**C**ytoplasmatic **S**perm **I**njection

Fatu x Suni



Oocyte collection in southern (SWR) & northern white rhinoceroses (NWR)

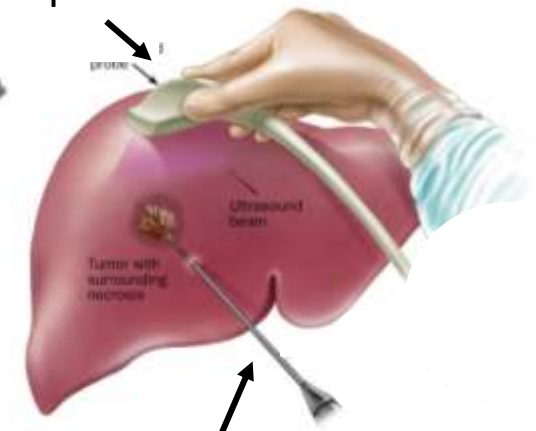
N° OPU	N° total females	Distribution reproductive status	N° total follicles	N° punctered follicles	N° recovered oocytes	N° injected MII	N° cleaved embryos	N° total blastocysts
82	26	10% fertile & 90% infertile	1513	1168	437	163	105	46 SWR 39 NWR

Embryo Transfer Program in wild surrogates

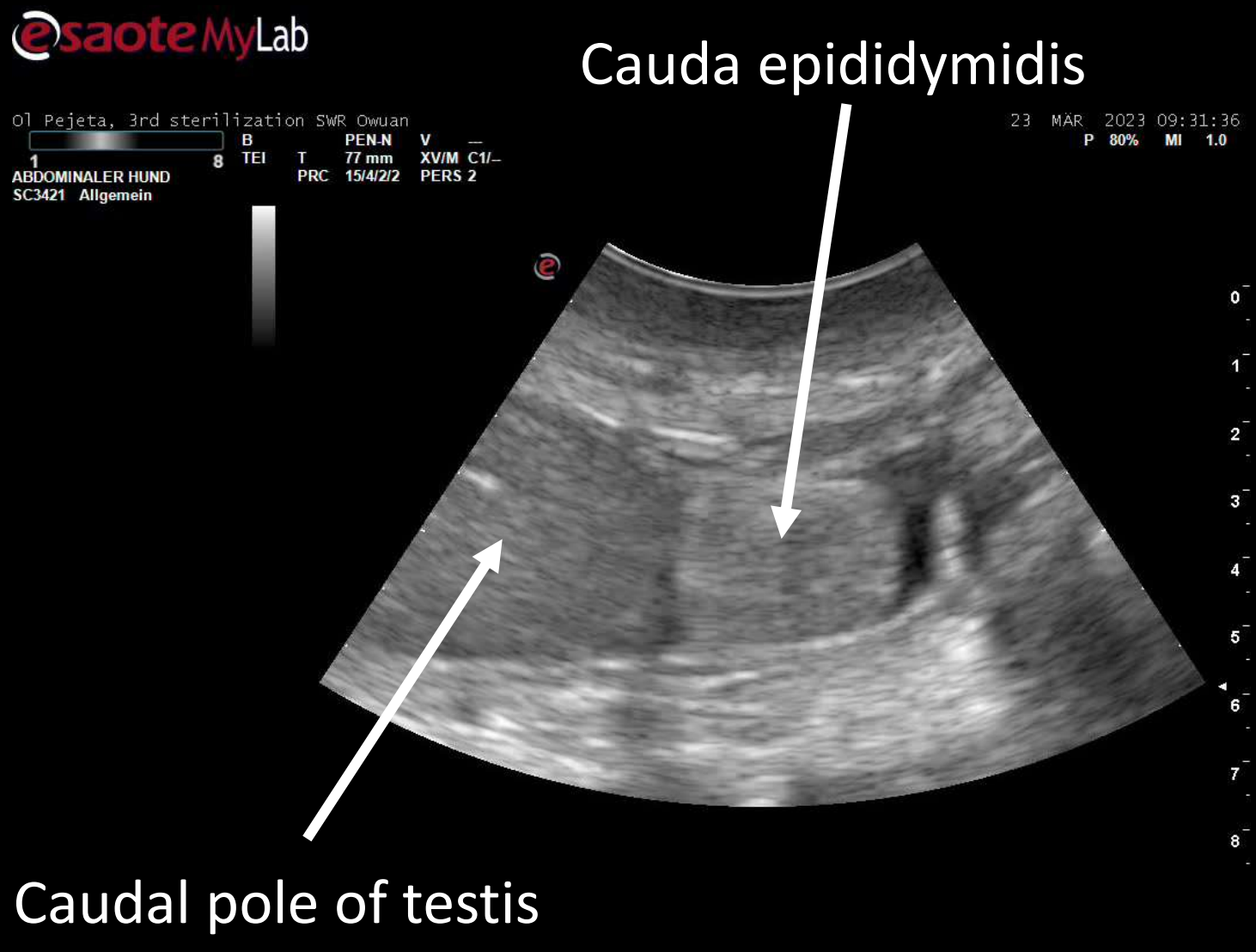
Timing the Transfer by Estrus Detection by a sterilized Teaser Bull

Non-surgical Teaser Bull preparation

ultrasound probe



water-cooled microwave ablation probe

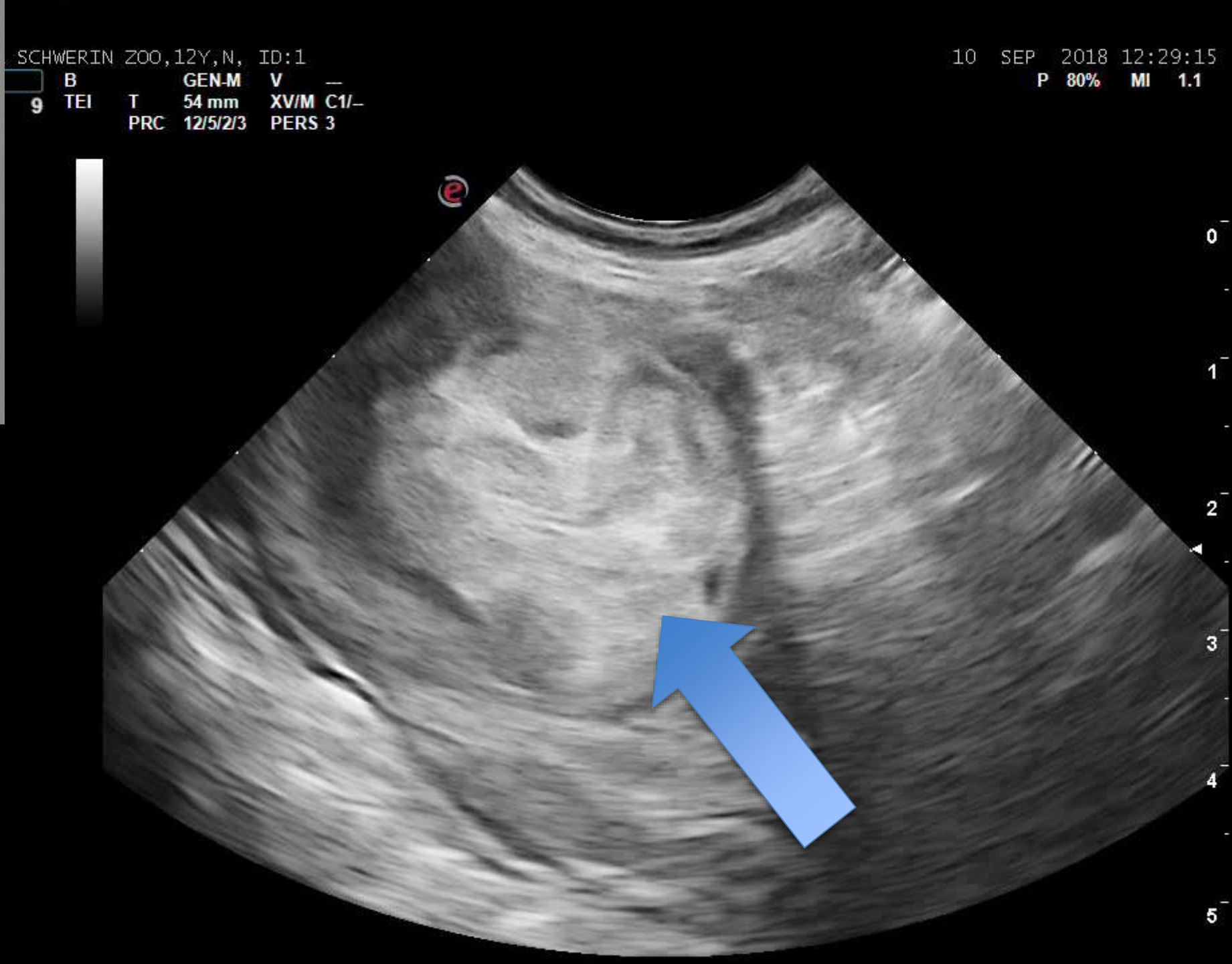


Targeting the epididymis with the probe

Ablation process for the tissue destruction


OXYGEN

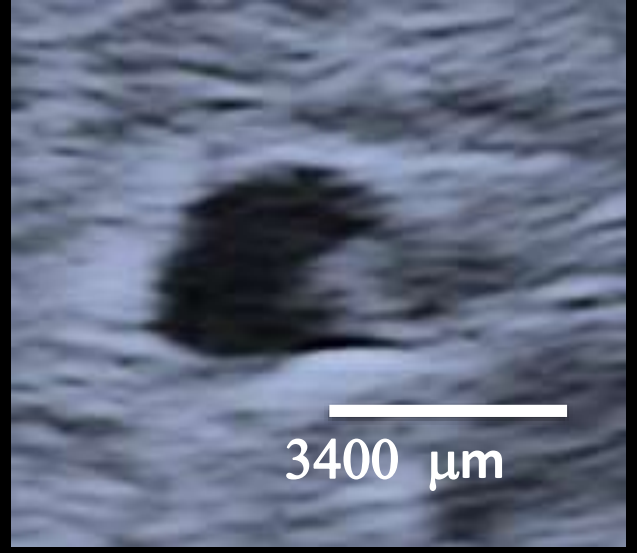
transrectal Embryo Transfer

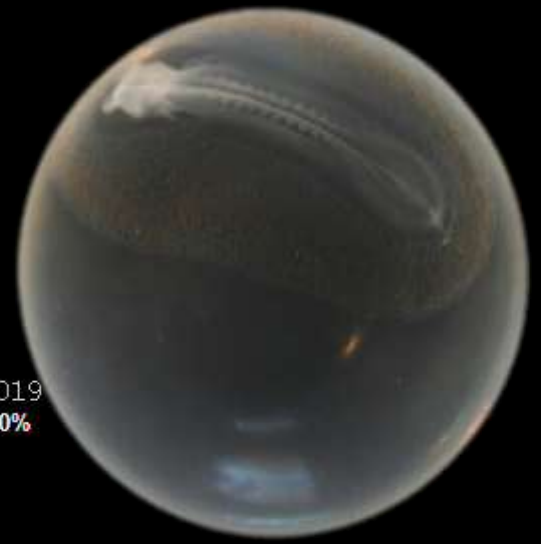

SCHWERIN ZOO,12Y,N, ID:1
10 SEP 2018 12:29:15
B GEN-M V —
9 TEI T 54 mm XV/M C1/–
PRC 12/5/2/3 PERS 3
P 80% MI 1.1
0
1
2
3
4
5


2022/01/31
DOG001_D05_0050
v007
1002 (1001 - 1128)


2022/01/31
DOG001_D05_0060
v004
1001 (1001 - 1231)


100 µm


3400 µm


23 MAI 2019
P 80%


0
1
2
3

First Rhino Fetus (70d) generated by Embryo Transfer

Photo of the Year 2024

The Guardian

Eur

'The sight of it is still shocking': 46 photos that tell the story of the century so far

IVF rhino foetus, 2023

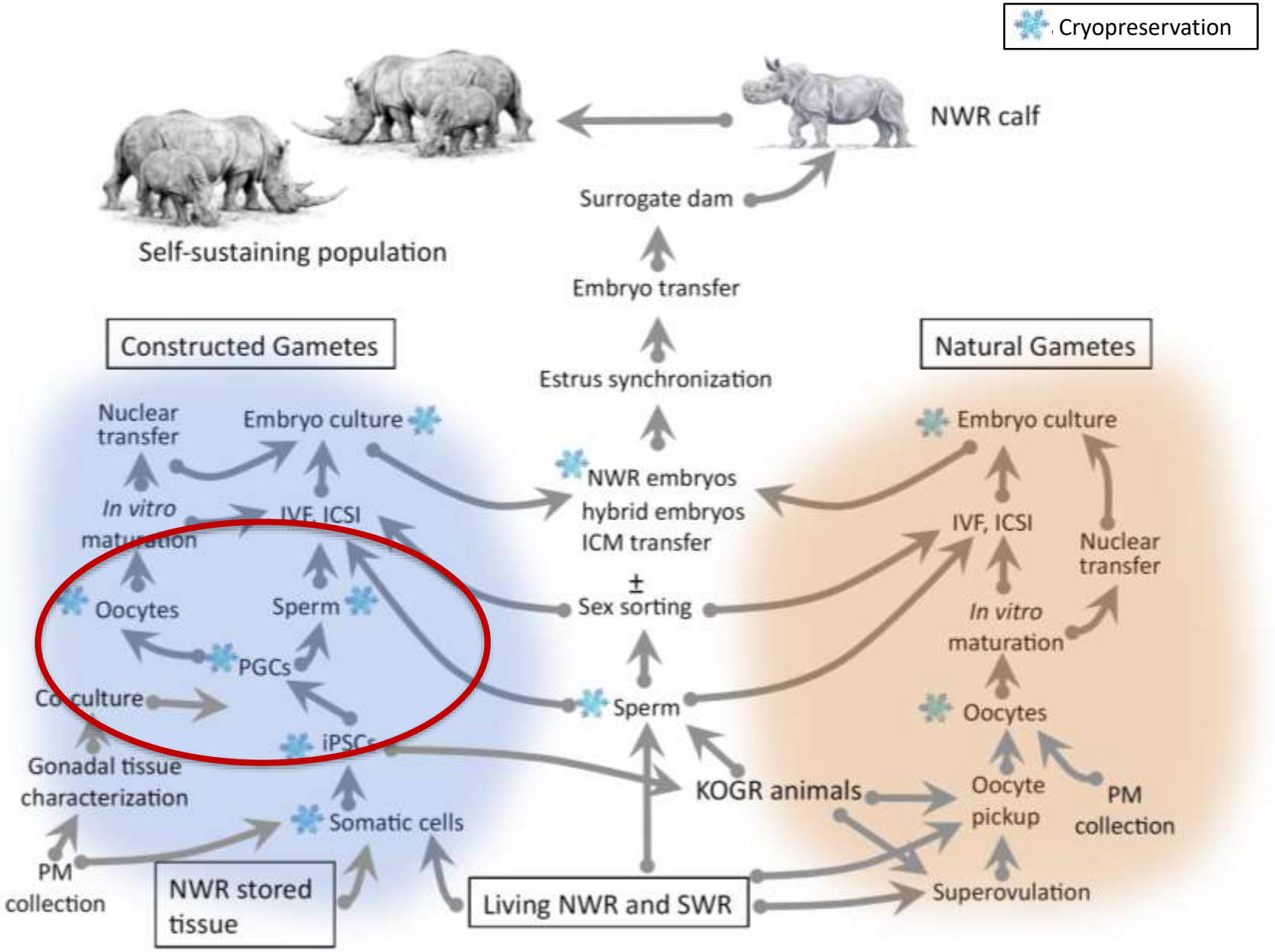
By Jon A Juárez

Photograph: Jon A Juárez

In March 2018, tragedy struck: Sudan, the last northern white male rhino, died. With only two females left, the species was functionally extinct. Then, a flash of hope: a foetus created using sperm taken from males before they died.

