Artificial Insemination in Endangered Species – Challenges and Successes






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Objectives of this Lecture

- Importance of wildlife research
- Role of artificial insemination in management of endangered species
- Tools for understanding male and female reproductive biology
- Semen collection and cryopreservation
- Artificial insemination techniques
- AI successes in endangered species
- Remaining challenges
- Conclusions

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Wildlife Research Challenges

- Lack of knowledge
- Small, geographically dispersed collections
- Animals - timid to dangerous
- Requires genetic management to retain viability

Usually 90% gene diversity for the next 100 years

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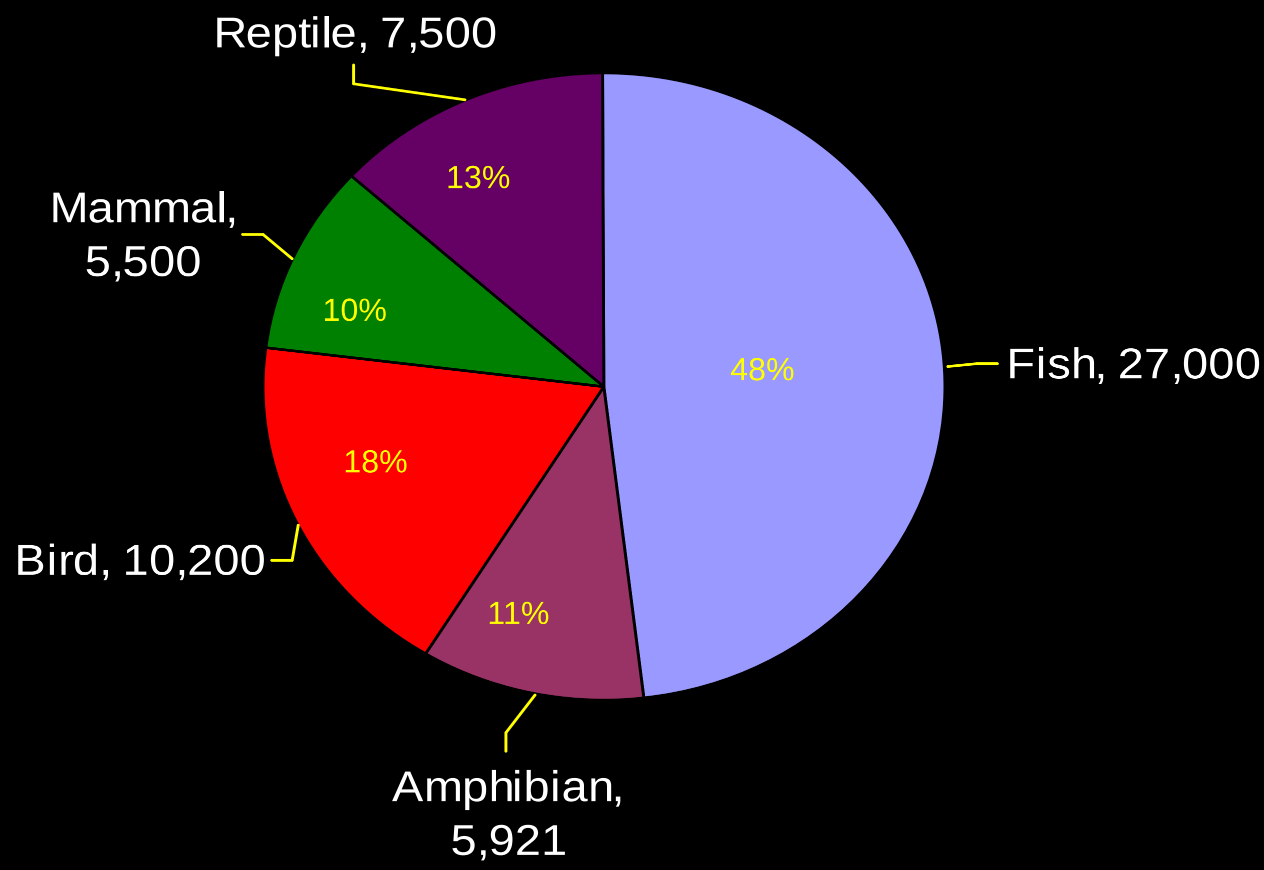
Numbers of Described Mammals, Reptiles, Fishes, Amphibians and Birds



