



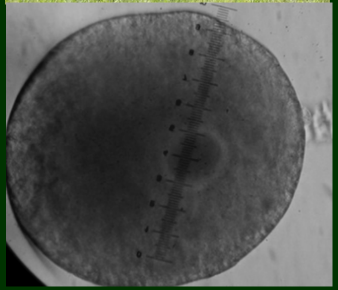

Fertility Preservation in Wild Animal Species - Recent Advances and Relevance to Human Reproductive Medicine

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I do not have anything to disclose

Learning Objectives

- Identify the diversity and complexity of reproduction in wild species, which represent an invaluable set of comparative data that can greatly enhance success in identifying reproductive mechanisms and infertility treatments in common species (including humans)
- Indicate the connection between the development of ART in wildlife and human reproductive medicine
- Assess the value of advances in human ART and fertility preservation to wildlife species preservation (development of new tools, similarity of some biological traits, sharing the same needs and the same environment)

Detrimental Human Activities

1/4 of mammal species
1/3 of amphibian species
1/2 of fish species
>1/2 of plant species

are threatened in their natural habitat

Soil biodiversity
60% of species on Earth
Risk of artificialization

Loss of biodiversity 'at large':
Less heritage breeds and crops due to selection of commercial breeds

Multidisciplinary Strategy to Save and Sustain Species
One Plan Approach

Protection of natural habitats = The highest priority
(ecology, bio-economics, bio-politics, social sciences)

↓ ⊕

Maintenance of genetic diversity
Propagation *in situ* and *ex situ* of small populations
Reinforcement/reintroduction in natural habitats

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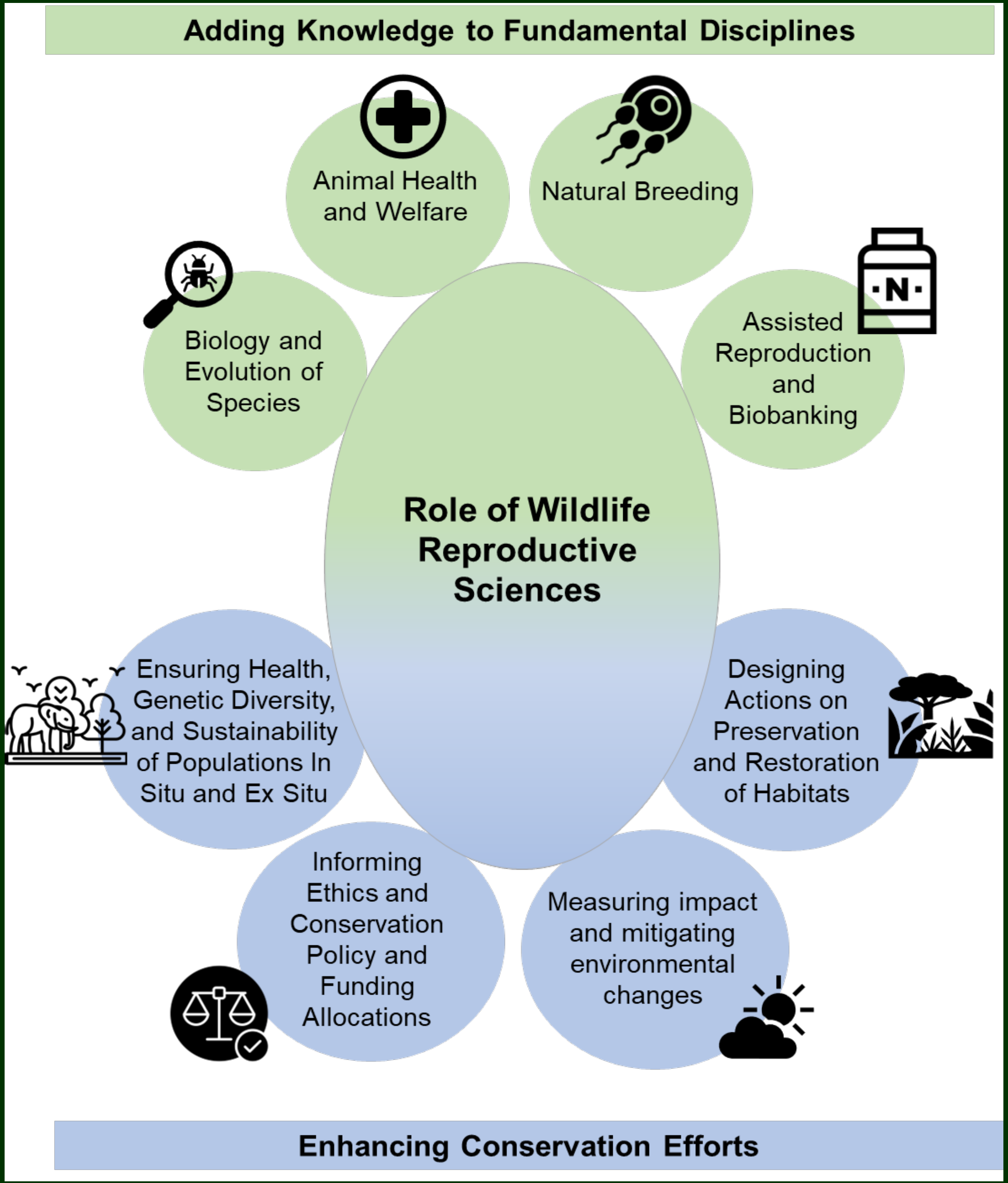
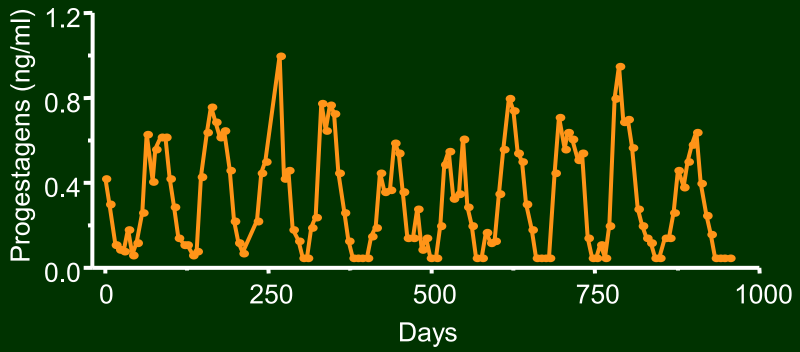
Understanding the biology of entire species
(field ecology, genetics, behavior, nutrition, husbandry, reproductive physiology, endocrinology, veterinary medicine)
New tools/technologies, bioinformatics, biomathematics


Adding Knowledge to Fundamental Disciplines
Animal Health and Welfare
Natural Breeding
Biology and Evolution of Species
Assisted Reproduction and Biobanking
N
Role of Wildlife Reproductive Sciences
Ensuring Health, Genetic Diversity, and Sustainability of Populations In Situ and Ex Situ
Designing Actions on Preservation and Restoration of Habitats
Informing Ethics and Conservation Policy and Funding Allocations
Measuring impact and mitigating environmental changes
Enhancing Conservation Efforts

From Fundamental Reproductive Sciences to Reproductive Biotechnologies

Fundamental/comparative studies



→ Scholarly knowledge and conservation actions *in situ* and *ex situ*
(enhancing natural mating, maintaining genetic diversity)

→ Development of Assisted Reproductive Technologies and
Genome Resource Banking
(overcoming mating difficulties, preserving fertility, maintaining genetic diversity)

Diversity and Complexity of Mammalian Reproduction

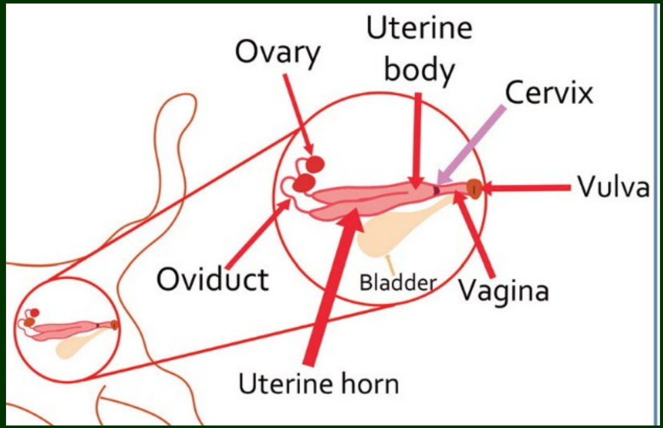
As many mechanistic differences in reproduction as there are species
Only few species (~250!) well described among 6,400 mammalian species

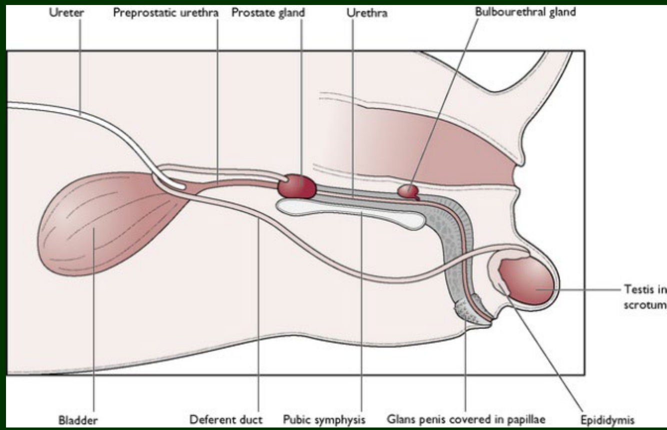
Diversity in fertility issues (teratospermia, aging, stress of captivity…)
Species-specificities in hormonal stimulations, *in vitro* culture, cryopreservation

Limitations when conducting studies in wild mammals:
Sheer lack of attention to wildlife reproduction
Small sample numbers
Complex working conditions
Lack of space, facilities, expertise and funding