Sadly, the mother died of an unrelated infection. But tests showed the tiny foetus would probably have survived. Said photographer Jon A Juárez, "Though the story is bittersweet, the foetus proves the science works. If we support scientists' efforts, we can still correct our course and make the planet a better place." *GS*


5Y-KZD
DREAMLINER
BOEING 787-8


Cryopreservation
NWR calf
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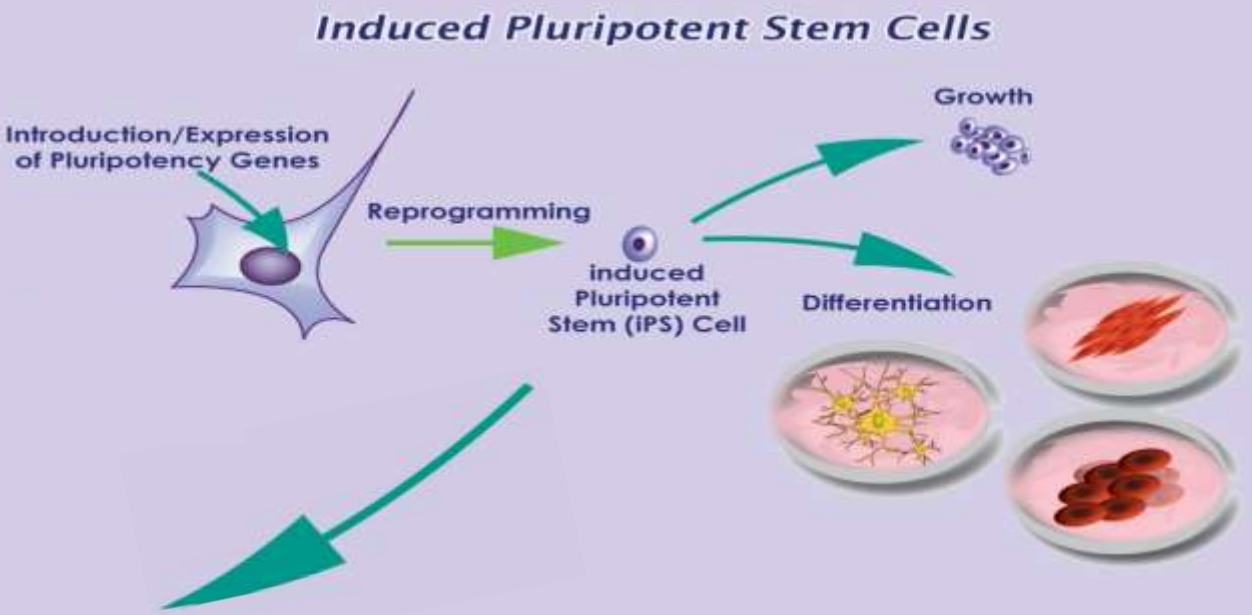
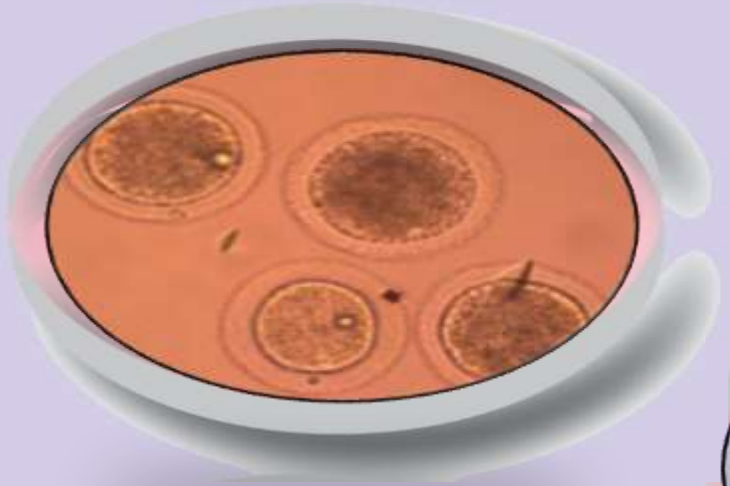
Self-sustaining population
Embryo transfer
Constructed Gametes
Natural Gametes
Estrus synchronization
Nuclear transfer
Embryo culture
NWR embryos
hybrid embryos
ICM transfer
Embryo culture
In vitro maturation
IVF, ICSI
IVF, ICSI
Nuclear transfer
±
Sex sorting
Oocytes
Sperm
In vitro maturation
PGCs
Co-culture
Sperm
Oocytes
iPSCs
Gonadal tissue characterization
KOGR animals
Oocyte pickup
PM collection
Somatic cells
PM collection
NWR stored tissue
Living NWR and SWR
Superovulation

In 2012, Dr. Yamanaka was awarded the Nobel Prize in Physiology or Medicine for his discovery that adult somatic cells can be reprogrammed into pluripotent cells. By introducing the genes for four factors that turn genes on and off, he induced the skin cells of adult mice to become like embryonic stem cells, whicl



In-Vitro-Gametogenesis

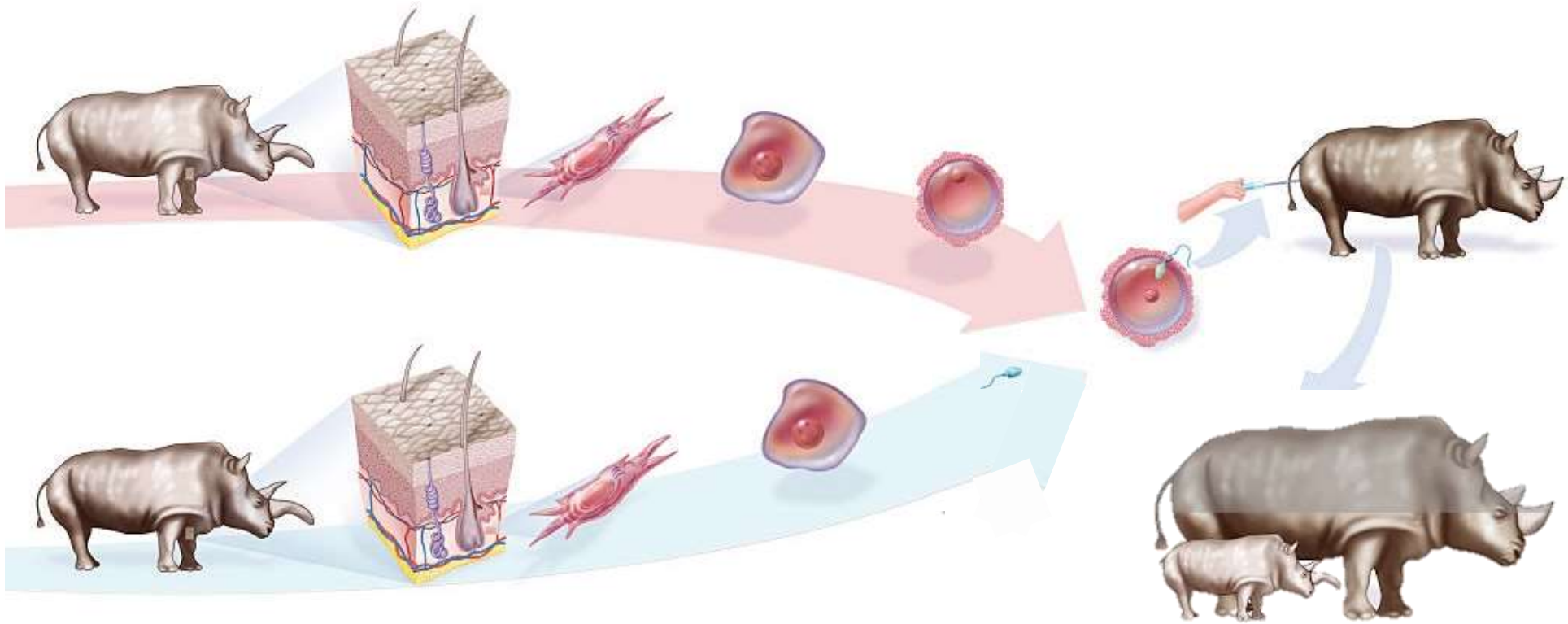


Dream?

Saito et al. Nature 471, 504-7 (2011)
Hayashi et al. Science. 338, 971-5 (2012)
Hikabe et al. Nature 539, 299–303 (2016)

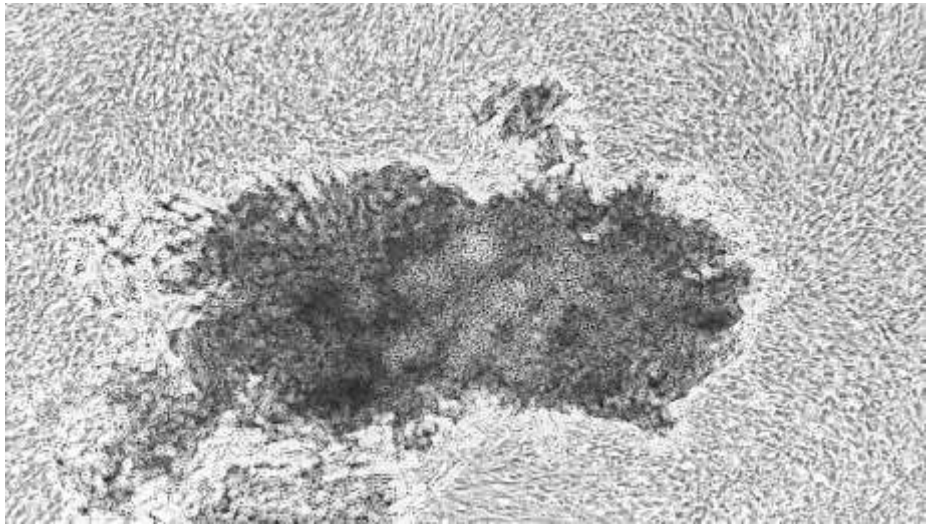
Induced Pluripotent Stem Cells (iPSC) of critical endangered rhino species

Transformation potential for In-Vitro-Gametogenesis
for producing eggs and sperm



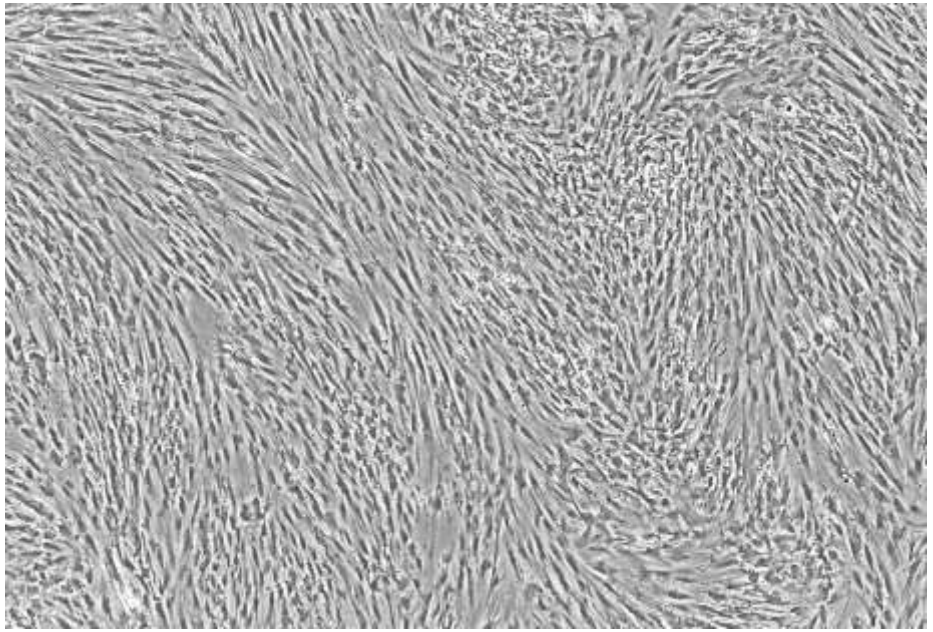
Fibroblast culture of deceased northern white rhino

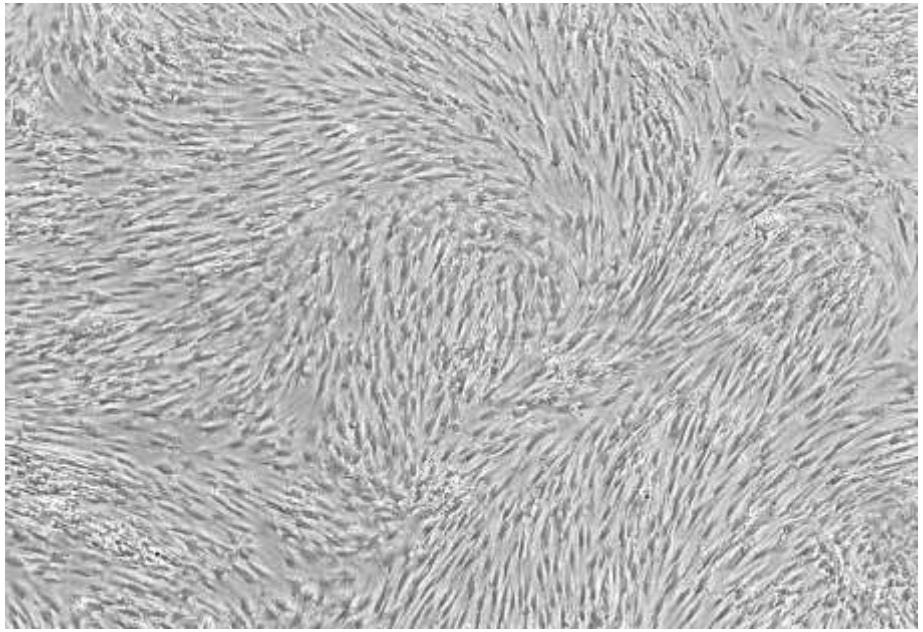
P0



NWR

P1





NWR

Pluripotent stem cells of the northern white rhino (NWR) are able to differentiate into **cardiomyocytes**

www.nature.com/scientificreports

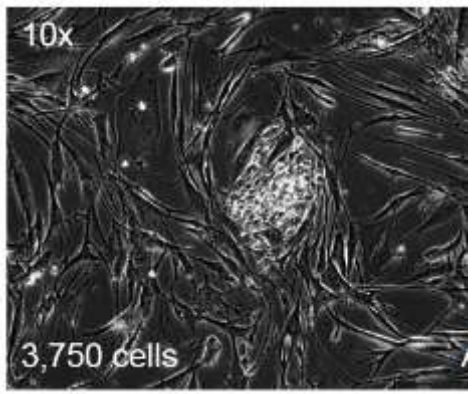
scientific reports

Check for updates

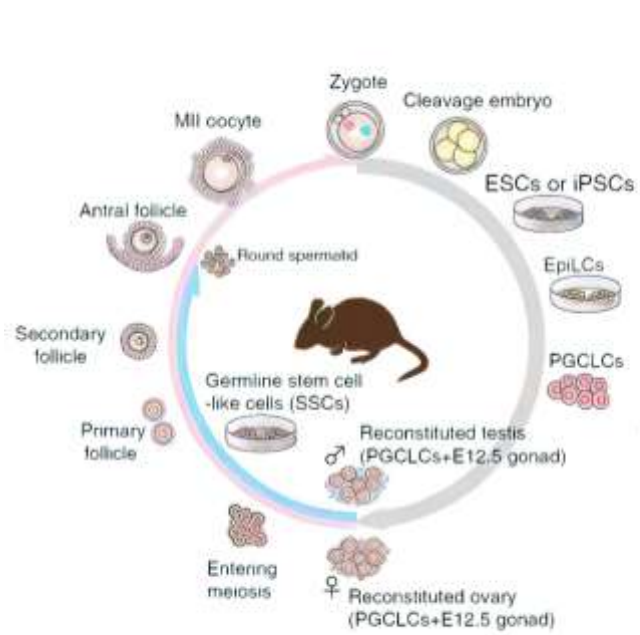
OPEN

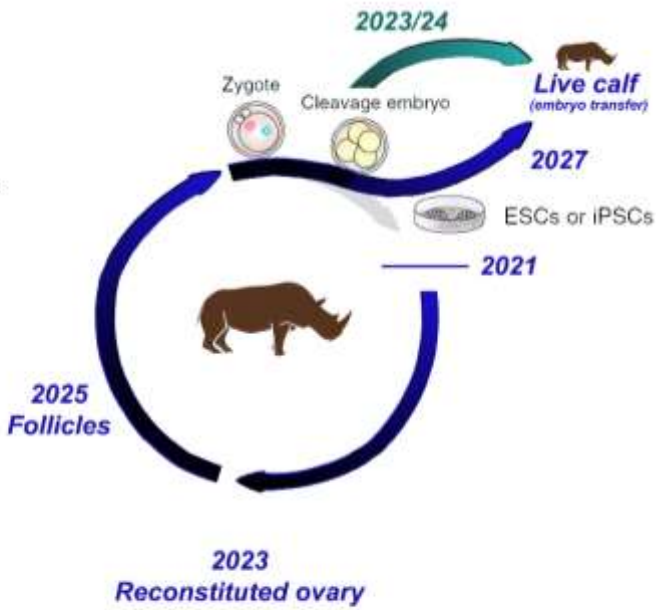
Naïve-like pluripotency to pave the way for saving the northern white rhinoceros from extinction