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Amazing Diversity in Reproduction

< 1% of annual lifespan reproducing

Sperm storage (mo)

No males required

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Reproductive Diversity Among Species

Species	Seasonality	Distinguishing features
Domestic dog	None	• Spontaneous ovulation
Bush dog	None	• Male reduces inter-estrous interval
Maned Wolf	Strict (Oct – Feb)	• Ovulation only in presence of a male
Gray wolf	Strict (Jan – Apr)	• Behavioral dominance regulates reproductive success (alpha female)
African wild dog	Strict (Apr – Jul)	• Reproduction in alpha female, behavioral and estrogen suppression of subordinates • Litter size up to 20 pups
Island Fox	Strict (Jan – Mar)	• Ovulation only in presence of a male

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Number of Reproduction Publications for Wildlife Species, Jan 1999 - Sep 2009

Journals	Total reproductive science manuscripts	Wildlife - related (%)
Animal Reproduction Sciences	1,434	125 (8.7)
Biology of Reproduction	3,991	286 (7.2)
Cryobiology	197	58 (29.4)
Journal of Mammology	36	32 (88.9)
Molecular Reproduction and Development	1,598	53 (3.3)
Reproduction	1,604	149 (9.3)
Reproduction of Domestic Animals	680	31 (4.5)
Reproduction Fertility and Development	650	134 (20.6)
Theriogenology	1,852	259 (13.9)
Zoo Biology	76	69 (90.8)
Total	12,118	1,196 (9.9)

90% of publications are on common, already-studied species

Wildt et al., 2009

Taking this Basic Information to Affect Species Management

- Consistency
- Contributing to maintaining gene diversity while moving the 'population needle'

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Role of Artificial Insemination in Managing Wildlife Species

- Management tool for enhancing conservation breeding
  - Behavioral incompatibility
  - Poor reproductive performance
  - Anatomical defects
- Maintain genetic diversity in small populations
  - Low number of founders
  - Limited gene pool in population
- Movement of gametes for conservation strategies
  - Species Survival Plans (SSPs)
  - Genome Resource Banks (GRBs)

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Information Critical for Developing Artificial Insemination

Male

- Seasonality
- Age
- Semen collection
- Gene diversity and ejaculate quality
- Cryopreservation

Female

- Seasonality
- Age
- Ovulation mechanism
- Control of ovarian function
- Time of insemination
- Site of insemination
- Sperm concentration
- Impact of stress and/or anesthesia

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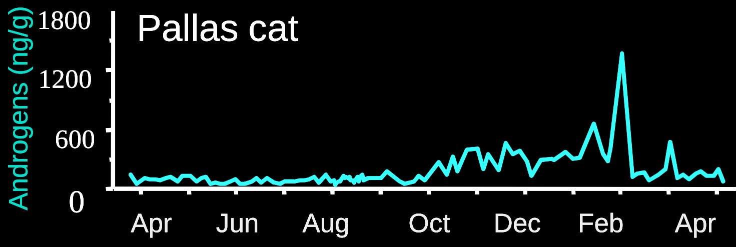
Male Reproduction

- Seasonality – Pallas cat (distinct seasonality), cheetah (seasonality not evident)
- Age – onset of puberty; impact on sperm DNA integrity
- Semen collection – electroejaculation; rectal massage (rarely artificial vagina)
- Low gene diversity – poor semen quality
- Cryopreservation – low post-thaw sperm survival