Diversity of Reproductive Mechanisms Within the Felid Family

36 wild species
All threatened, vulnerable or endangered
From 1.5 kg to 300 kg

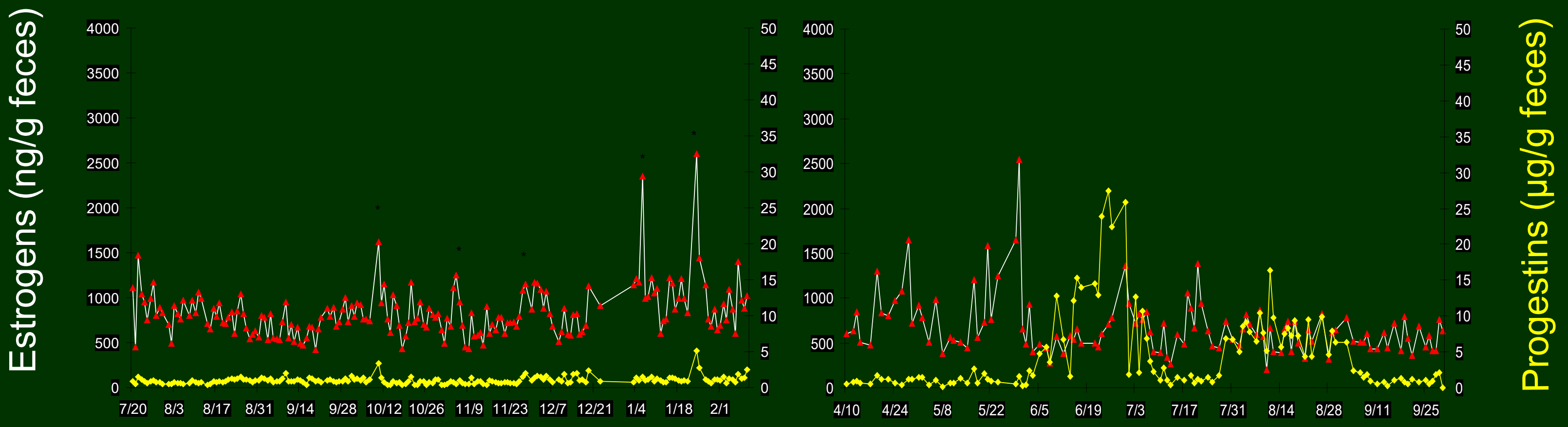


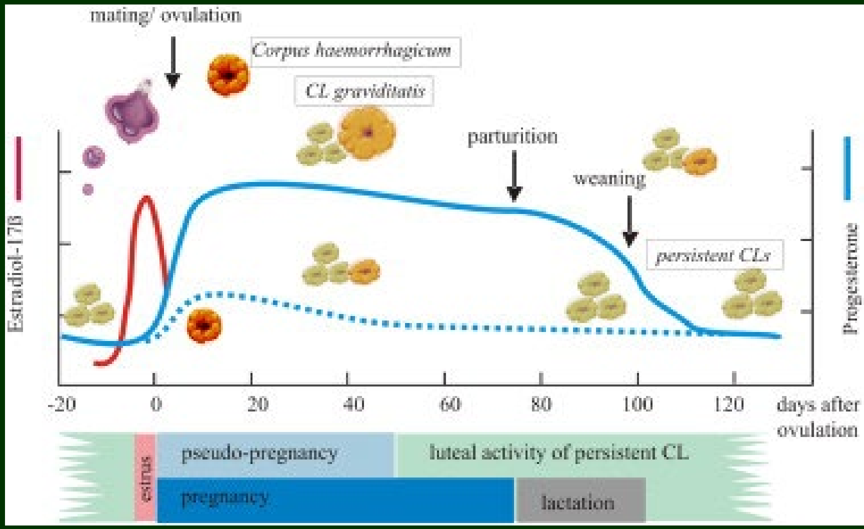


Ovulations induced by mating
(cheetah, tiger)

Spontaneous ovulations
(clouded leopard, fishing cat)

Persistent Corpora Lutea
(lynx)





Non-invasive monitoring of ovarian cycles in the absence of mating

Reproductive Aging in Wild Animal Species: Extreme Scenarios

Semelparity (antechinus)
(death because of reproduction)

Reproductive aging followed
by post-reproductive life

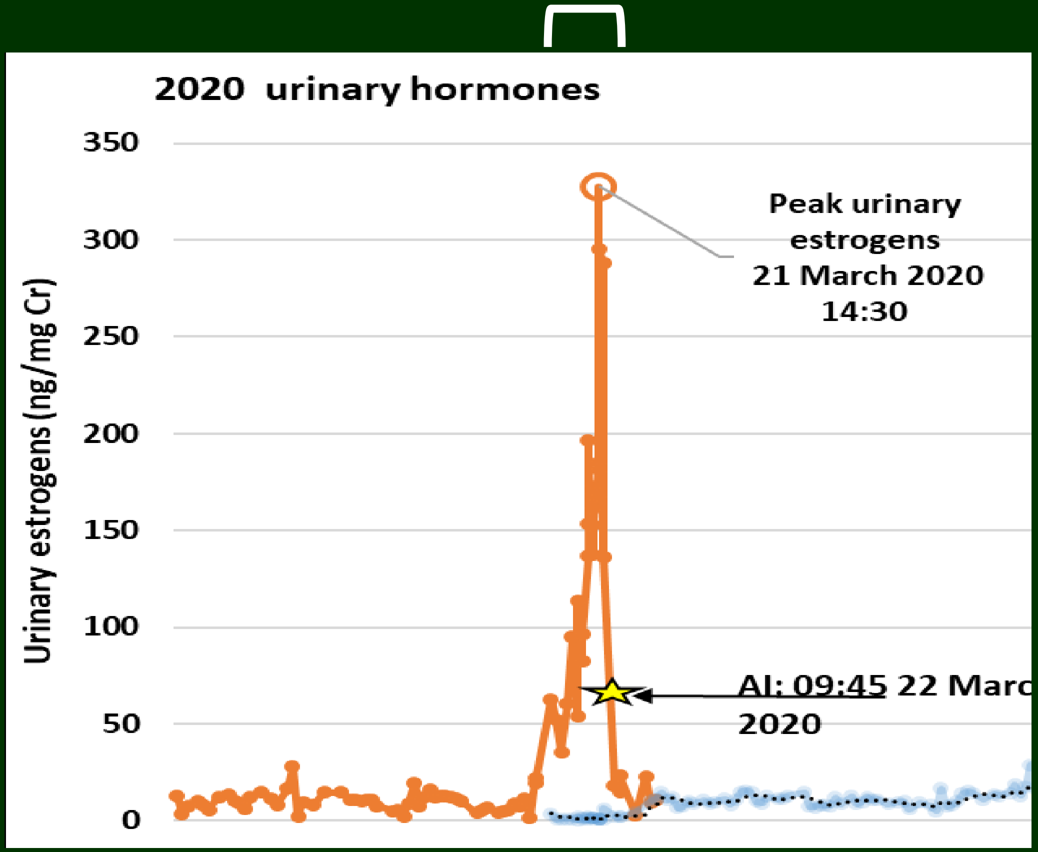
No reproductive aging

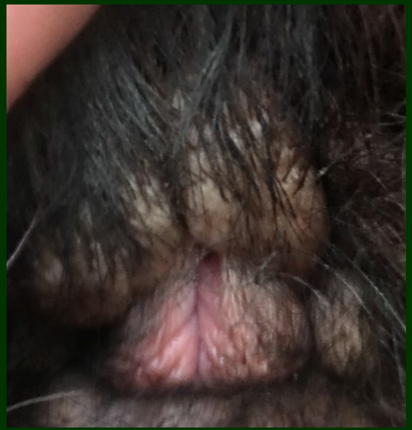
Reproduction of the Giant Panda (*Ailuropoda melanoleuca*)

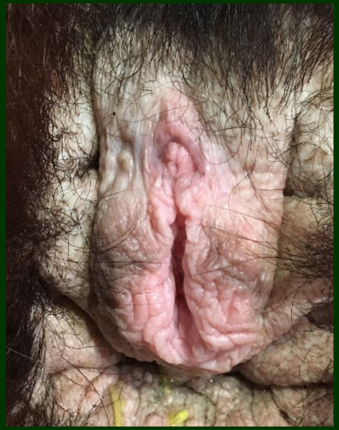



Studying and Assisting Reproduction in the Giant Panda

Single estrus
of 7-10 days
(March-May)