Vera Zywitza[1], Ejona Rusha[2], Dmitry Shaposhnikov[2], Jorge Ruiz-Orera[3], Narasimha Telugu[1], Valentyna Rishko[2], Masafumi Hayashi[4], Geert Michel[5], Lars Wittler[6], Jan Stejskal[7], Susanne Holtze[8], Frank Göritz[8], Robert Hermes[8], Jichang Wang[9], Zsuzsanna Izsvák[9], Silvia Colleoni[10], Giovanna Lazzari[10,11], Cesare Galli[10,11], Thomas B. Hildebrandt[8,12], Katsuhiko Hayashi[4], Sebastian Diecke[1,14✉] & Micha Drukker[2,13,14✉]



BioRescue






CSIRO PUBLISHING

Reproduction, Fertility and Development, 2021, 33, 95–101

https://doi.org/10.1071/RED20265

Artificially produced gametes in mice, humans and other species

Katsuhiko Hayashi[A,G], Cesare Galli[B,C], Sebastian Diecke[D] and Thomas B. Hildebrandt[E,F]


ScienceAdvances

BACK TO VOL. 8, NO. 49

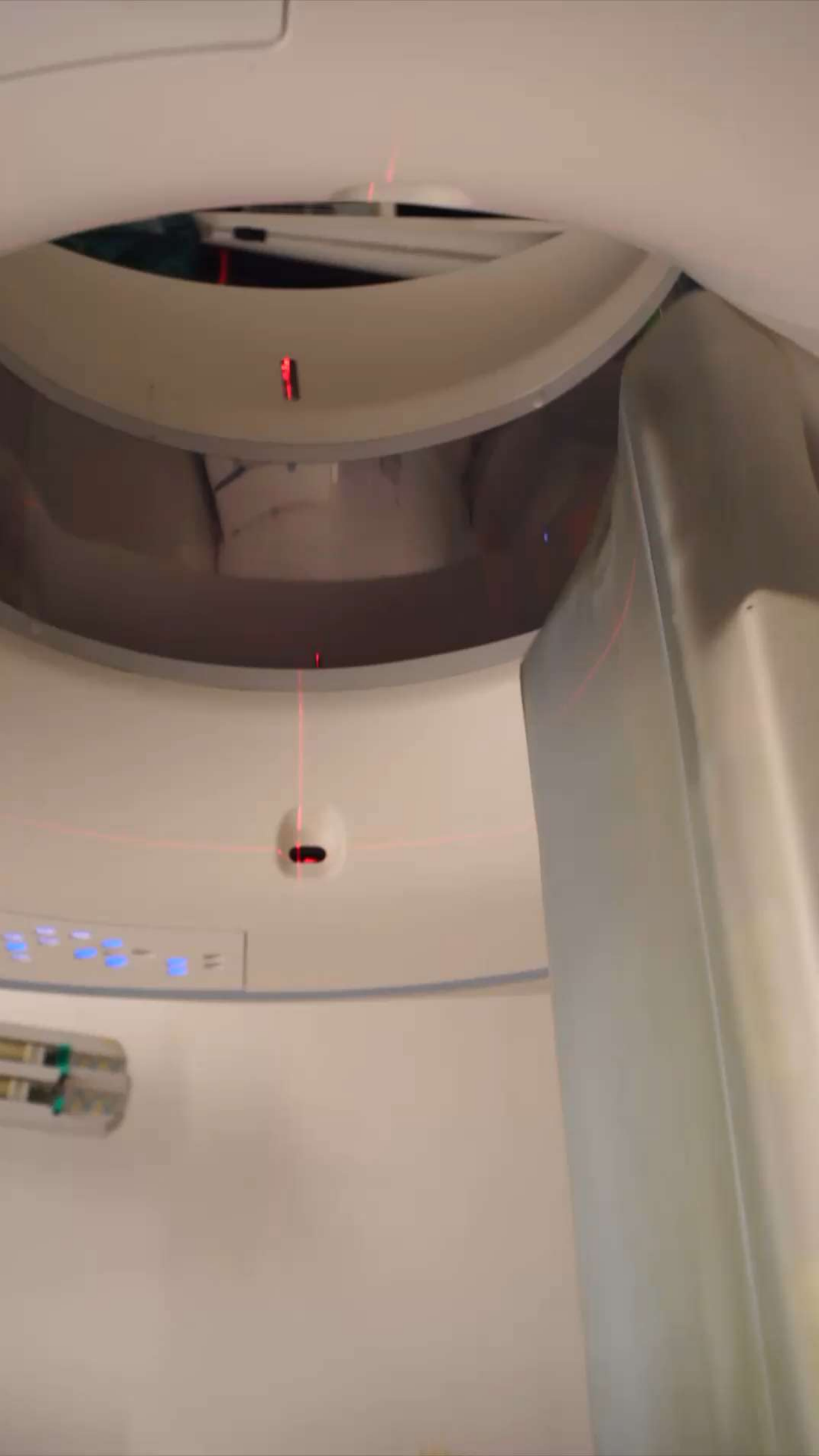
RESEARCH ARTICLE | LIFE SCIENCES

Robust induction of primordial germ cells of white rhinoceros on the brink of extinction

fewer Authors Info & Affiliations

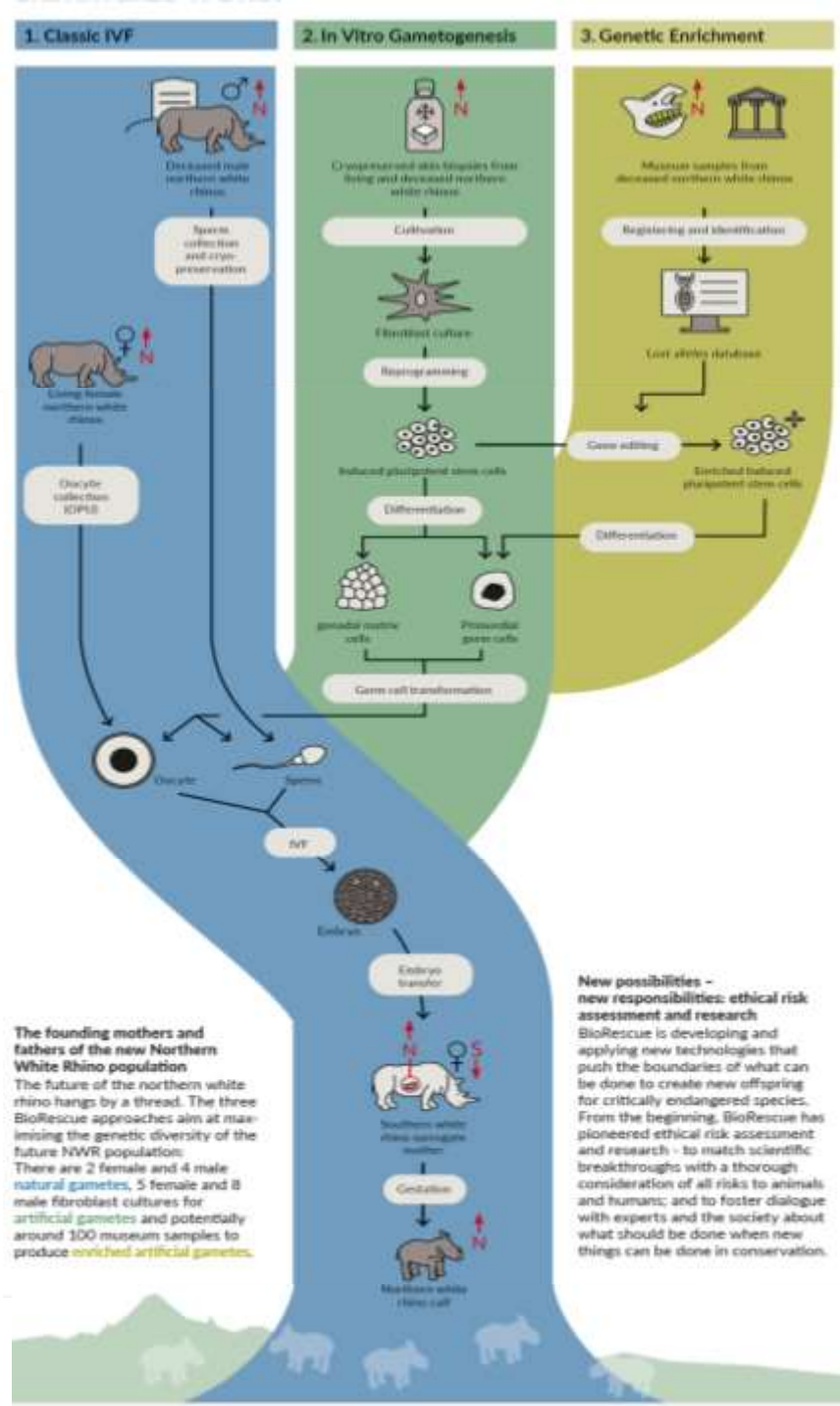
SCIENCE ADVANCES

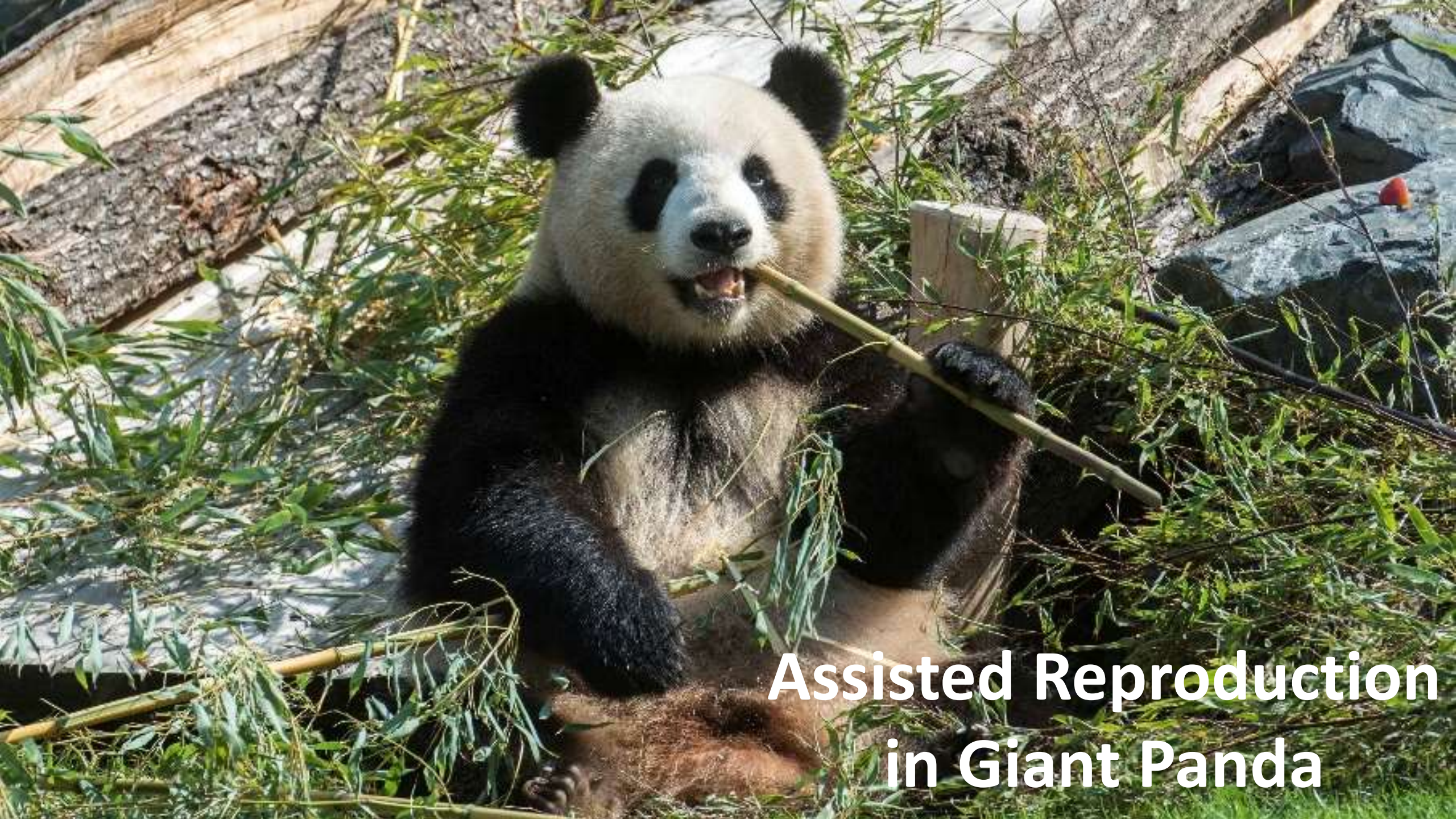
ESC & iPSC derived of the northern white rhino as basis for the *In Vitro Gametogenesis* (adaptiert von Hayashi et al., 2021 in *Reproduction, Fertility and Development*)



THREE INTEGRATED ASSISTED REPRODUCTIVE STRATEGIES FOR THE CONSERVATION OF HIGHLY ENDANGERED SPECIES

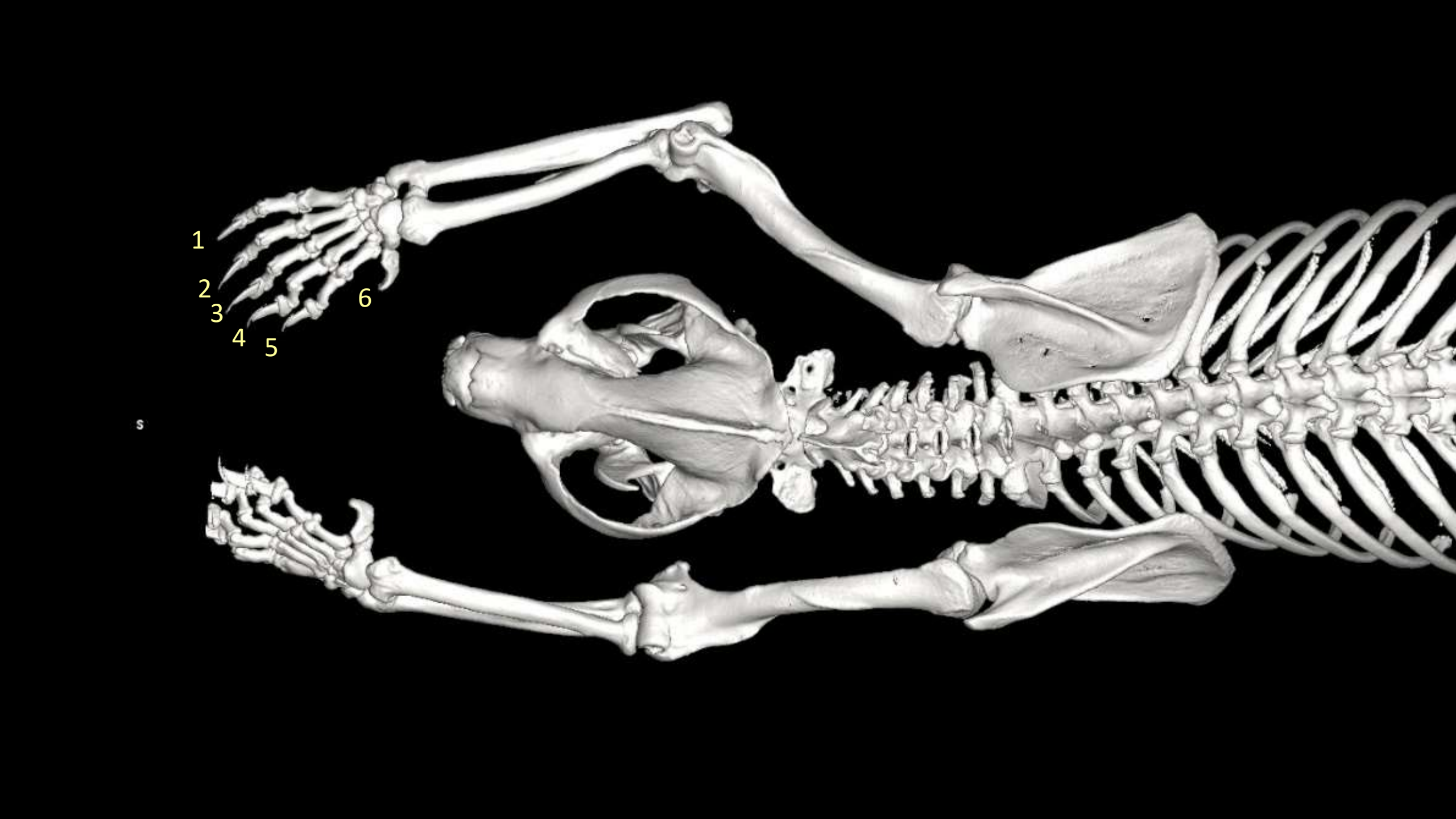
BioRescue





Assisted Reproduction in Giant Panda


WL: 40 WW: 350
R
P
S
L


1
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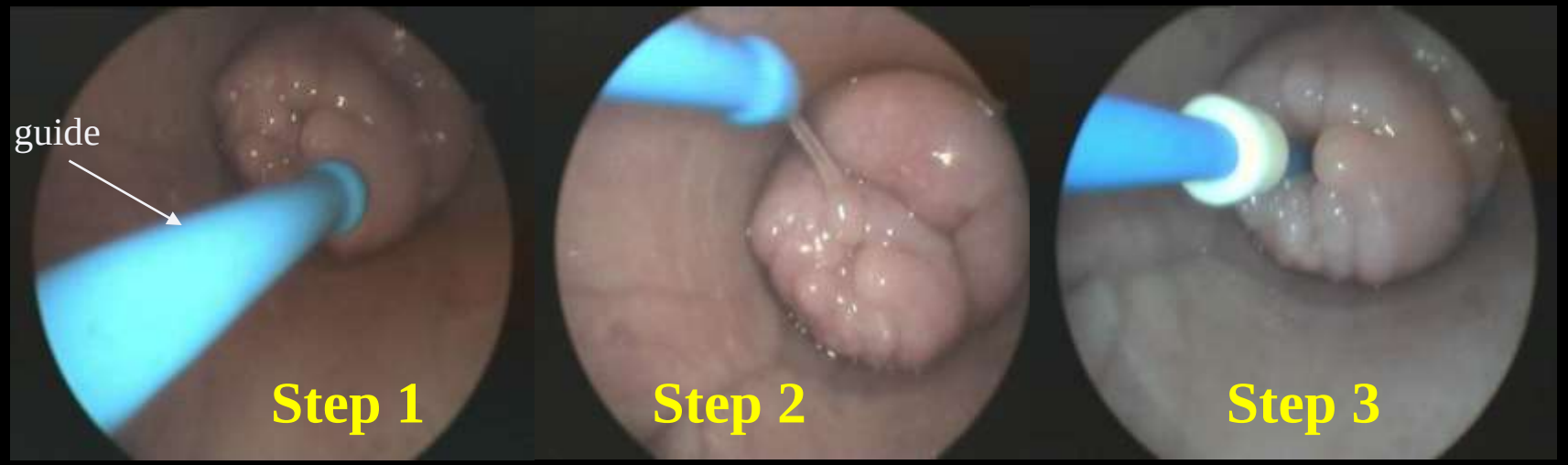
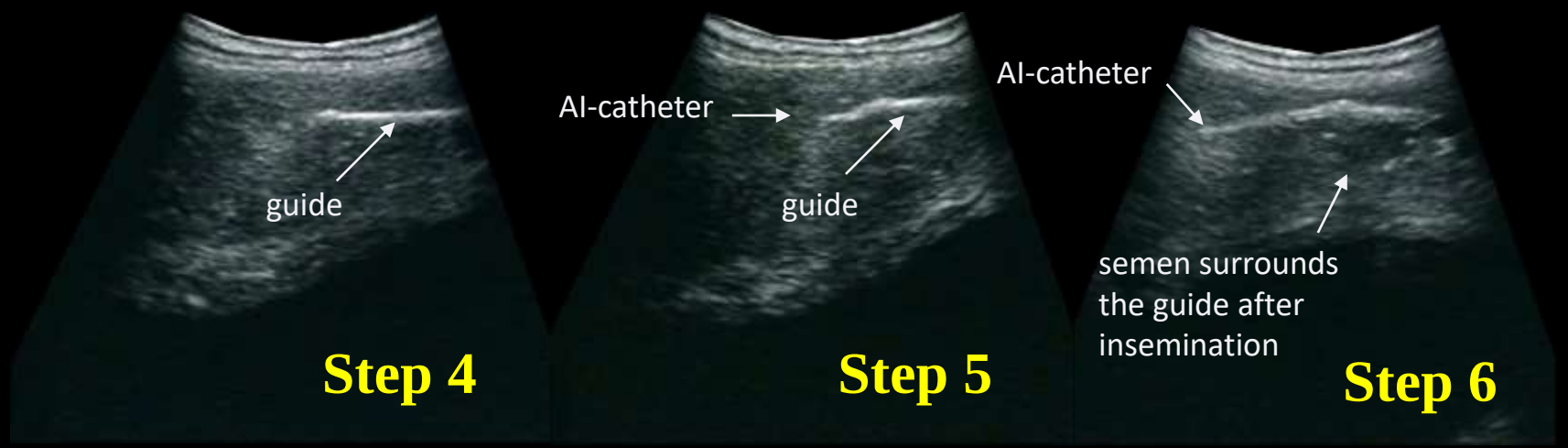
6

Endoscopic/ultrasound-guided artificial insemination

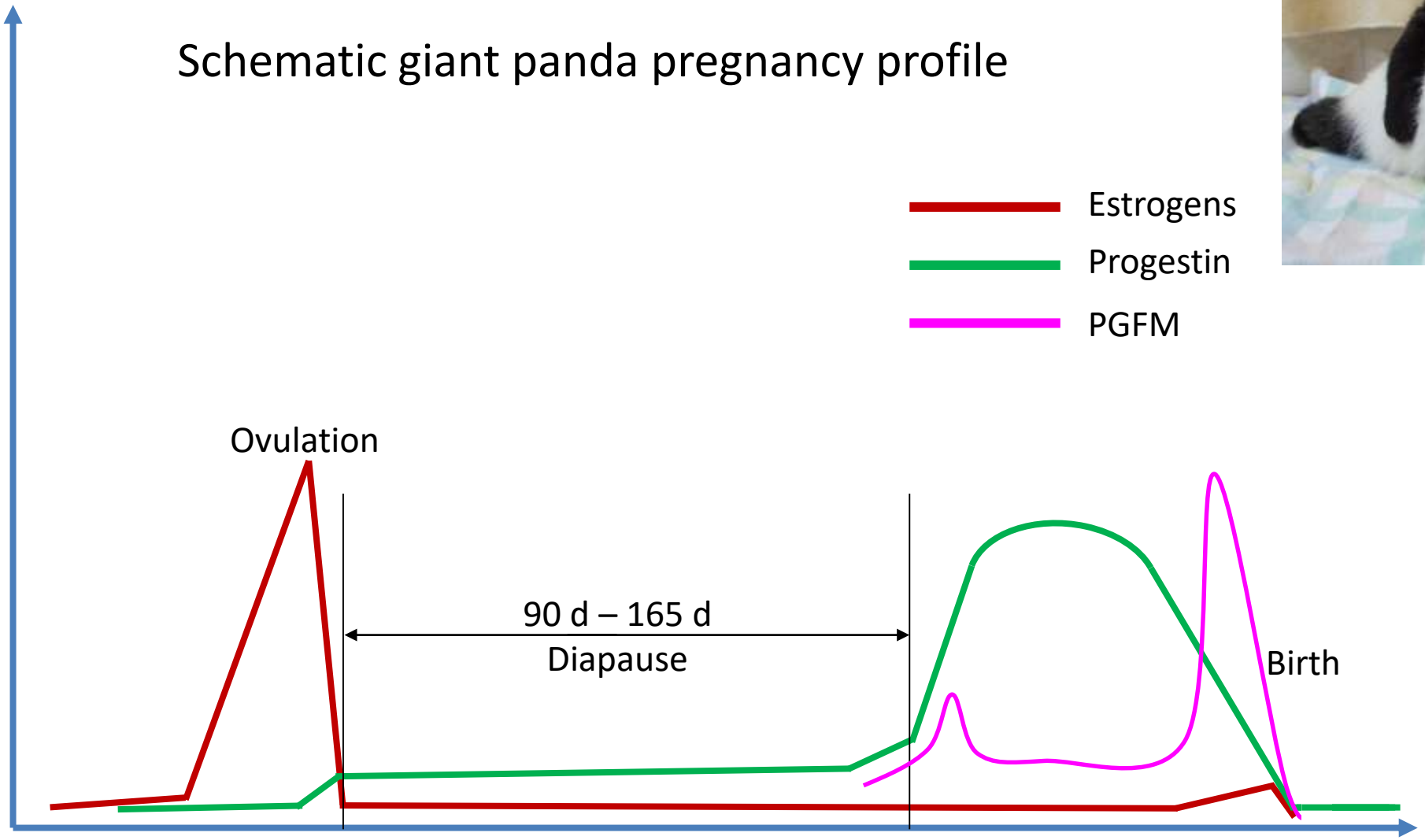
endoscopy



ultrasound

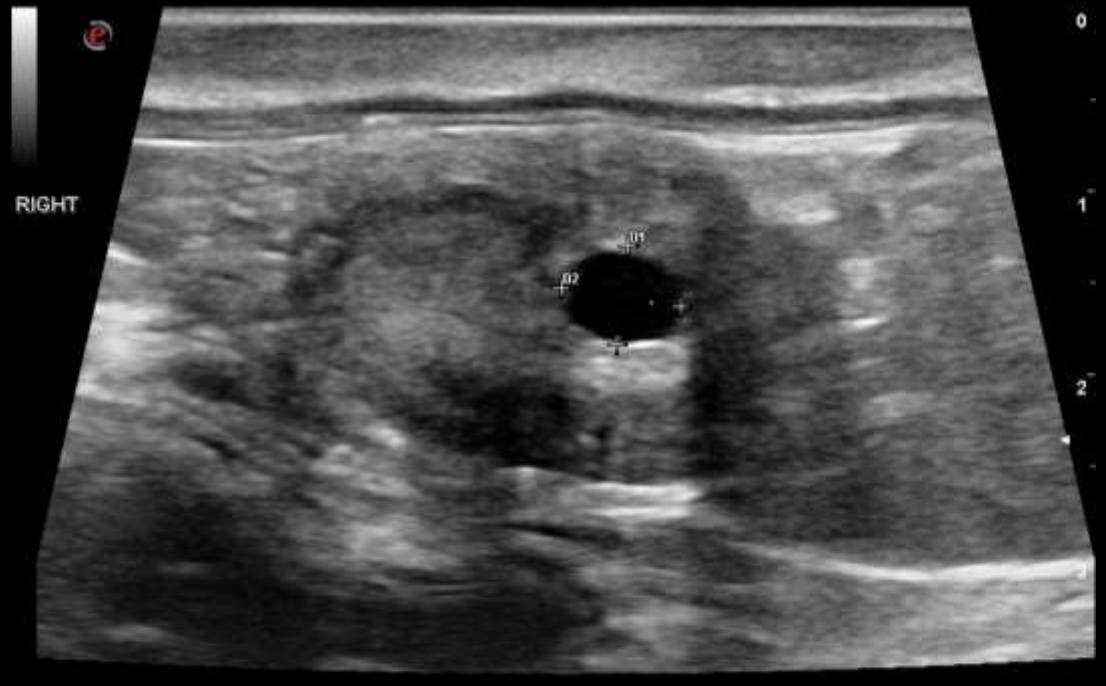


Schematic giant panda pregnancy profile



Is there a cross-talk between embryo(s) and uterus?
Does the uterus know it is pregnant?