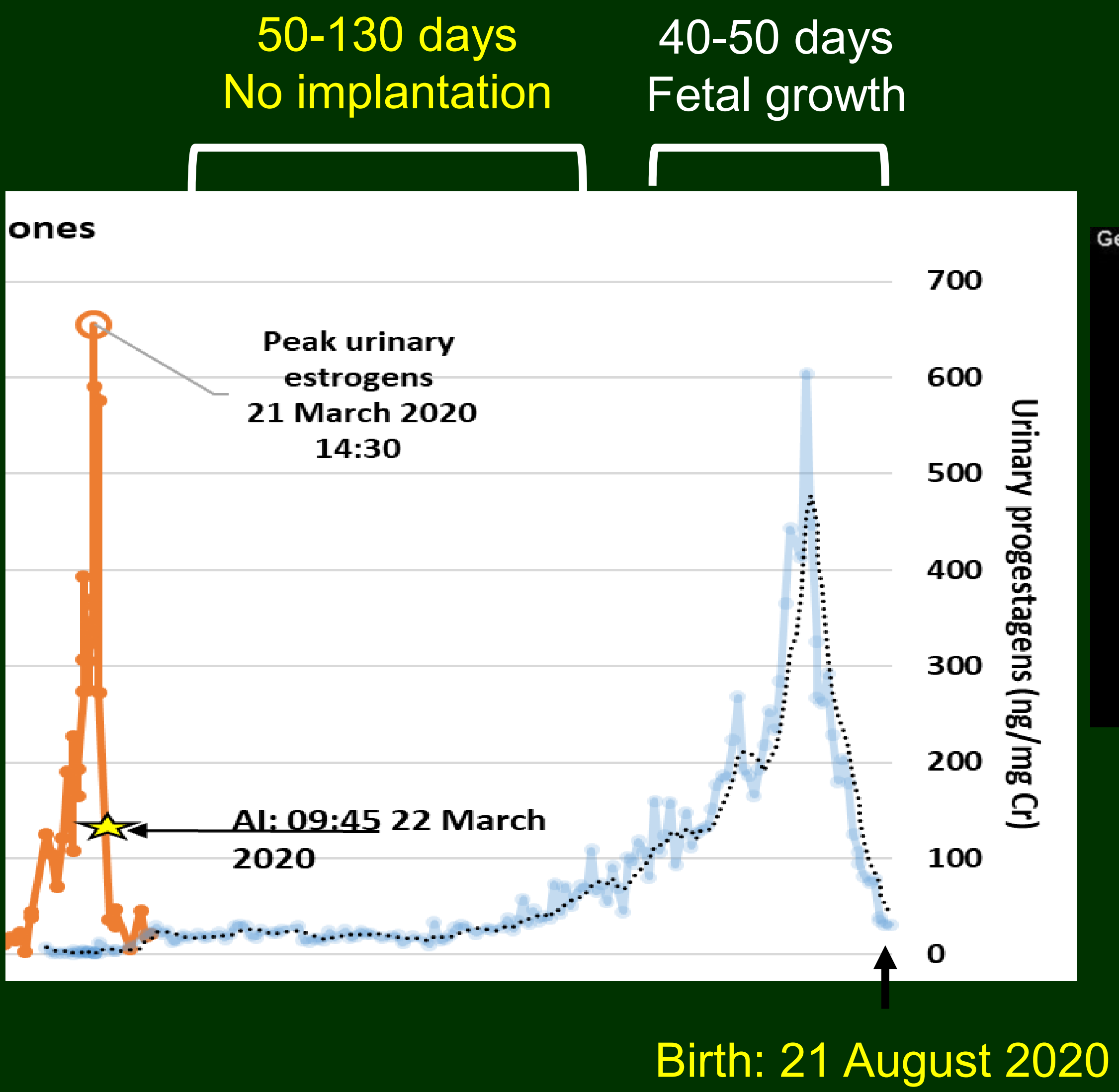


Artificial Insemination in the Giant Panda

Trans-cervical artificial insemination:
~18h and 30h post-ovulation

Insemination dose (1.5 ml):
800 millions of motile sperm
From fresh or <u>frozen</u> samples

Non-Invasive Monitoring of Pregnancy in the Giant Panda

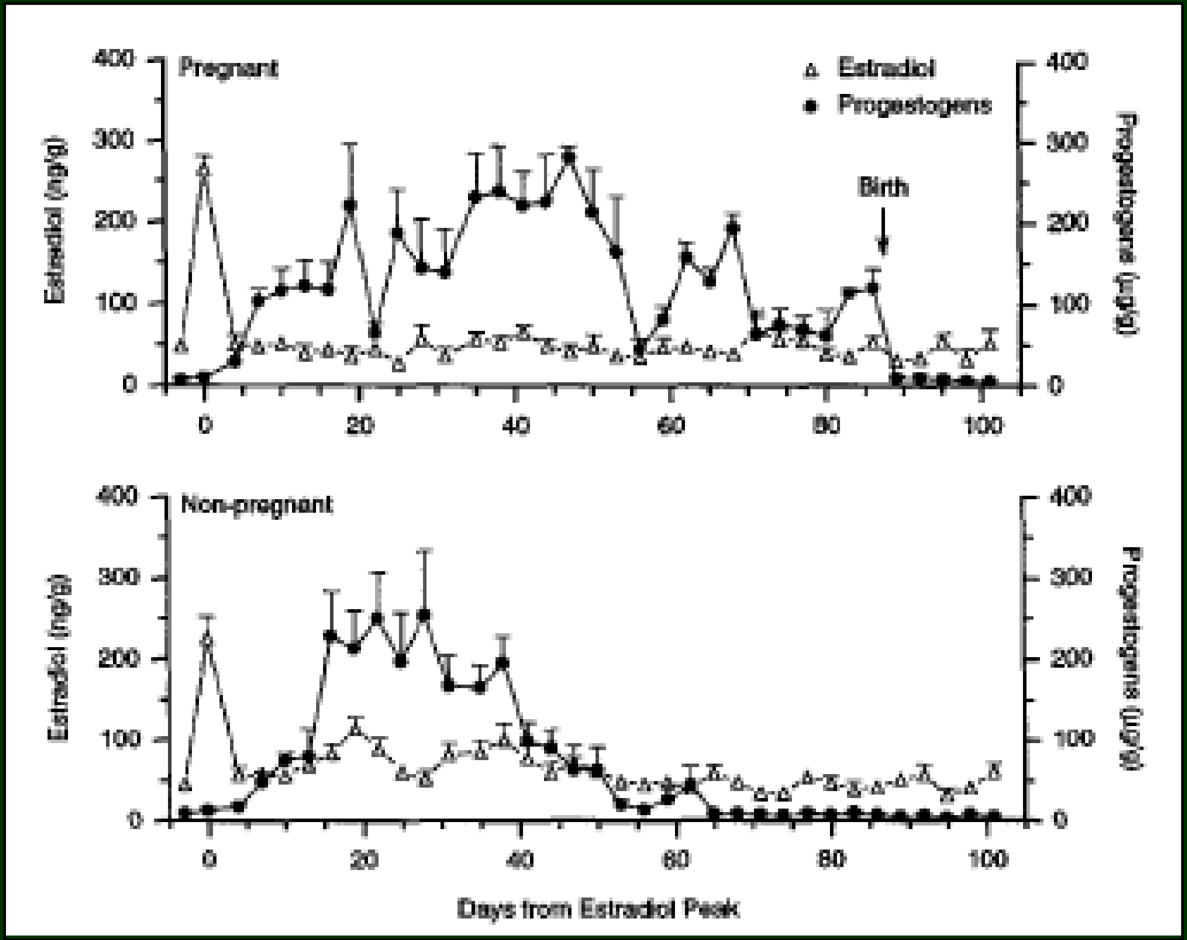


Non-Invasive Monitoring of Pregnancy and Birth Timing by Ultrasonography

Transabdominal or transrectal ultrasonography without sedation in trained animals (Eld's deer, cheetah, elephants…)

Only way to confirm pregnancy in giant panda (late stage)

Non-Invasive Monitoring of Pregnancy and Birth Timing by Endocrinology



In cats, difficult to diagnose pregnancy based on progestagens
Similar hormone concentrations between pregnant and non-pregnant females
If hormones are still elevated after 60 or 70 days, female is likely pregnant

Species-Specific Pregnancy Hormones

	Alpaca	Sheep	Goat	Rhino	Sea Lion	Dog	Pig	Tapir	Cow	Dolphin	Beluga	Orca	Walrus	Horse	Donkey	Hyena	Human
Pregnenolone	<1	0	0	2	0	<1	8	3	<1	<1	<1	0	<1	5	8	16	2
Progesterone (P)	3	4	6	5	19	14	12	2	4	9	9	9	11	1	2	188	121
17αOH-P	0	0	0	<1	1	<1	0	<1	<1	<1	<1	<1	<1	<1	0	2	6
20αOH-P	<1	5	0	--	<1	<1	0	<1	<1	7	6	26	<1	--	<1	0	20
5α-dihydroprogesterone (DHP)	<1	0	<1	2	9	6	3	<1	<1	2	3	3	2	28	20	74	6
Allopregnanolone (3α-DHP)	<1	0	3	<1	0	<1	3	2	<1	<1	2	2	0	11	11	54	2
20α-DHP	0	0	0	16	<1	0	0	0	76	3	1	4	<1	97	0	0	9
3β,20α-DHP	<1	0	<1	7	0	0	<1	1	0	91	43	48	0	208	129	0	<1
Dehydroepiandrosterone	0	0	0	0	0	0	0	2	<1	0	0	0	6	0	0	0	4
Androstenedione	0	0	0	<1	<1	0	0	<1	<1	1	4	3	<1	0	0	2	3
Testosterone	0	0	0	0	0	0	0	<1	0	<1	<1	<1	2	0	0	1	1
Estrone	<1	0	<1	0	0	0	<1	0	<1	0	0	0	0	0	0	7	14

Conley et al., 2021

Limitations of Non-Invasive Monitoring of Pregnancy by Endocrinology

Need for species by species and hormone by hormone approach for developing effective non-invasive monitoring

Early pregnancy is difficult to detect through non-invasive technique (no distinction between cyclic hormonal patterns and early pregnancy)

Emerging Approaches

Non-steroid indicators of early pregnancy (immunoglobulins in cheetahs)
Excreted cell-free DNA (bears)
Excreted extra-cellular vesicles

Need for more non-invasive indicators of healthy pregnancy

Current Status of Assisted Reproduction in Wild Species

'Success stories' with artificial insemination (fresh or frozen semen)

Births after in vitro fertilization and embryo transfer (anecdotal)

Cryopreservation of oocytes and gonadal tissues (under development)