Wauters et al., 2019


RIGHT
01
02
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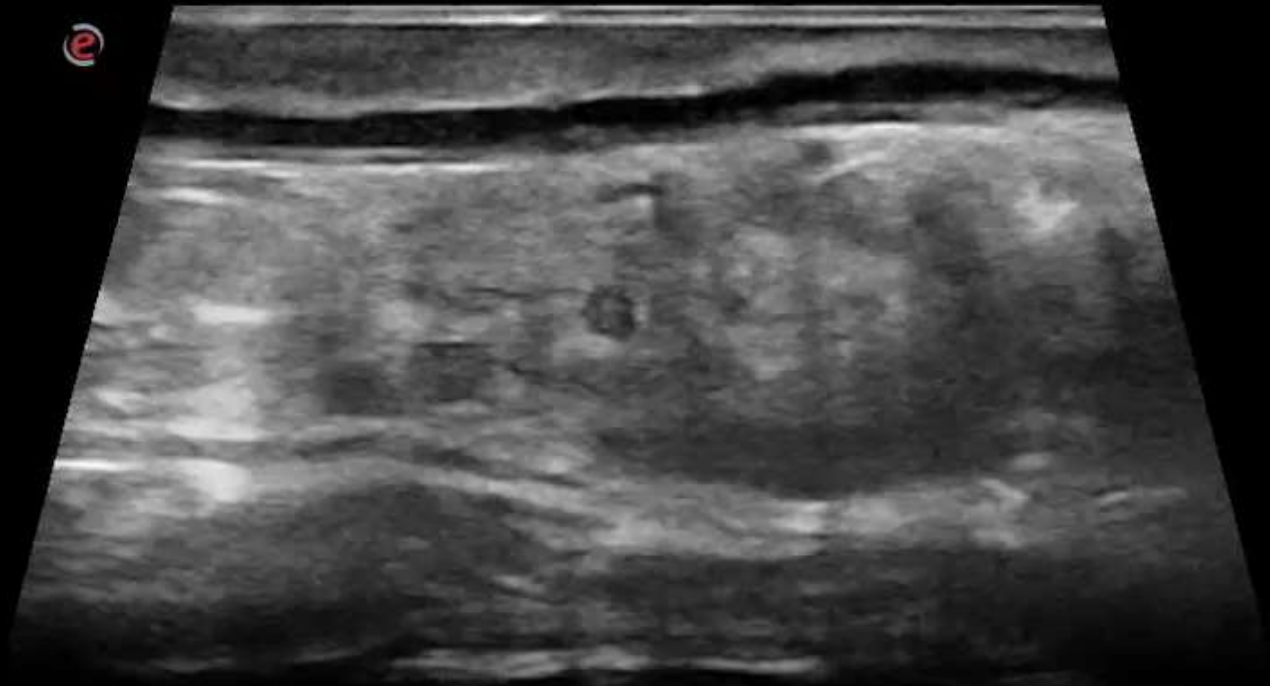
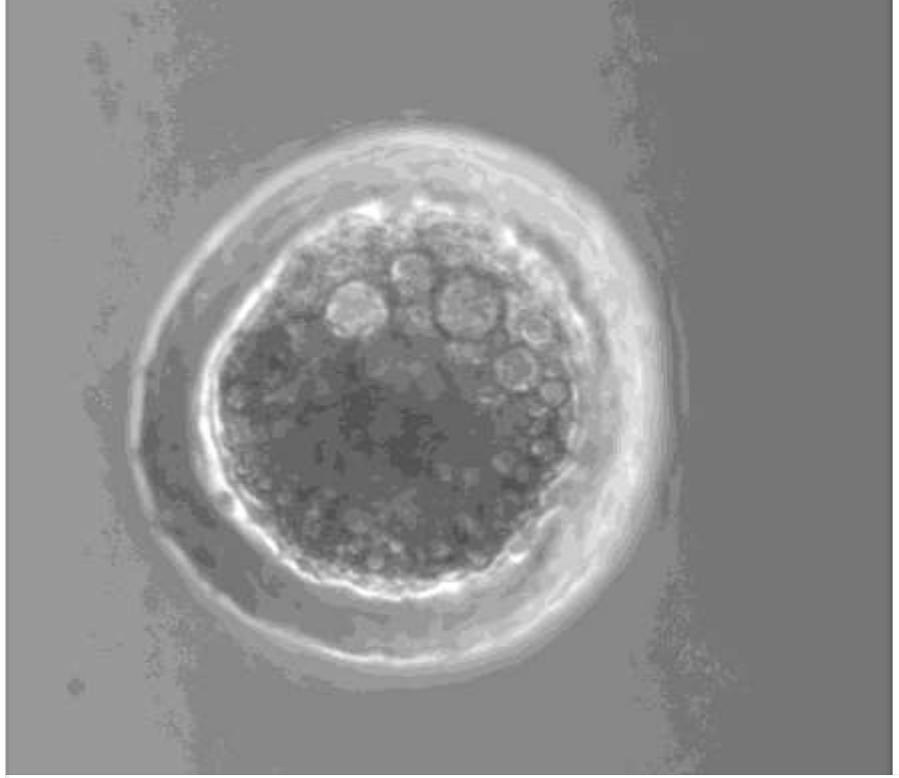
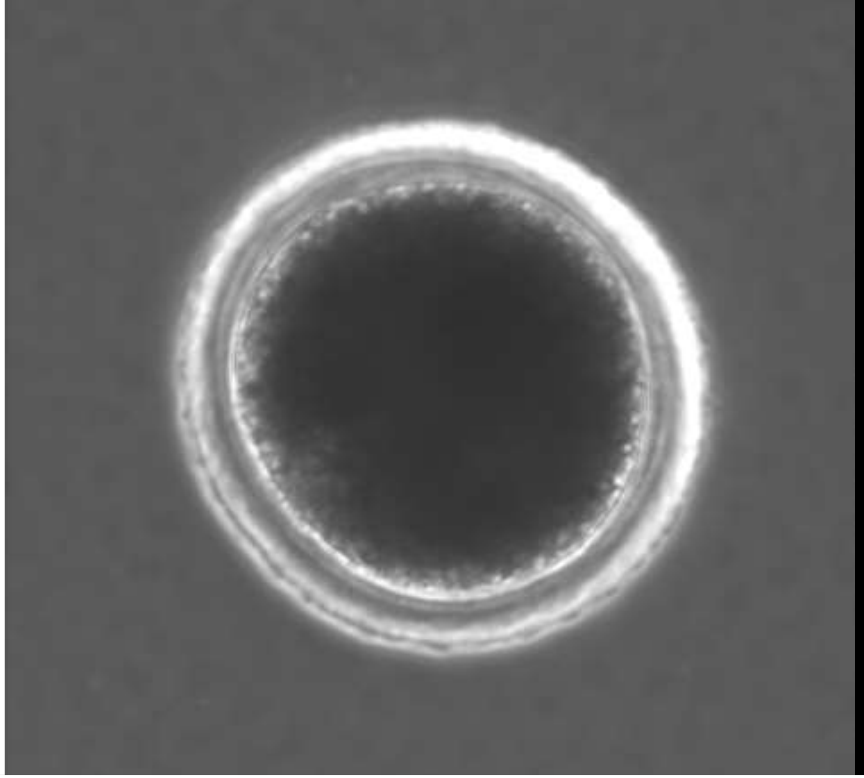
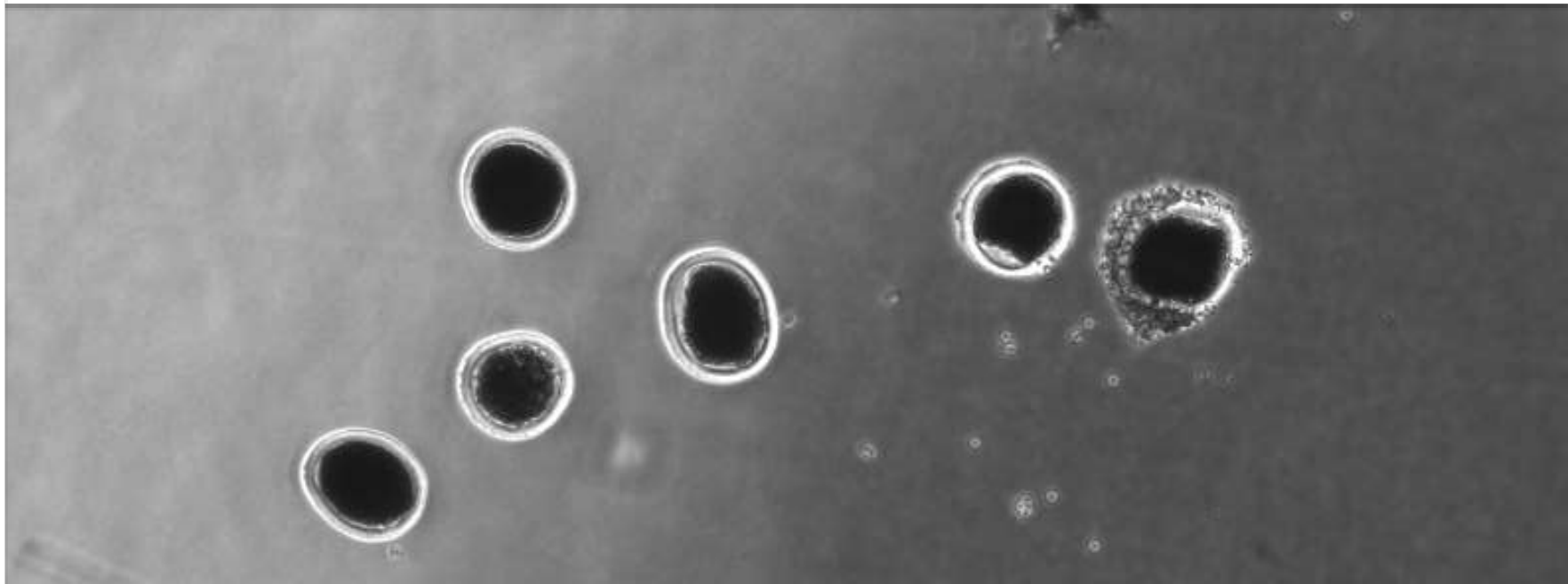
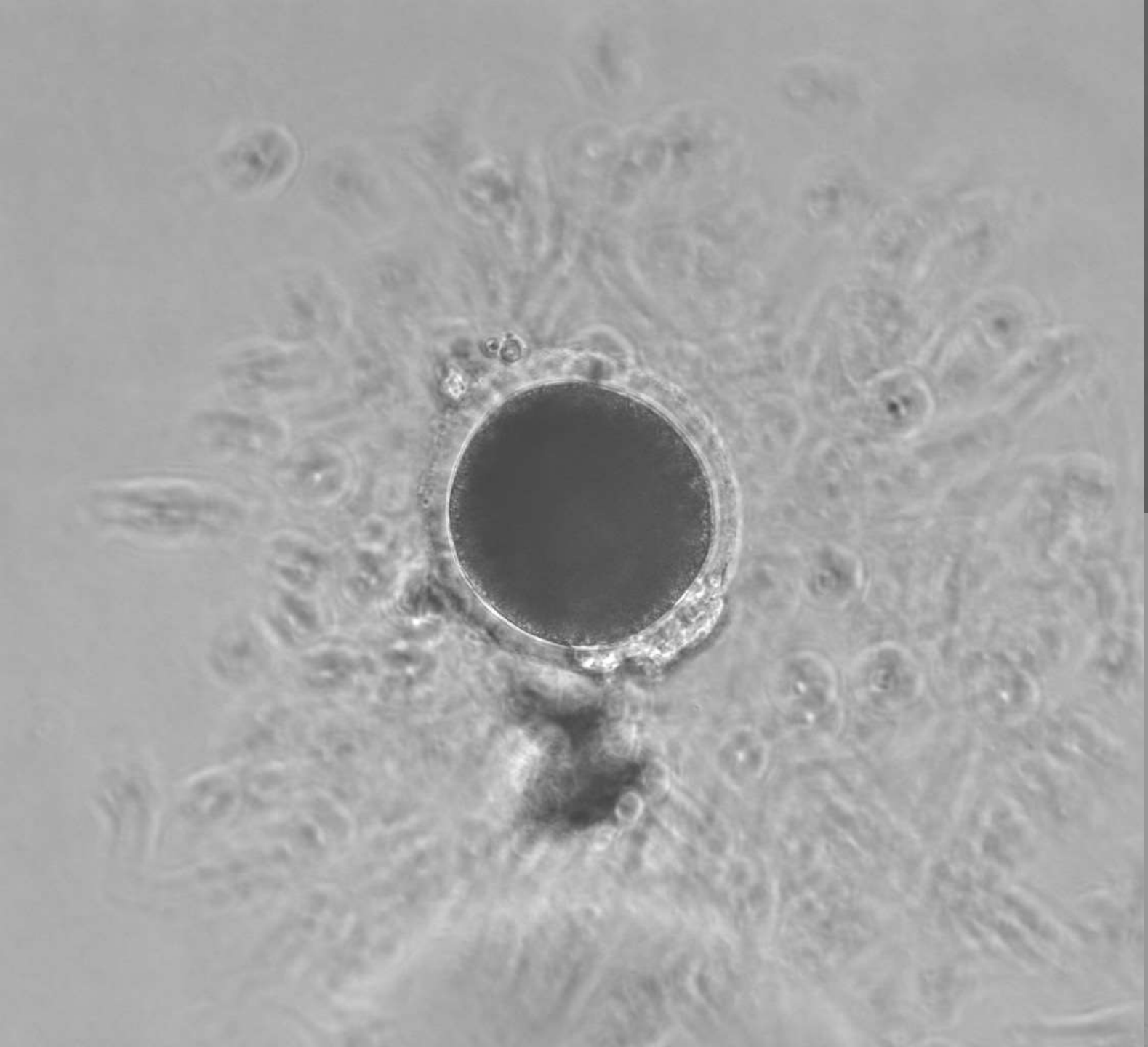
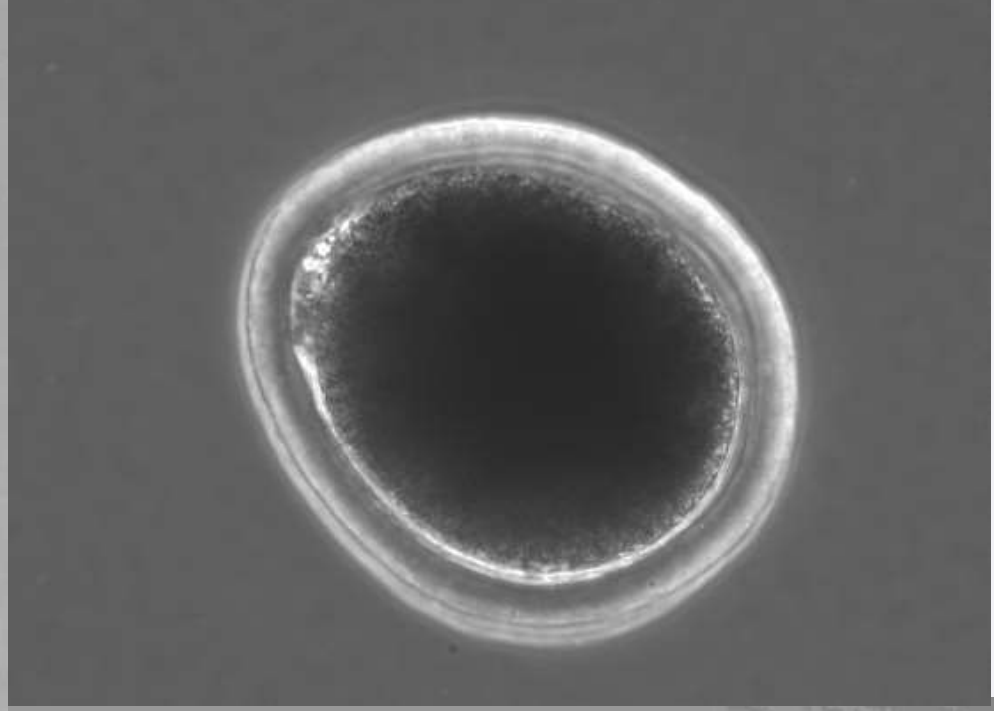
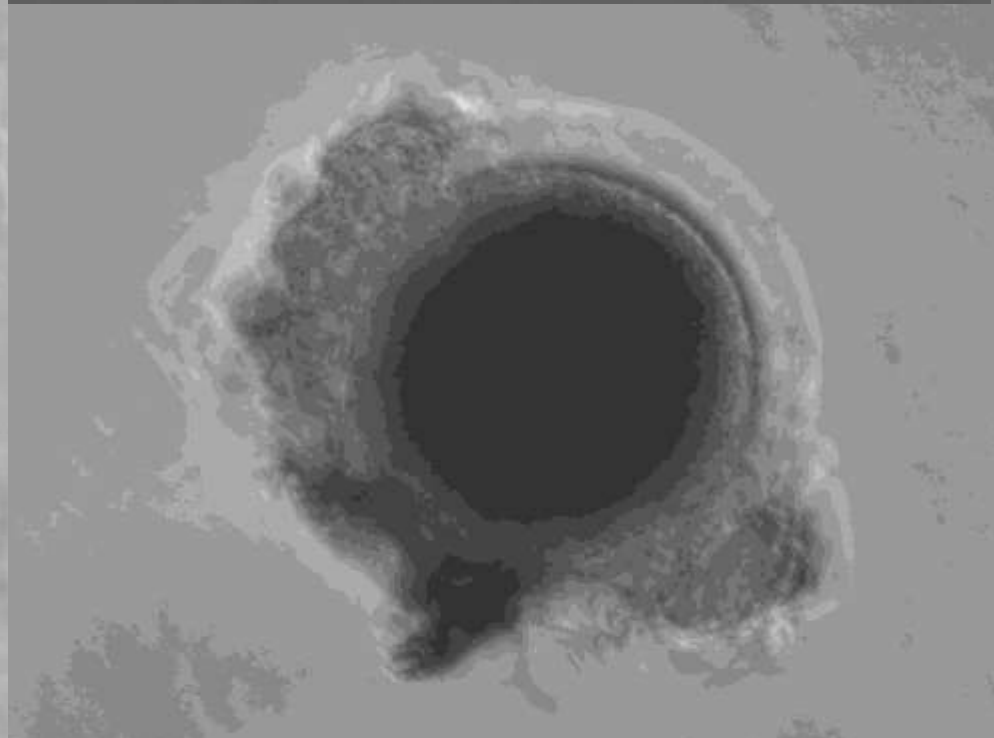
1
2



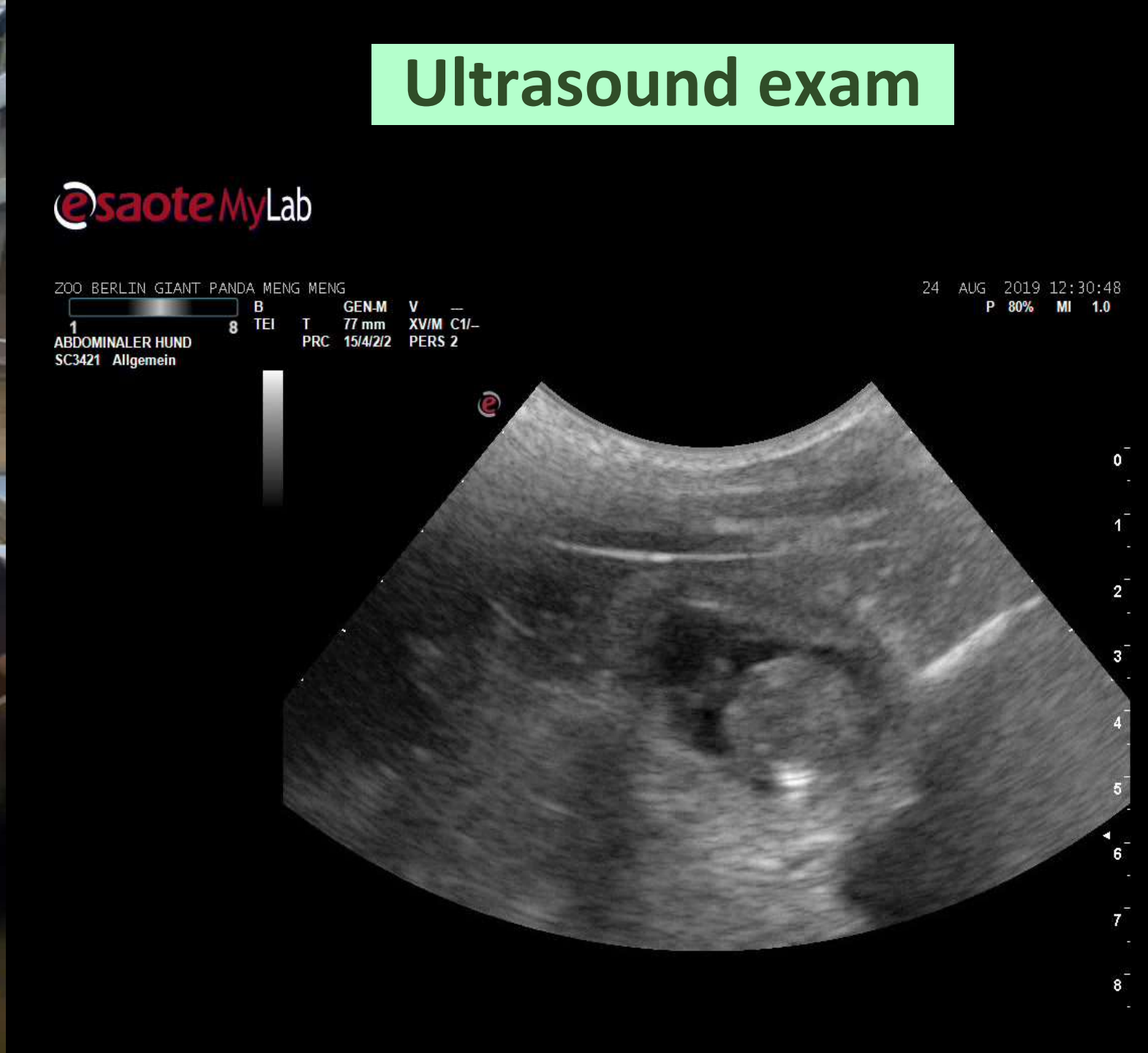






Ultrasound exam



Cubs’ development

energy-rich, green milk


TIERPARK
HAGENBECK

Elephants have the longest gestation in the animal kingdom with an avarage of 660 days

Mouse: 20d

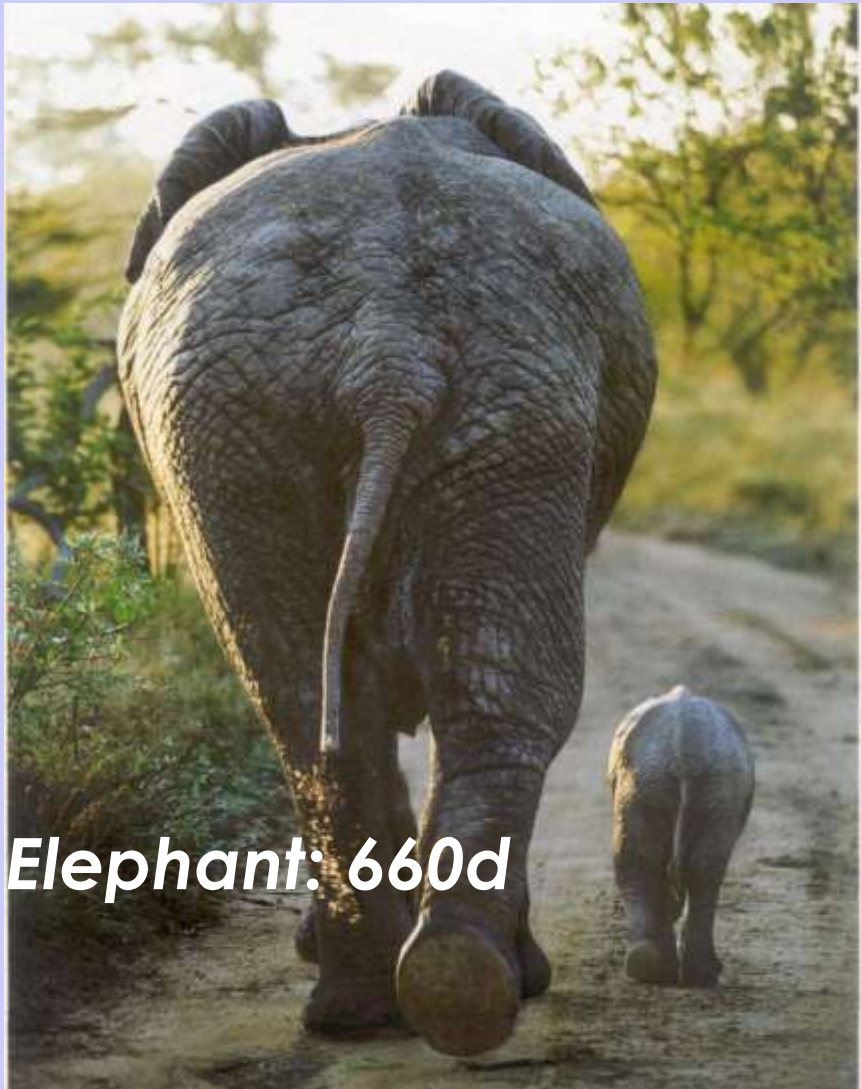
Dog: 62d

Sheep: 140d

Human: 260d

Horse: 340d

Rhino: 450d



ELSEVIER

Animal Reproduction Science 53 (1998) 309–314

ANIMAL REPRODUCTION SCIENCE

Short communication

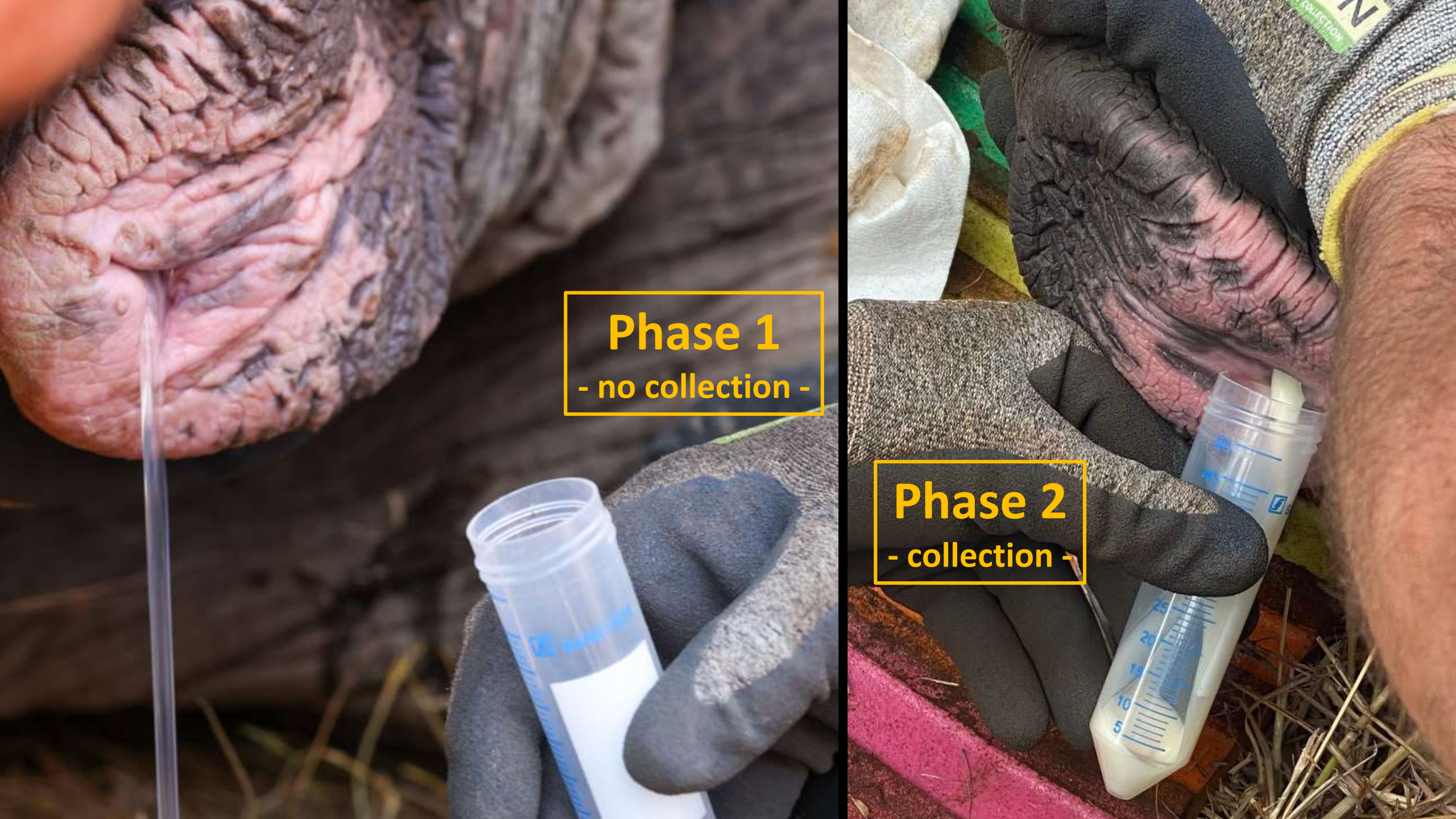
Manual collection and characterization of semen from Asian elephants (*Elephas maximus*)

D.L. Schmitt [a,*], T.B. Hildebrandt [b]

[a] *Department of Agriculture, Southwest Missouri State University, Springfield, MO 65804, USA*
[b] *Department of Reproductive Strategies, Institute for Zoo Biology and Wildlife Research, D-10315 Berlin, Germany*

Semen manual collection

Semen samples with motility up to 90% are optimal for cryo-preservation


Phase 1
- no collection -
Phase 2
- collection -


Frozen Dumbo 1
2009


16
FROZEN DUMBO


Frozen Dumbo 3
2023


EW 12-ack9


Frozen Dumbo 2
2010

Iqhwa
Born in Tiergarten Schönbrunn, Vienna
September, 2013

First elephant produced from frozen-thawed semen

Iqhwa means in Zolu-language: Ice

1th elephant oocyte collected by OPU in a living elephant cow

Elephant fetus, day 119d pc

SCIENCE ADVANCES | RESEARCH ARTICLE

NEUROPHYSIOLOGY

Elephant facial motor control

Lena V. Kaufmann[1,2], Undine Schneeweiß[1], Eduard Maier[1], Thomas Hildebrandt[3]*†, Michael Brecht[1,4]*†

We studied facial motor control in elephants, animals with muscular dexterous trunks. Facial nucleus neurons (~54,000 in Asian elephants, ~63,000 in African elephants) outnumbered those of other land-living mammals. The large-eared African elephants had more medial facial subnucleus neurons than Asian elephants, reflecting a numerically more extensive ear-motor control. Elephant dorsal and lateral facial subnuclei were unusual in elongation, neuron numerosity, and a proximal-to-distal neuron size increase. We suggest that this subnucleus organization is related to trunk representation, with the huge distal neurons innervating the trunk tip with long axons. African elephants pinch objects with two trunk tip fingers, whereas Asian elephants grasp/wrap objects with larger parts of their trunk. Finger "motor foveae" and a positional bias of neurons toward the trunk tip representation in African elephant facial nuclei reflect their motor strategy. Thus, elephant brains reveal neural adaptations to facial morphology, body size, and dexterity.