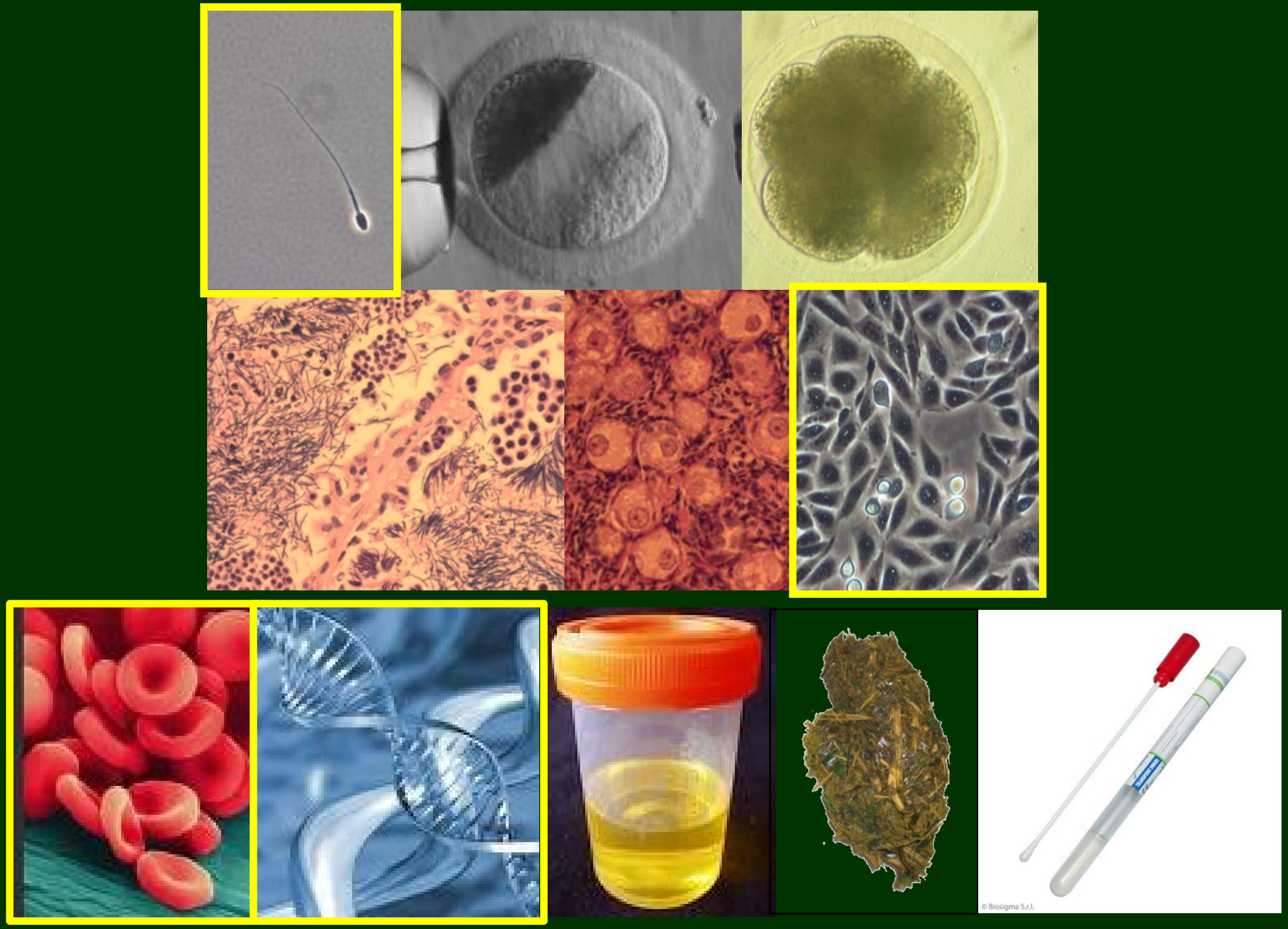
Good Progress but Urgent Need for More Fertility Preservation Options

Preservation and use of gonadal tissues
In vitro production of gametes from gonadal tissues or stem cells
Minimal cost, field-friendly preservation methods (vitrification, desiccation)




Current Status of Genome Resource Banking

Organized collection, storage and use of biomaterials for the purpose of species conservation and biomedical science



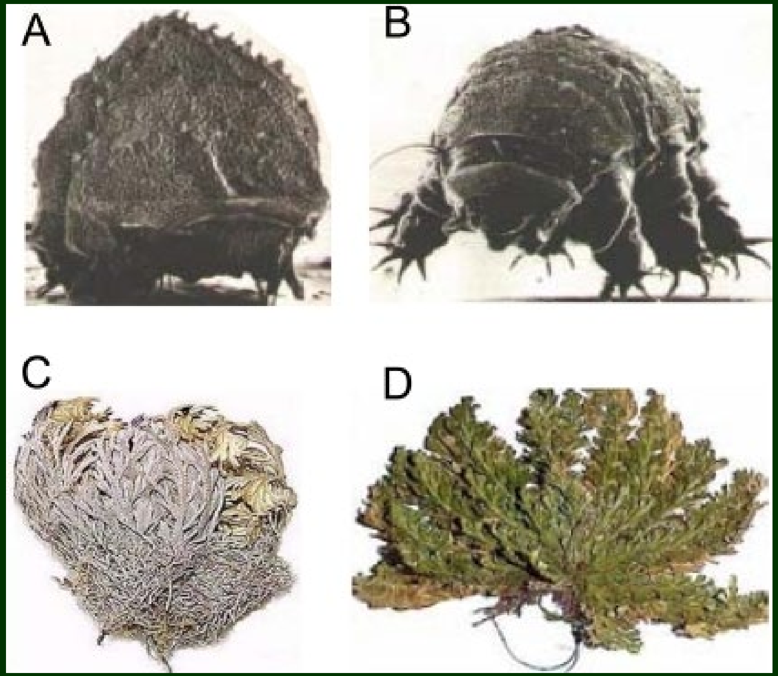
Avoid Over-Simplification in Cryobiology

Germplasm cryopreservation methods developed in cattle industry do not work for other species

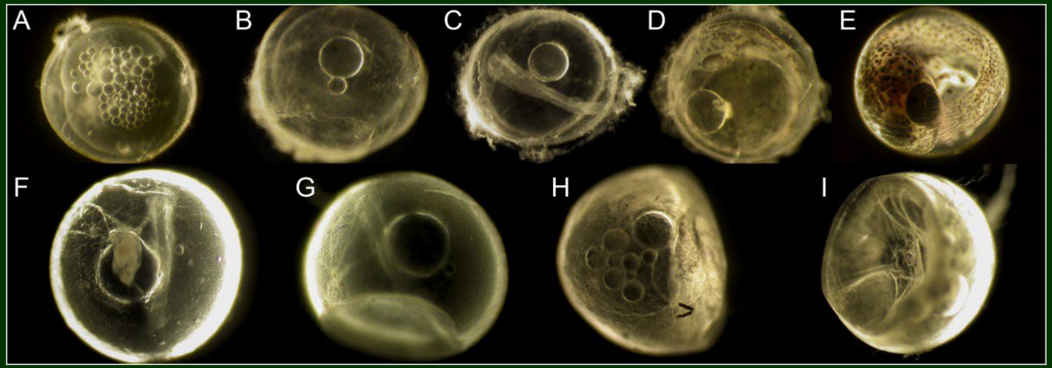
Species-specificities in cryoprotectant tolerance, resilience to cold and freezing temperatures

Species-specificities of thawing/warming conditions (Laser warming)

Exploring New Preservation Options
Lessons from Nature – From Plants to Killifish



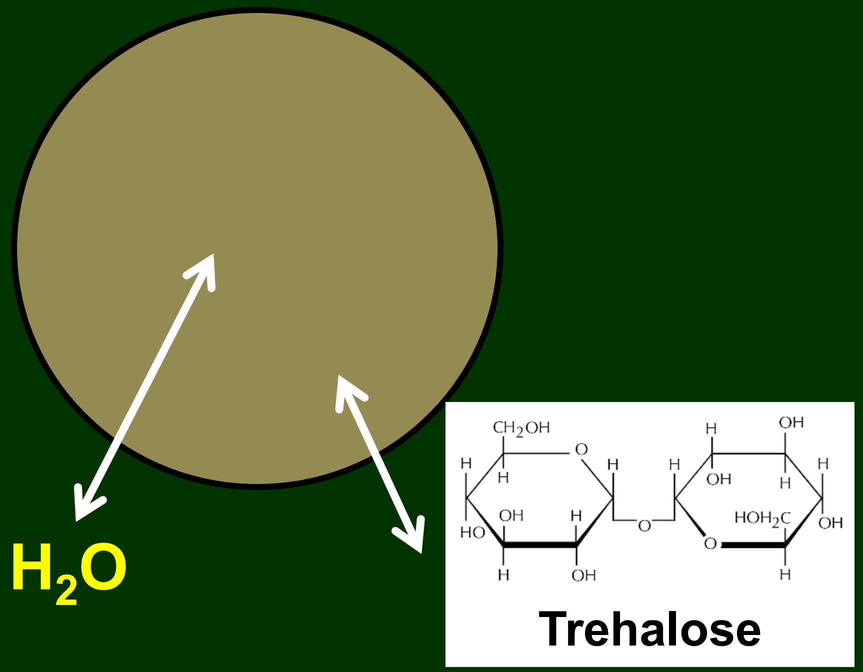
Suspending life at non-freezing temperatures

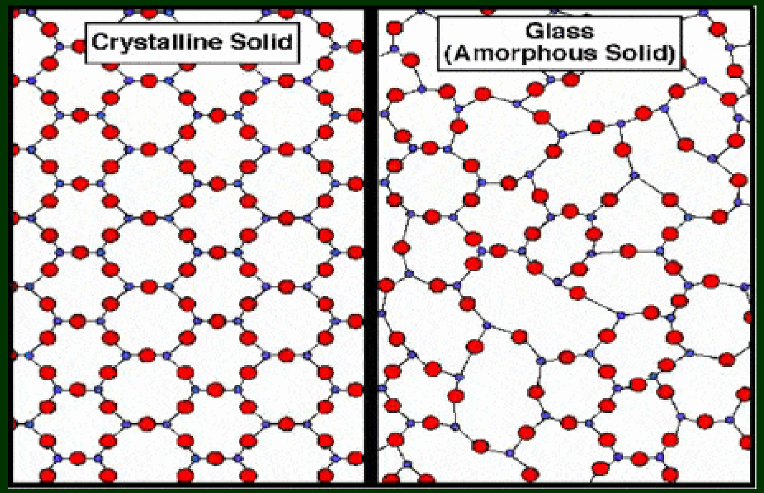


Natural protection from dry, hot, cold, or high salinity environments

'Xeroprotectants': intracellular production of trehalose, Late Embryogenesis Abundant proteins, Heat Shock Proteins, Intrinsically Disordered Proteins, Antioxidants

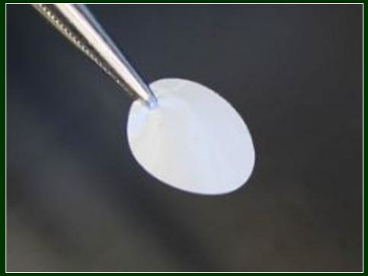
Principles of Dehydration for Germplasms