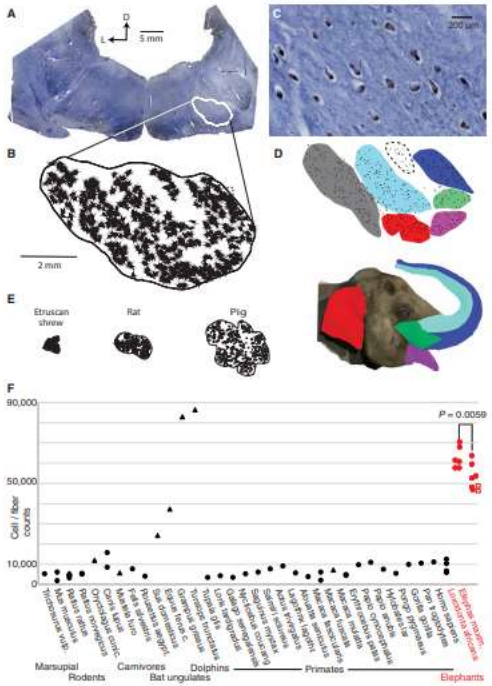


Fig. 1. Extraordinary size and neuron number of the elephant facial nucleus. (**A**) Micrograph of a coronal Nissl-stained 60-µm brainstem section of the adult Asian elephant cow Burma. The section stems from midlevel of the facial nucleus (white outline). D, dorsal; L, lateral. (**B**) Somata drawing and facial nucleus outline. (**C**) Micrograph [dashed area in (B)]. (**D**) Top: Facial subnuclei of the section in (B) highlighted in color. Bottom: Color-coded musclelotopy suggestion. (**E**) Somata drawings of facial nuclei of various mammals, scale as (B). (**F**) Facial nucleus neuron number in mammals. Species averages are given in black [dots data (*5*); triangles data (*6*)]. In red, data are given for individual African and Asian elephant facial nuclei; filled symbols (cell counts), empty symbols (facial nerve fiber counts), squares (stillborn elephants), dots (adult elephants) (see fig. S1). Photo credit (D): Michael Brecht, Humboldt-Universität zu Berlin; Zoologischer Garten Berlin, Berlin, Germany.

communications biology

ARTICLE

https://doi.org/10.1038/s42003-023-04965-0 OPEN

The functional anatomy of elephant trunk whiskers

Nora Deininger[1], Undine Schneeweiß[1], Lena V. Kaufmann[1,2], Lennart Eigen[1], Celina Spenseger[1], Ben Gerhardt[1], Susanne Holtze[3], Guido Fritsch[3], Frank Göritz[3], Rolf Becker[4], Andreas Ochs[4], Thomas Hildebrandt[3] & Michael Brecht[1,5]✉

Behavior and innervation suggest a high tactile sensitivity of elephant trunks. To clarify the tactile trunk periphery we studied whiskers with the following findings. Whisker density is high at the trunk tip and African savanna elephants have more trunk tip whiskers than Asian elephants. Adult elephants show striking lateralized whisker abrasion caused by lateralized trunk behavior. Elephant whiskers are thick and show little tapering. Whisker follicles are large, lack a ring sinus and their organization varies across the trunk. Follicles are innervated by ~90 axons from multiple nerves. Because elephants don't whisk, trunk movements determine whisker contacts. Whisker-arrays on the ventral trunk-ridge contact objects balanced on the ventral trunk. Trunk whiskers differ from the mobile, thin and tapered facial whiskers that sample peri-rostrum space symmetrically in many mammals. We suggest their distinctive features—being thick, non-tapered, lateralized and arranged in specific high-density arrays—evolved along with the manipulative capacities of the trunk.

Growth Curves

CRL –Crown-Rump-Length

PROCEEDINGS OF THE ROYAL SOCIETY B

Proc. R. Soc. B (2007) **274**, 323–331
doi:10.1098/rspb.2006.3738
Published online 31 October 2006

Foetal age determination and development in elephants

Thomas Hildebrandt[1,*], Barbara Drews[1], Ann P. Gaeth[2], Frank Goeritz[1], Robert Hermes[1], Dennis Schmitt[3], Charlie Gray[4], Peter Rich[4], Wolf Juergen Streich[1], Roger V. Short[2,5] and Marilyn B. Renfree[2]

[1]*Institute for Zoo and Wildlife Research, Alfred-Kowalke-Straße 17 10315 Berlin, Germany*
[2]*Department of Zoology, and* [5]*Faculty of Medicine, University of Melbourne, Melbourne, Victoria 3010, Australia*
[3]*Department of Agriculture, Missouri State University, Springfield, MO 65897, USA*
[4]*African Lion Safari, Cambridge, Ont, Canada N1R 5S2*

Elephants have the longest pregnancy of all mammals, with an average gestation of around 660 days, so

TH – Thorax Diameter

BPD – Biparietal Distance

FL – Femur Length

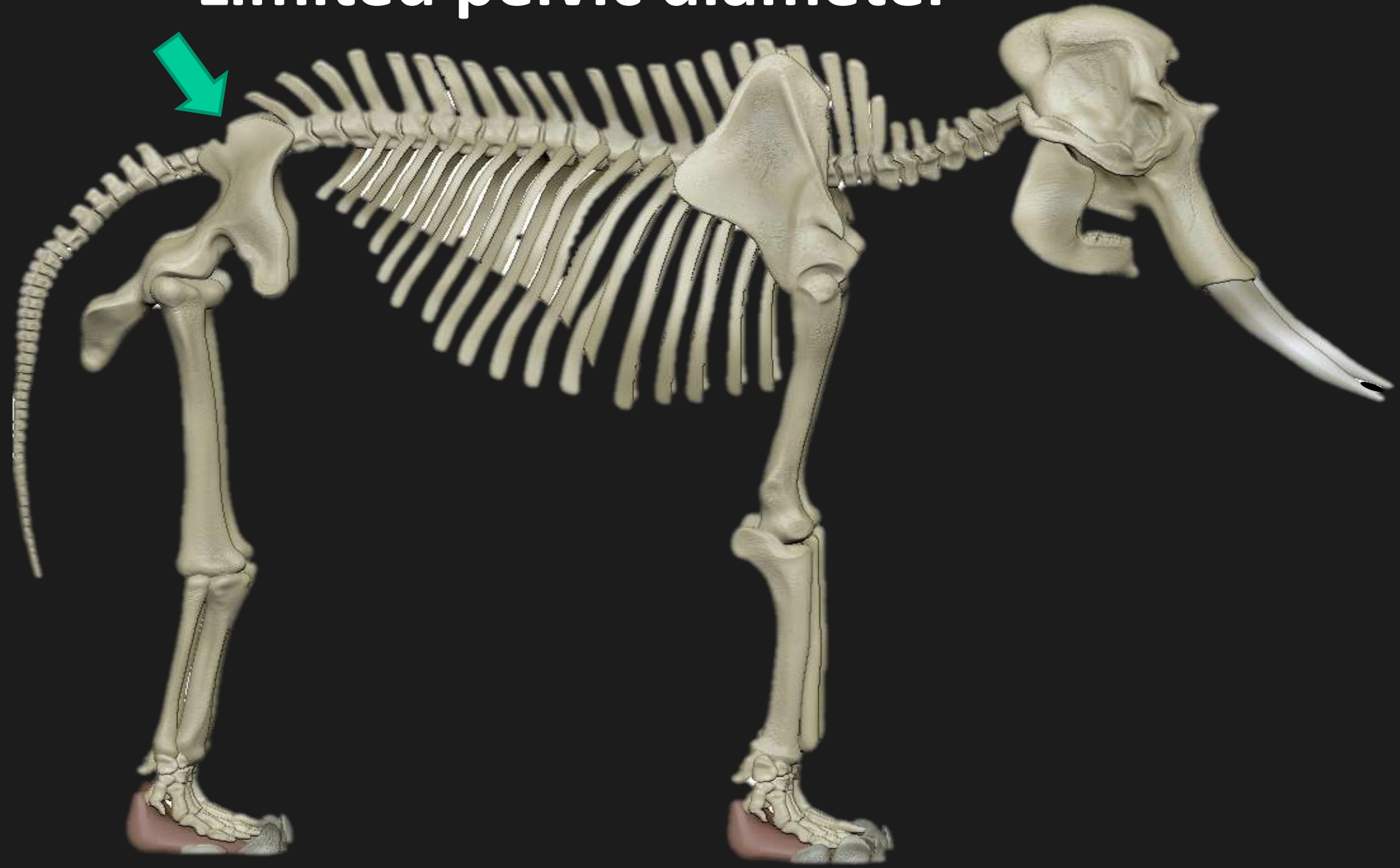
mm

days p.o.

0 50 100 150 200 250

fully ossified skull
after 640d pregnancy

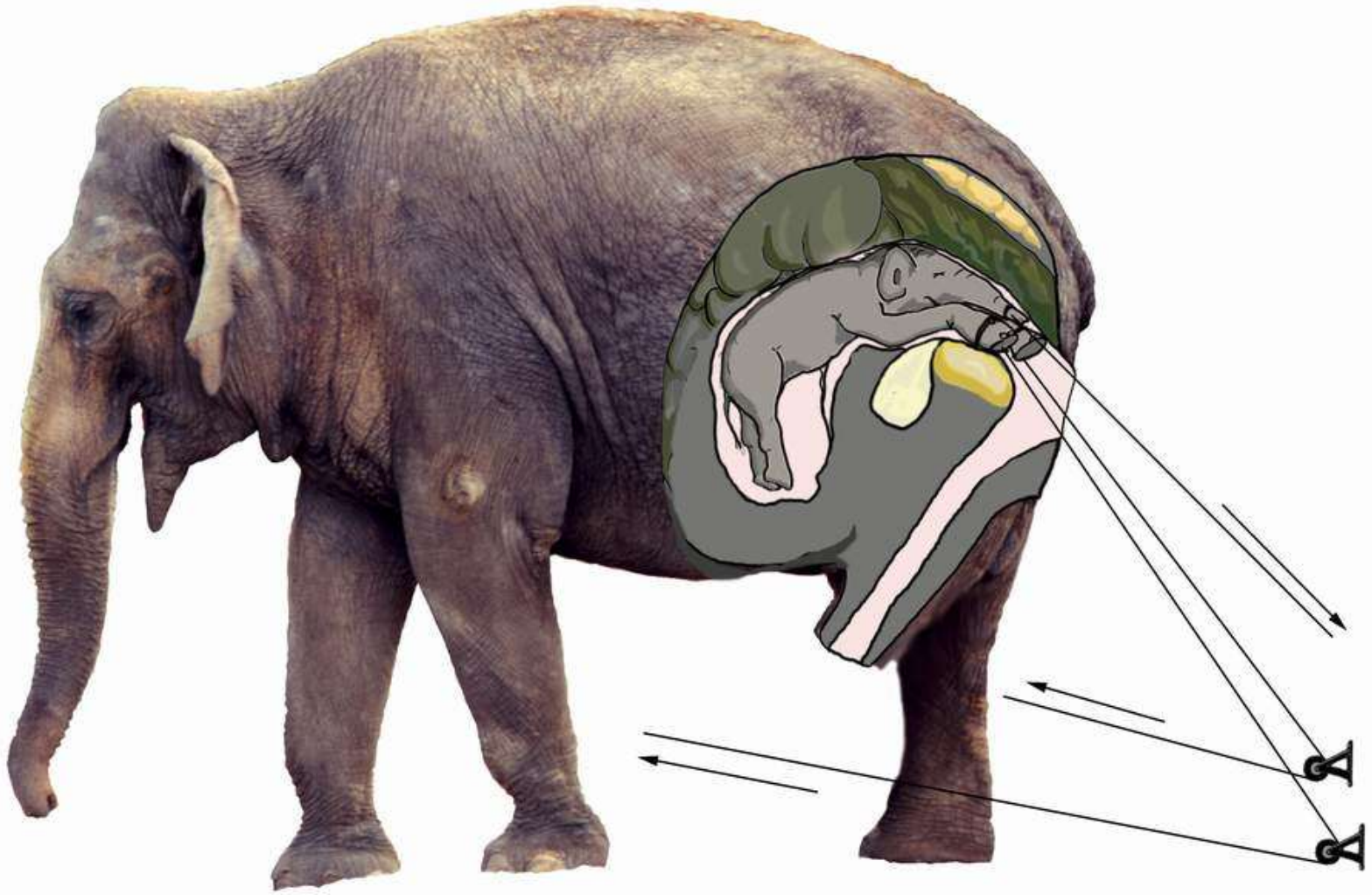
Limited pelvic diameter



Malposition in a normal sized calf

Courtesy Bengt Roken

Schematic diagram of the extraction set-up using episiotomy



Dissected Asian elephant fetus (6 parts) after fetotomy

Acknowledgement

We like to thank the Dvur Kralove Zoo for its financial support and the following zoological institutions and their staff for the active involvement in this rhino conservation programme:

Budapest Zoo
Charite & Max-Planck-Institute Berlin
Chorzow Zoo
Dvur Kralove Zoo
Longleat
Montpellier Zoo
Parc Pairi Daiza
Salzburg Zoo
San Diego Zoo Global
Schwerin Zoo
Thoiry Zoo
Staff Aventea

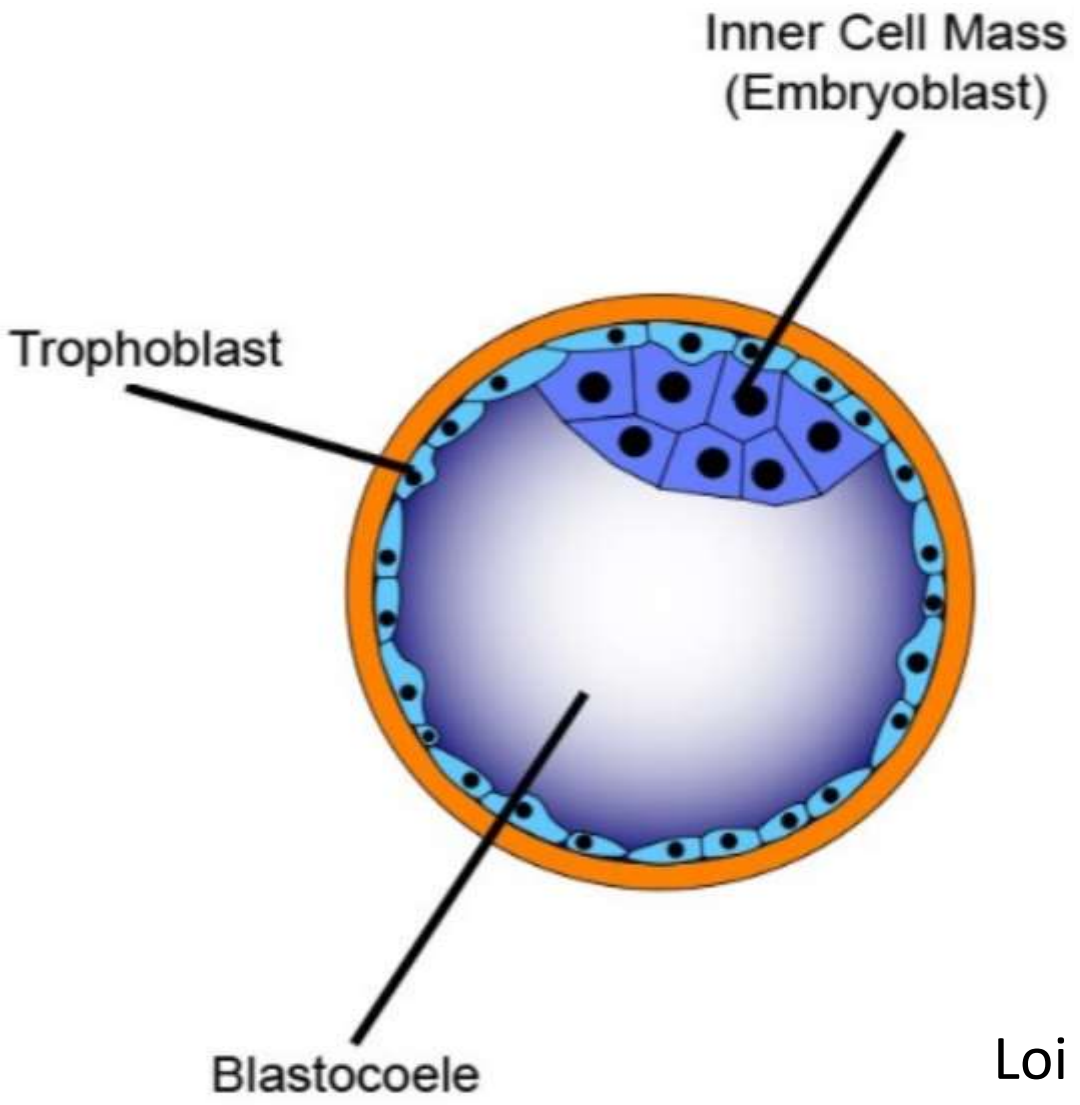
- Interspecies Chimera -
Alternative Recipients?

nature COMMUNICATIONS
Article | OPEN

An extra-uterine system to physiologically support the extreme premature lamb

Partridge et al., 2017, Nature Communication 8, article number:15112

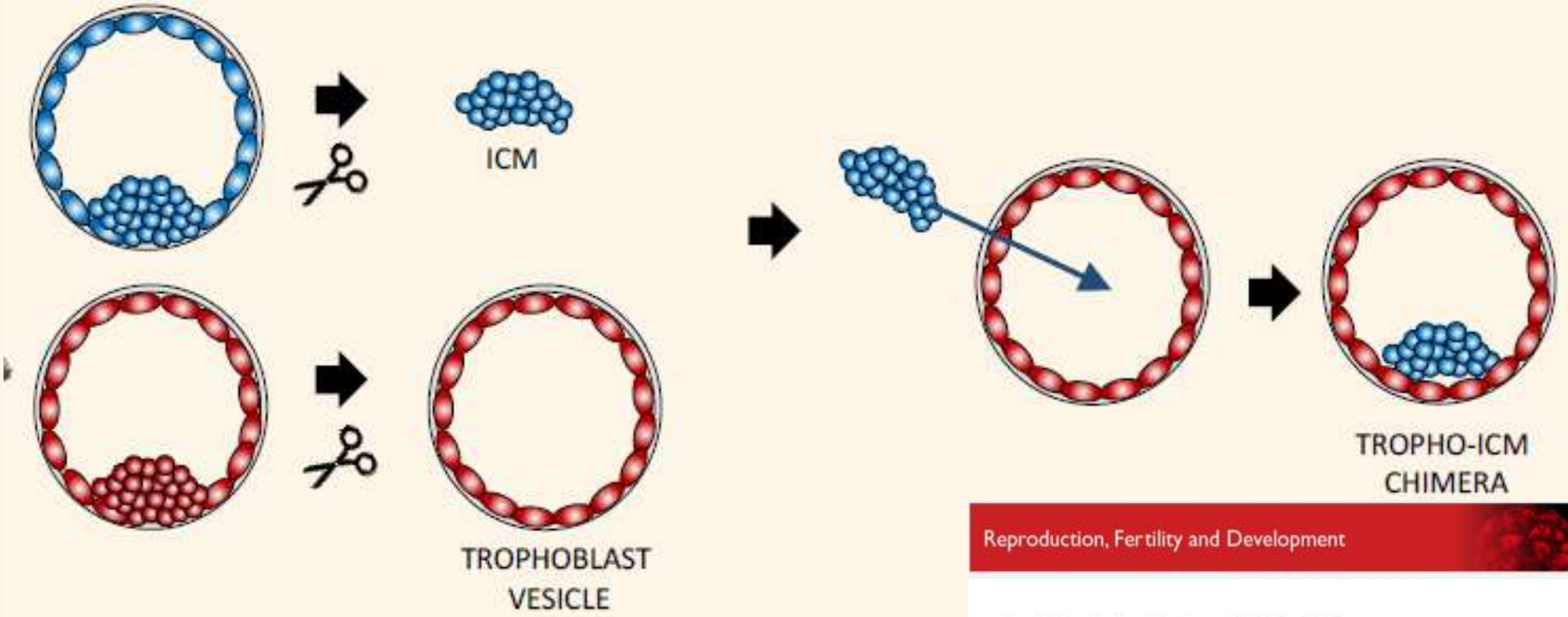
inner cell mass transfer



Loi et al. J Reprod Develop, 2018

INTER-SPECIES TROPHO-ICM CHIMERAS

Injection of ICM into trophoblast vesiscles



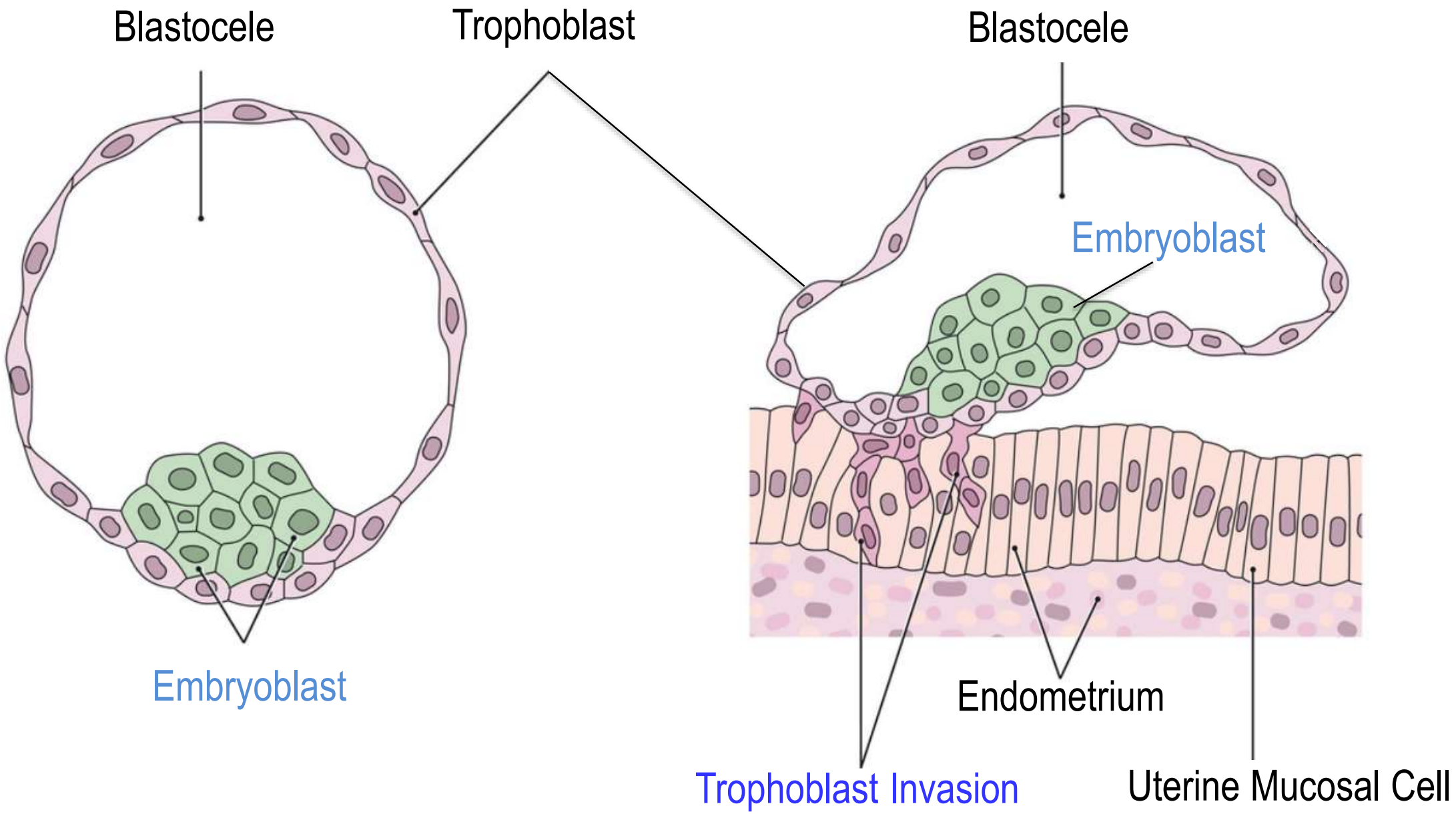
Reproduction, Fertility and Development

Journal of Reproduction and Development, Vol. 64, No 2, 2018

—Original Article—

Development to term of sheep embryos reconstructed after inner cell mass/trophoblast exchange

Pasqualino LOI[1], Cesare GALLI[2], Giovanna LAZZARI[2], Kazutsugu MATSUKAWA[3], Josef FULKA, Jr[4], Frank GOERITZ[5] and Thomas B. HILDEBRANDT[5]


Blastocele
Trophoblast
Blastocele
Embryoblast
Embryoblast
Endometrium
Trophoblast Invasion
Uterine Mucosal Cell

Induced pluripotent stem cells from highly endangered species

Inbar Friedrich Ben-Nun[1], Susanne C Montague[1], Marlys L Houck[2], Ha T Tran[1], Ibon Garitaonandia[1], Trevor R Leonardo[1], Yu-Chieh Wang[1], Suellen J Charter[2], Louise C Laurent[1,3], Oliver A Ryder[2] & Jeanne F Loring[1,3]

For some highly endangered species there are too few reproductively capable animals to maintain adequate genetic diversity, and extraordinary measures are necessary to prevent extinction. We report generation of induced pluripotent stem cells (iPSCs) from two endangered species: a primate, the drill, *Mandrillus leucophaeus* and the nearly extinct northern white rhinoceros, *Ceratotherium simum cottoni*. iPSCs may eventually facilitate reintroduction of genetic material into breeding populations.

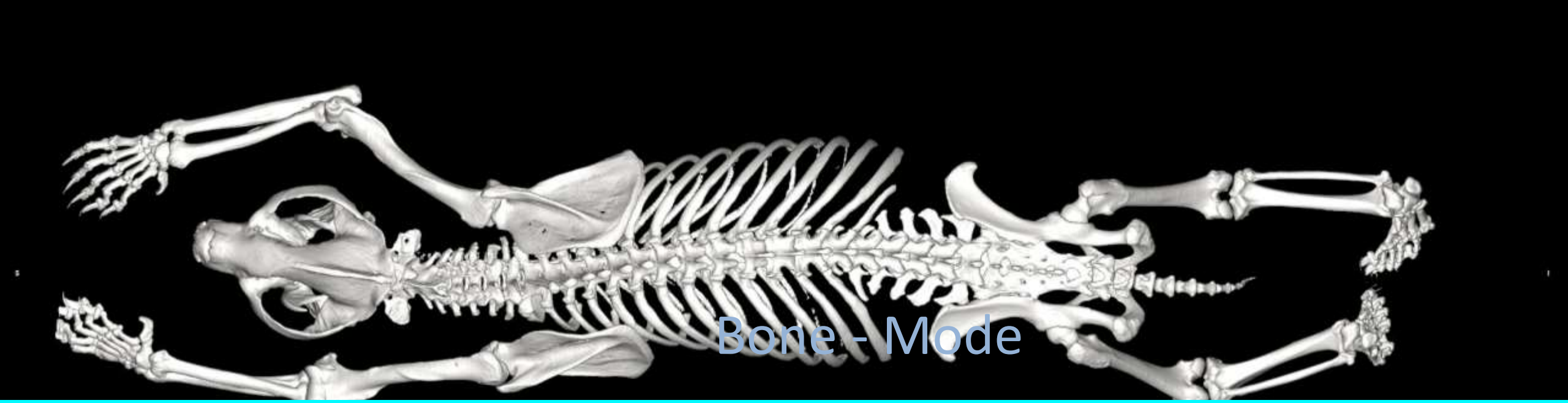
Induced pluripotent stem cells (iPSCs) are generated from somatic cells by direct molecular reprogramming and, as are embryonic stem cells, are capable of unlimited expansion and differentiation into multiple cell types. iPSCs have been generated from somatic cells of humans[1,2] and research animals such as mouse[3] and rhesus monkey[4]. We adapted iPSC technology to reprogram rare animal species, in an effort to use this technology to preserve genetic material of endangered species. The San Diego Zoo Institute for Conservation Research maintains an extensive collection of frozen fibroblast cultures, called the Frozen Zoo, comprised of viable cells of over 8,600 individual vertebrates of ~800 species. We report here to our knowledge the first generation of iPSCs from the endangered primate, the verge of extinction. An estimated population of 2,230 individuals in 1960 has been reduced to only seven living individuals today[7]. Two wild-born northern white rhinoceroses currently reside in the San Diego Zoo Safari Park. The other five (one wild-born and four born in captivity) were kept until recently at the Zoo Dvůr Králové in the Czech Republic. In an effort to save the species, four rhinoceroses with reproductive potential were relocated from the Zoo Dvůr Králové to the Ol Pejeta Conservancy in Kenya in 2009. However, efforts to encourage natural reproduction have so far been unsuccessful, and the last birth of a northern white rhinoceros was in 2000. Because of the critical status of both the drill and northern white rhinoceros populations, we chose these species for reprogramming to iPSCs that might aid conservation efforts.

To reprogram the primate cells, we used retroviral vectors carrying human sequences of the reprogramming factors *POU5F1* (also known as *OCT4*), *SOX2*, *KLF4* and *MYC* (also known as *cMYC*) on a cryopreserved fibroblast cell line from a male drill (named Loon) sampled at 15 years of age (now dead). We generated four iPSC lines (named drill A C, H and J) from the fibroblasts (**Fig. 1**). We initially selected these lines by their unique morphology that is characteristic of embryonic stem cells and iPSCs derived from other species (**Fig. 1a**). Drill iPSCs were karyotypically normal, as determined by comparison with the source fibroblasts (Online Methods, **Fig. 1b** and **Supplementary Fig. 1**). Genomic PCR revealed that the drill iPSCs carried the exogenous sequences for the reprogramming genes (**Supplementary Fig. 2**). Because we found that northern white rhinoceros fibroblasts could not be transduced with amphotrophic retroviruses, we used retro-VSV.G viruses (**Supplementary Fig. 3**) carrying the same four reprogramming factors, on a cryopreserved fibroblast cell line initiated from the youngest living northern white rhinoceros, a female named Fatu, born in 2000 and sampled at ten years of age (currently residing in the Conservatory in Kenya). We generated three rhinoceros iPSC lines (northern white rhinoceros (NWR) A, B and C) from this cell line (**Fig. 2**). We also selected these lines by their characteristic morphology (**Fig. 2a**). NWR iPSCs

IVF-Lab
at the Institute for Zoo & Wildlife Research

>30 species processed


aguti
dromedary
hippo
Sumatran tiger
gerenuk
Asian lion
tree kangaroo
walrus
seal
leopard
wallaroo kangaroo
brown bear
naked mole rat



Bone – Surface - Mode

CONSERVATION

improving population dynamic

global responsibility & societal investment

Northern white Rhino iPSCs are pluripotent & differentiate into three germ layers