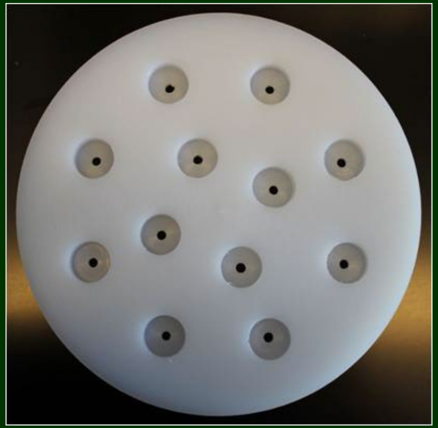




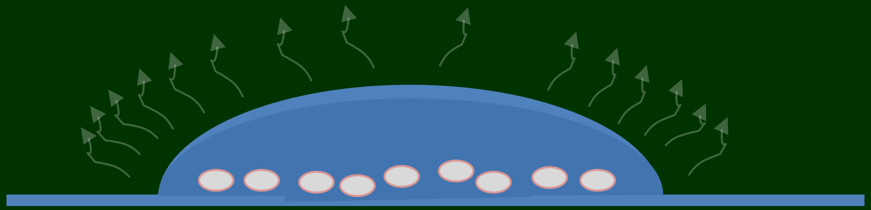
- Incorporation of trehalose (via membrane permeabilization, endocytosis of nanoparticles)
- Dehydration (via different methods) until glass state is reached (desiccation)
- Storage at supra-zero temperatures (in controlled humidity) for several weeks
- Rapid rehydration and reanimation

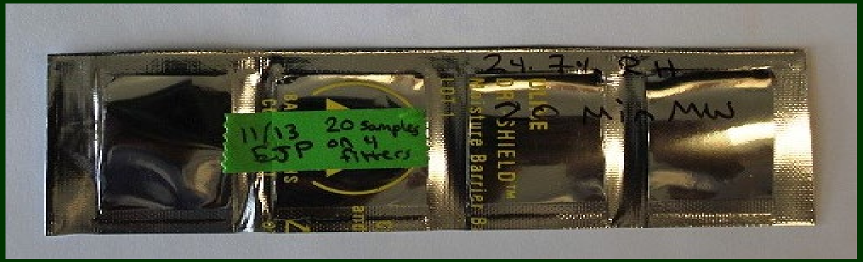
Microwave-Assisted Drying and Storage



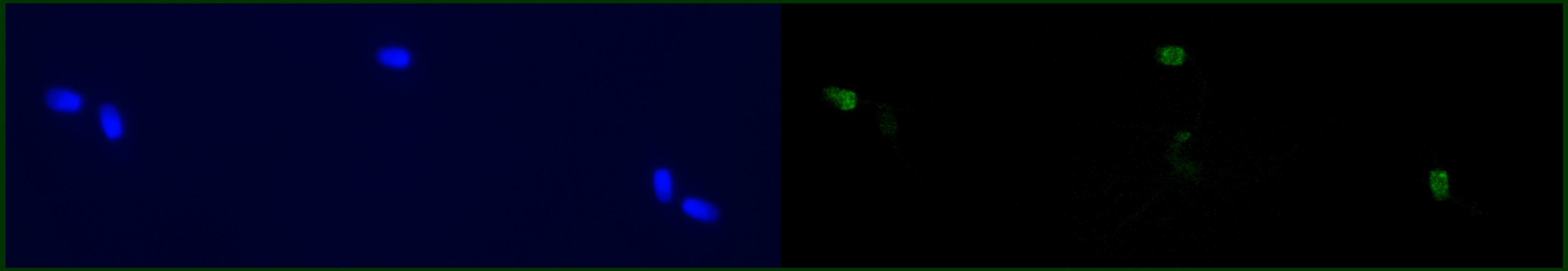


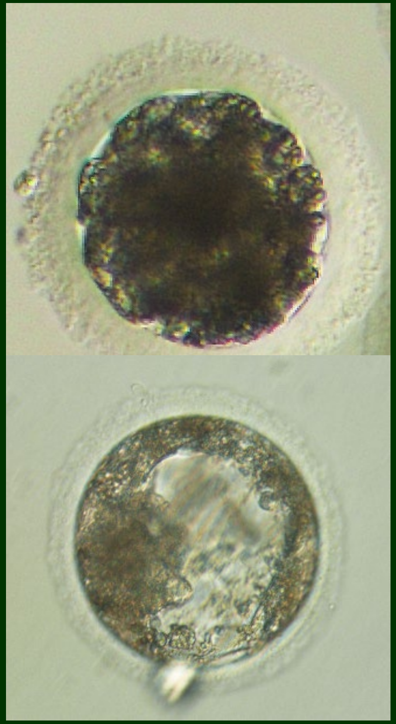
Pulses at 20% power in CEM SAM system (600 W microwave)





Cat Sperm Desiccation





Loss of sperm motility
No morphological changes
<15% sperm DNA damage regardless of the storage time

60% of *in vitro* fertilization after sperm injection
15% of morulae
7% of blastocyst formation

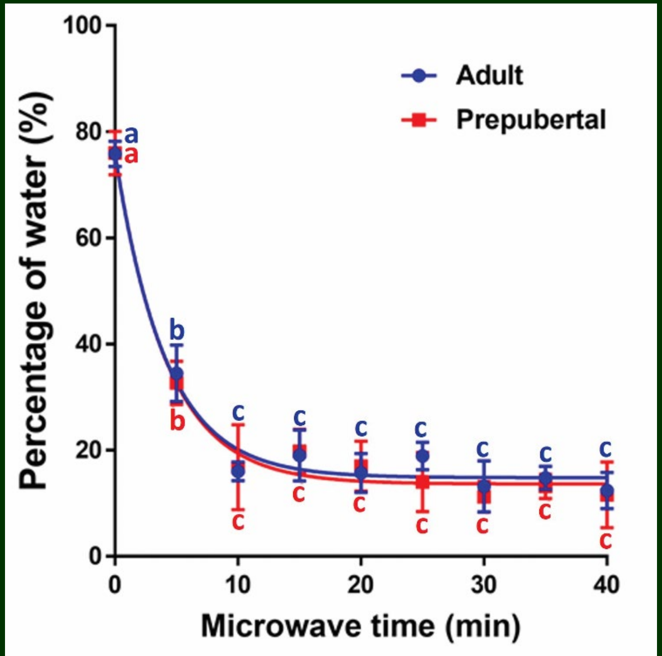
Intercontinental shipment at non-cryogenic temperatures is possible

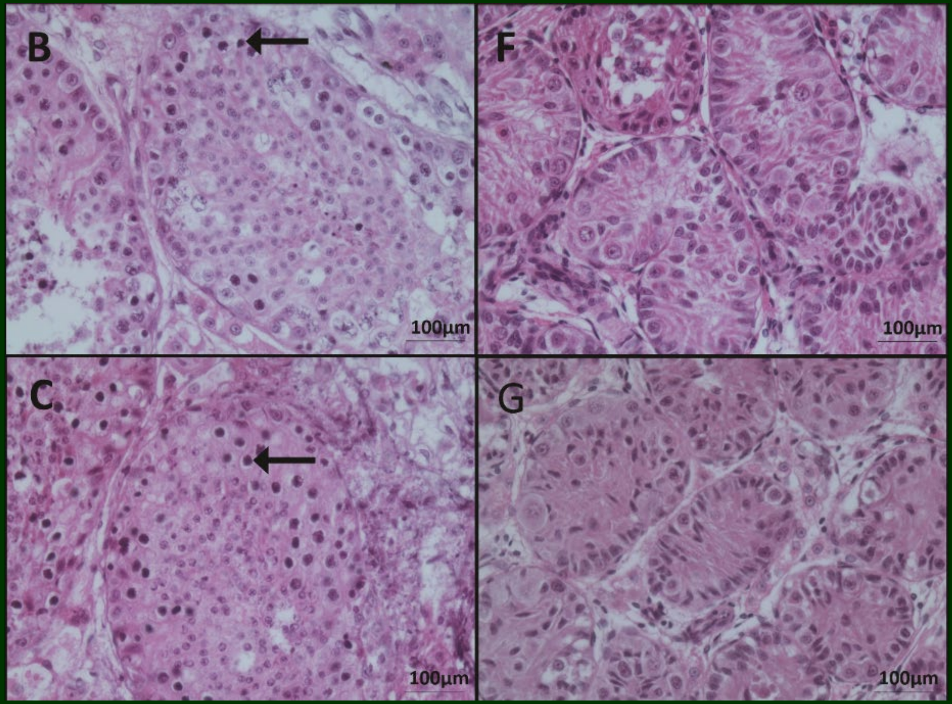
Lee PC, Zahmel J, Jewgenow K, Comizzoli P. J Assist Reprod Genet. 2022 Jan;39(1):141-151.

Cat Testicular Tissue Desiccation

Reversible membrane permeabilization with digitonin (tissue biopsies)
Exposure to 1M trehalose for 10 min
Microwave-assisted drying

60% of morphologically intact seminiferous tubules after 20 min of drying
50% of viable cells after 20 min of drying
No increase in DNA fragmentation



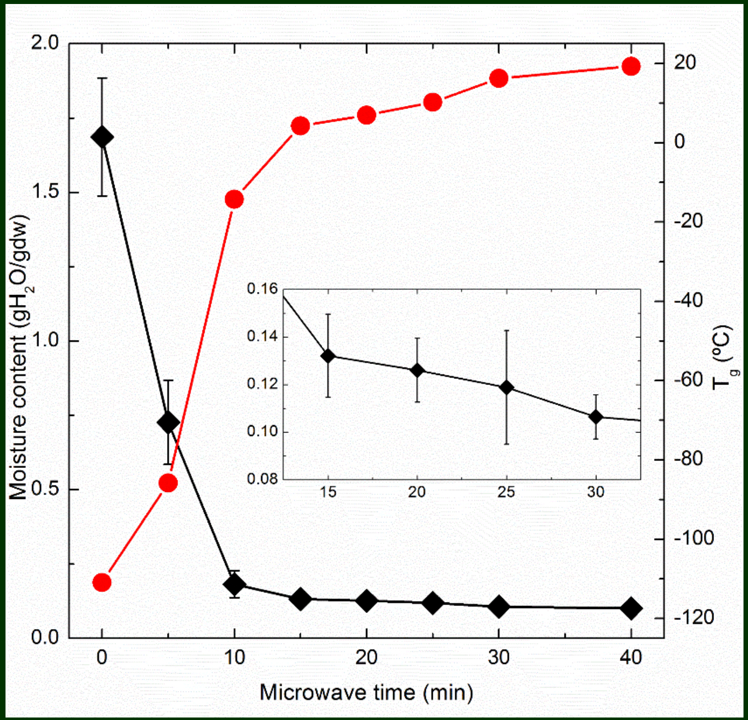


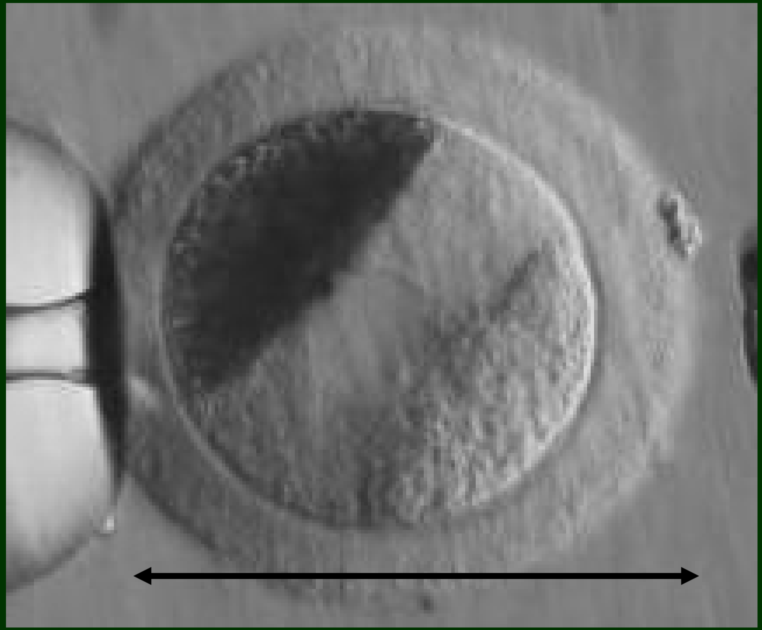
Silva HVR, da Silva AM, Lee PC, Brito BF, Silva AR, da Silva LDM, Comizzoli P. Biopreserv Biobank. 2020 Oct;18(5):415-424.

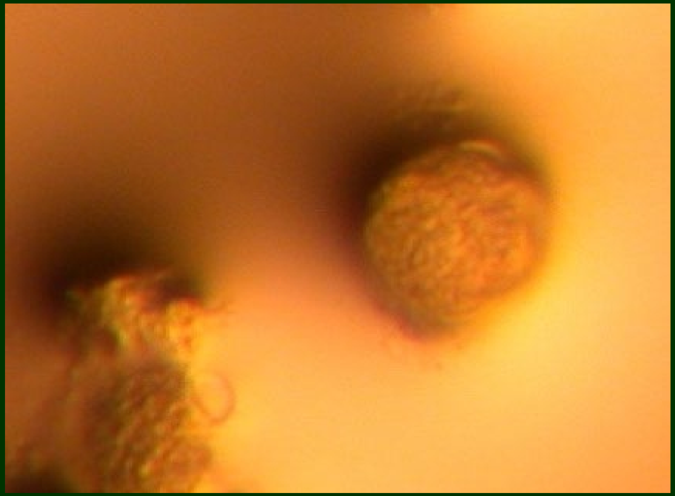
Preservation of Germinal Vesicles in the Cat Model Using Microwave-Assisted Dehydration

Large oocyte volume
Focus on the GV

Hemolysin treatment before trehalose exposure (1.5M for 10 min)







Trehalose glass

DNA Integrity of Dried Germinal Vesicles in the Cat Model

(Elliott et al., 2015)

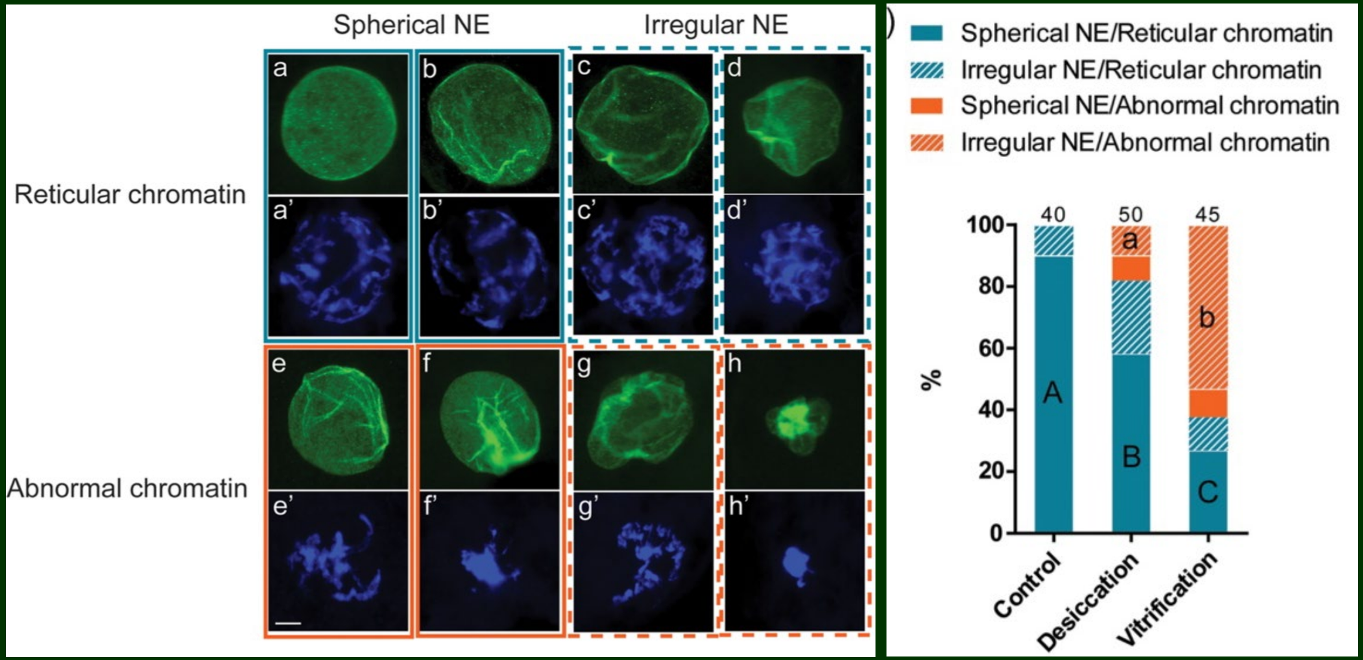
MW time (min)	n	Absent (%)*	TUNEL+ (%)**	Intact (%)£
0	42	4.8 [ab]	7.5 [a]	88.1 [ab]
5	36	8.3 [ab]	6.1 [a]	86.1 [ab]
10	53	3.8 [ab]	7.8 [a]	88.7 [ab]
15	46	2.2 [a]	6.7 [a]	91.3 [a]
20	52	3.8 [ab]	12.0 [a]	84.6 [ab]
25	33	9.1 [b]	10.0 [a]	81.8 [b]
30	32	6.3 [ab]	10.0 [a]	84.4 [ab]
40	43	7.0 [ab]	32.5 [b]	62.8 [c]

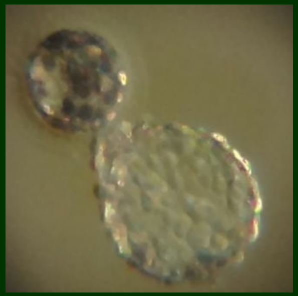
Storage time	Storage temperature	n	Absent (%)*	TUNEL+ (%)**	Intact (%)£
0 day		8	12.5 [a]	14.3 [ac]	75.0 [b]
2 days	4°C	10	10.0 [a]	33.3 [bc]	60.0 [acd]
	Ambient	10	0.0 [b]	30.0 [bc]	70.0 [bc]
1 week	4°C	28	25.0 [cd]	33.3 [bc]	50.0 [a]
	Ambient	24	20.8 [ad]	31.6 [bc]	54.2 [ad]
2 weeks	4°C	27	25.9 [cd]	35.0 [b]	48.1 [a]
	Ambient	18	22.2 [ad]	28.6 [bc]	55.6 [ad]
4 weeks	4°C	17	23.5 [cd]	30.8 [bc]	52.9 [a]
	Ambient	18	11.1 [a]	25.0 [bc]	66.7 [bcd]
8 weeks	4°C	21	9.5 [a]	21.1 [c]	71.4 [bc]
	Ambient	20	30.0 [cd]	14.3 [ac]	60.0 [acd]

Chromatin Configuration and Nuclear Envelope Integrity of Dried Germinal Vesicles in the Cat Model

(Graves-Herring 2013; Lee et al. 2019)

Compared to vitrification: better integrity of chromatin and nuclear envelope

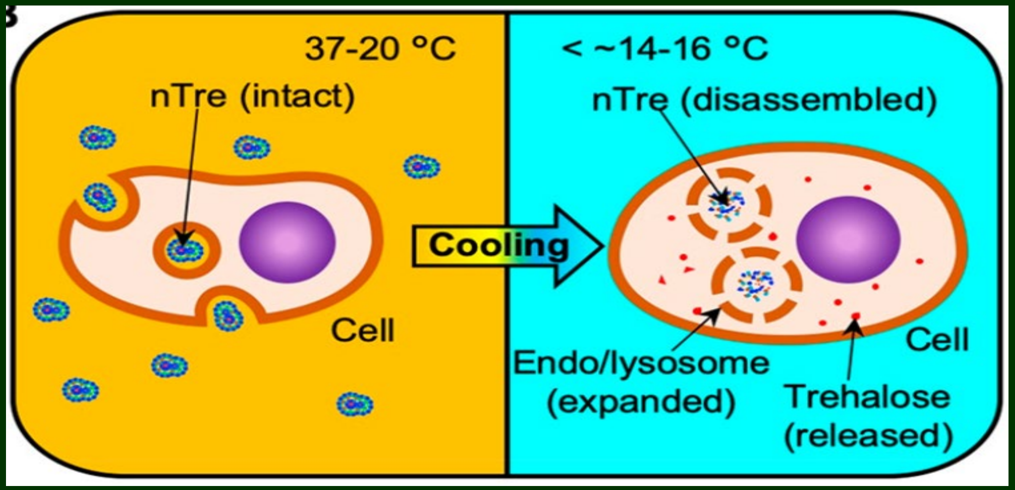


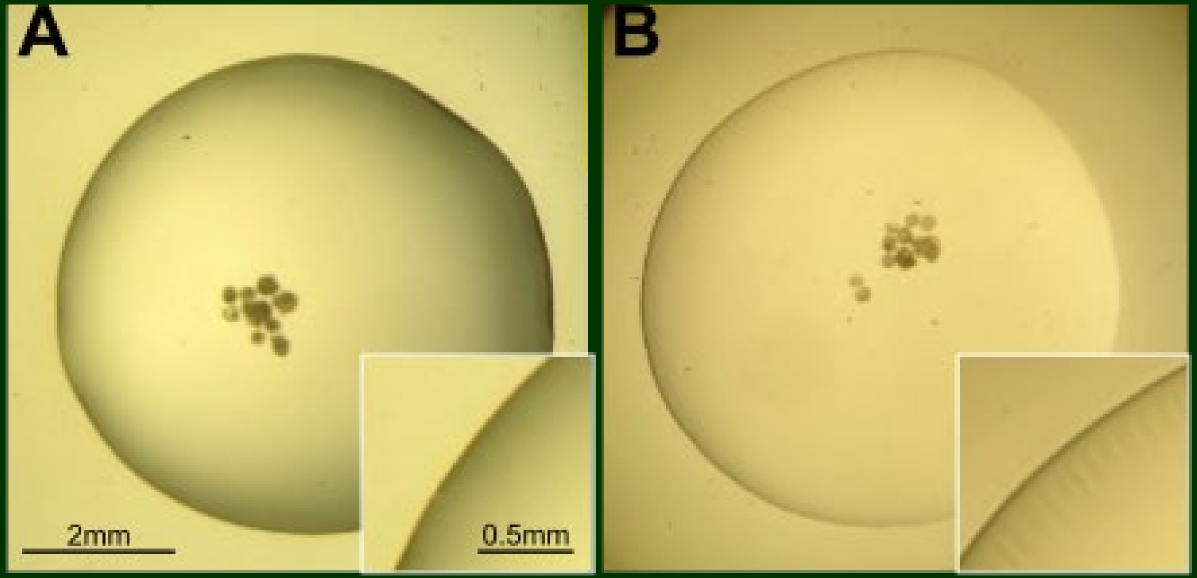


55% of resumption of meiosis to metaphase II after injection into a recipient fresh cytoplast
60% of *in vitro* fertilization
10% of blastocyst formation

Dehydration of Cumulus-Oocyte Complexes in the Cat Model
(Lee et al., 2023)

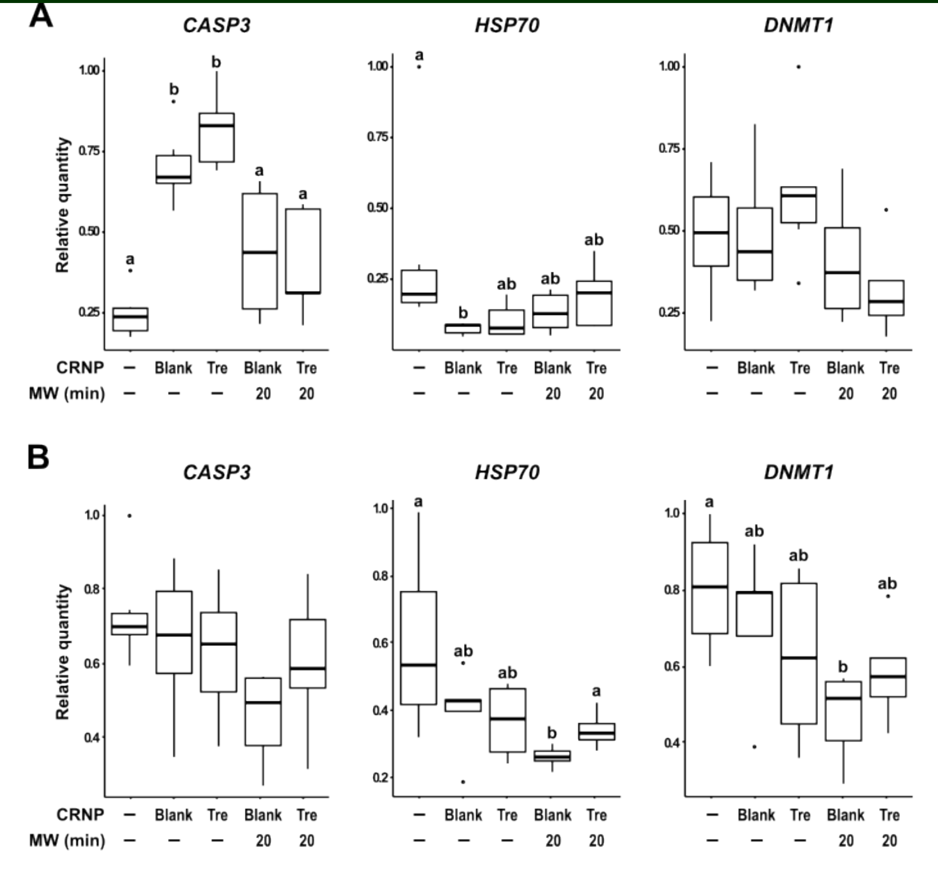
Incorporation of trehalose via natural endocytosis of cold-responsive nanoparticles (CRNP)





Dehydration of Cumulus-Oocyte Complexes in the Cat Model
(Lee et al., 2023)

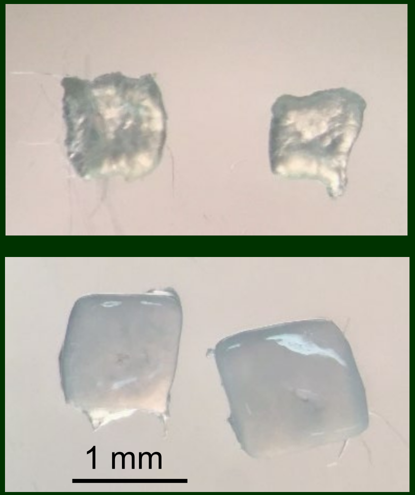
Drying time	CRNP	Total number of COCs (n)	MII (%)	Cleavage (%)[#]	8-cell (%)	Blastocyst (%)
0 min	Blank	33	66.7%[a]	77.3%[a]	77.3%[a]	18.2%
	Trehalose	36	61.1%[a]	81.8%[a]	77.3%[a]	13.6%
15 min	Blank	47	51.1%[ab]	75.0%[a]	75.0%[a]	8.3%
	Trehalose	46	41.3%[b]	68.4%[ab]	68.4%[ab]	15.8%
20 min	Blank	50	38.0%[b]	42.1%[b]	36.8%[b]	0.0%
	Trehalose	47	40.4%[b]	68.4%[ab]	57.9%[ab]	10.5%
30 min	Blank	44	0.0%[c]	-	-	-
	Trehalose	47	0.0%[c]	-	-	-

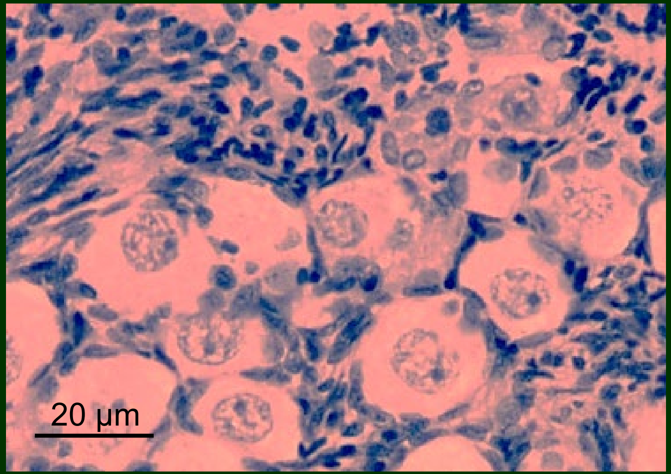


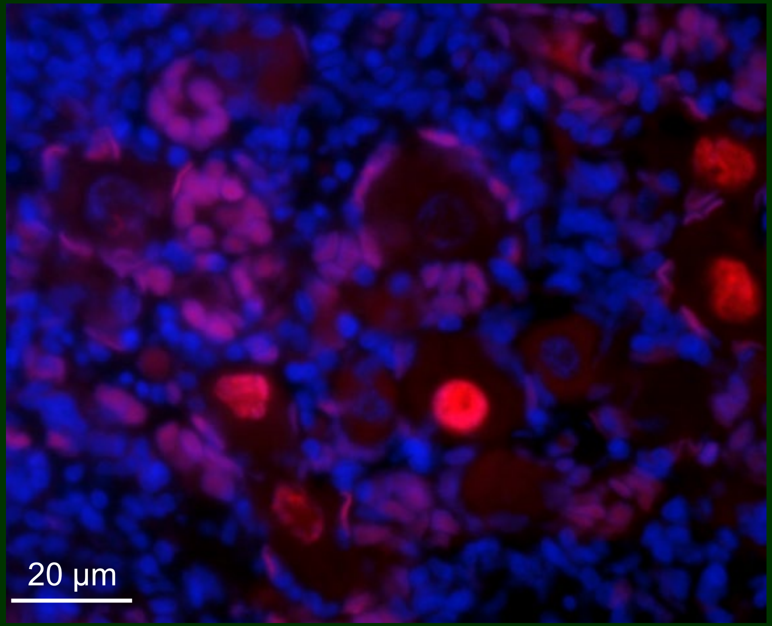
Ovarian Tissue Dehydration
(Lee et al., 2019; Amelkina et la., 2025)

Reversible membrane permeabilization with digitonin (tissue biopsies)
Exposure to 0.5M trehalose for 10 min
Microwave-assisted drying

50% of intact follicles regardless of the drying time (5 to 30 min)
50% of DNA integrity regardless of the drying time (5 to 30 min)
20% of viable and transcriptionally active follicles at 15 min (compatible with non-cryogenic storage)







New Preservation Strategies at Ambient Temperatures

Complexity

Simplicity

Easy processing, storage, transport, and even use of biomaterials in field conditions and at a lower cost

Initiation of a ‘Dry GRB’ at the National Zoo

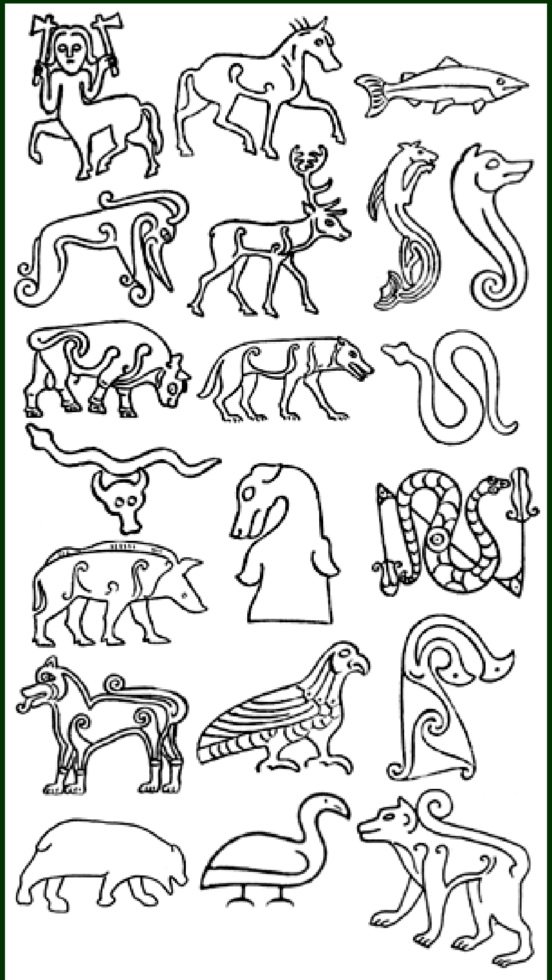
Creating Bridges between Human and Wild Animal Reproductive Science

Traditional Animal Models for Human Reproductive Medicine

80% of research funding
Convenient
Huge amount of knowledge
Several limitations

Expensive
Sometimes irrelevant

Value of Non-Traditional Animal Models



The more species we explore the better we can understand and address complex issues in human fertility

Individuals from wild animal populations are largely heterozygotic (like human populations)

Patient to patient variations (anatomy, physiology, pathology)

Vast amount of genetic data as well as phenotypes are accessible and usable

Value of Non-Traditional Animal Models

Hot topics	Human	Felids	Canids	Elephants	Rhino.	Ungulates	Birds
Ovarian hyperstim.	✔	✔					
Inconsistent ART	✔	✔	✔		✔	✔	✔
Hyperprolactinemia	✔			✔			
Obesity	✔	✔	✔	✔	✔	✔	✔
Precocious puberty	✔			✔	✔		
Reproductive aging	✔	✔	✔	✔	✔		
Stress and reprod.	✔	✔	✔	✔	✔	✔	✔

‘One Reproductive Health’

We share the same goals

We share the same environment

We share the same needs:

New preservation options

Field-friendly methods of preservation

Sample storage at minimal cost

Inspiring Advances in Human Assisted Reproduction


LATE SPECIAL

Evening News

Meet Louise, the world's first test-tube arrival

SUPERBABE

Wide-eyed Louise Brown pictured in hos
she was born. Today she's doing well.

In vitro fertilization in 1978
ICSI in 1992
Millions of babies

Value of Human Reproductive Sciences for Animal Conservation

Inspiring advances in human reproductive medicine applicable to wild species


Drugs, equipment, tools, techniques

Examination of fertility
Hormone monitoring

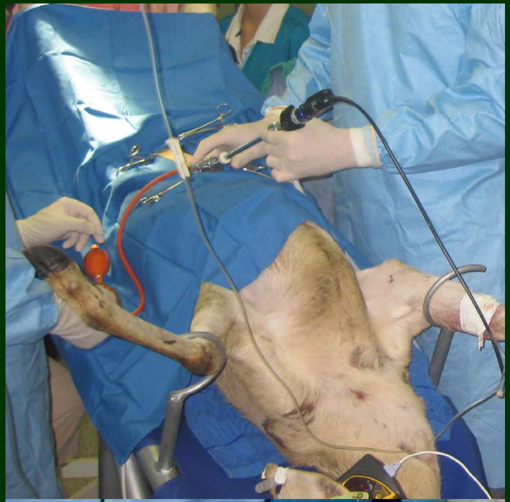
Studies on puberty and aging

Contraception

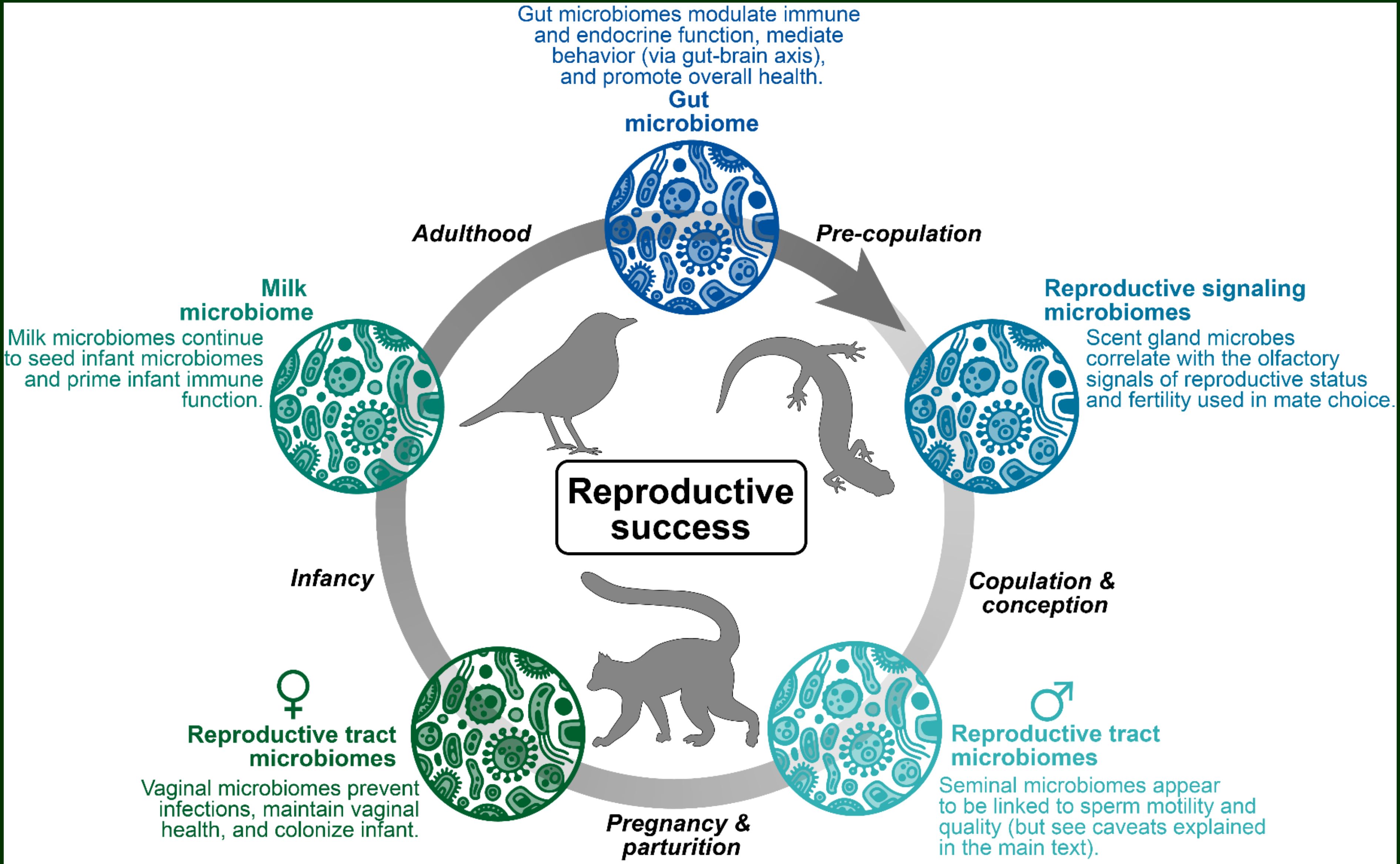
Biobanking

Fertility preservation

Reproductive microbiomes




Reproductive Biology and Microbiomes



Conclusions – Take-Home Messages

Need more fundamental knowledge about biodiversity and reproduction biology

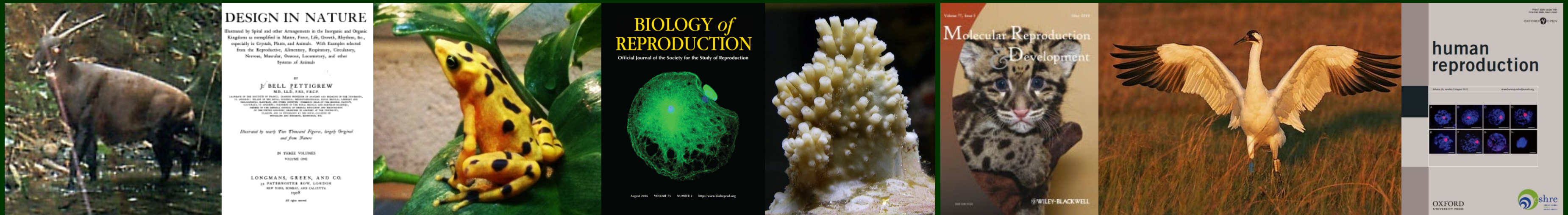
Comparative value of wild species and human fertility preservation

Work at the intersection of disciplines to develop new solutions

Keep understanding and mitigating cryo-damages in gametes and gonadal tissues

Stresses induced by dehydration vs. cryopreservation are different and might be easier to overcome

New opportunities and new challenges associated with the dehydration of cells and tissues for long-term storage at non-freezing temperatures



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THE ZOOLOGICAL PARK ORGANIZATION UNDER THE ROYAL PATRONAGE OF H.M. THE KING

Chulalongkorn University

THAILAND CLOUDED LEOPARD CONSORTIUM

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Turning Discovery Into Health

Leibniz Institute for Zoo and Wildlife Research