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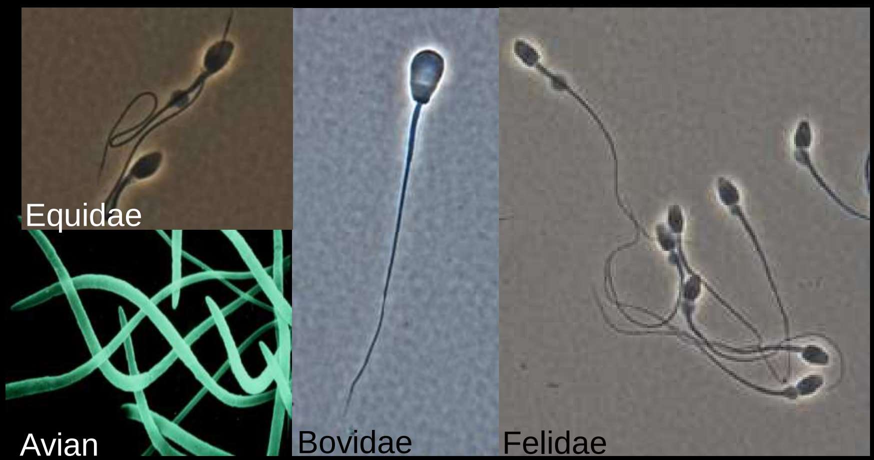
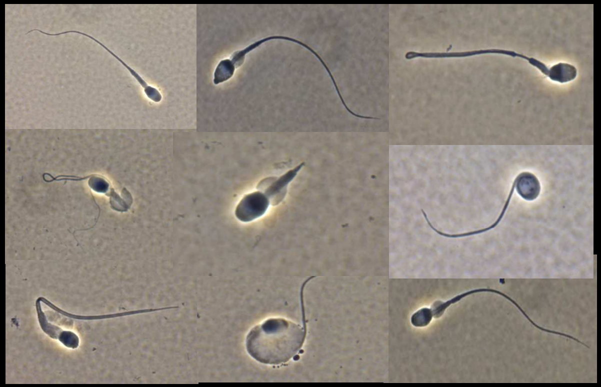
Major Challenges Working with Endangered Species Sperm

Diversity in sperm morphology

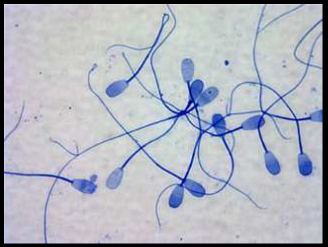


High incidence of abnormal sperm



Poor sperm cryosurvival

	Motility	Acrosome Integrity
Clouded leopard	~30%	~20%
Black footed ferret	~30%	~30%
Elephant	~50%	~60%



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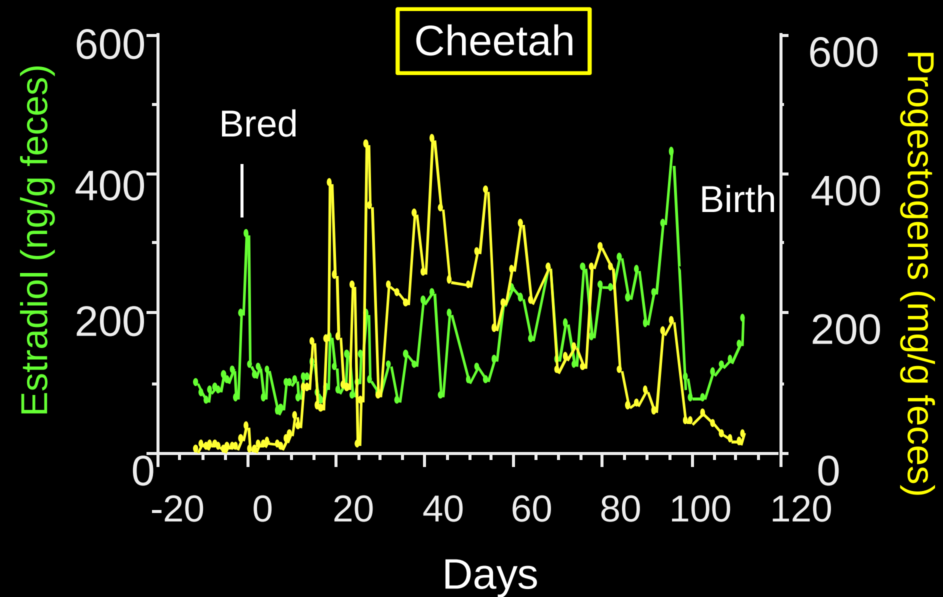
Tools for Understanding Female Reproductive Biology

- Non-invasive hormone monitoring (feces, urine, saliva, hair)
- Ultrasonography
- Laparoscopy



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Female Reproduction – Control of Ovarian Function

- Felids (eCG-hCG; eCG-GnRH; pFSH-pLH)
- Canids (GnRH agonist)
- Bovids (CIDR + $PGF_{2\alpha}$; $PGF_{2\alpha}$ at 12 d interval)
- Cervids (CIDR for 12-14 d +/- PMSG)
- Equids (Altrenogest + GnRH)





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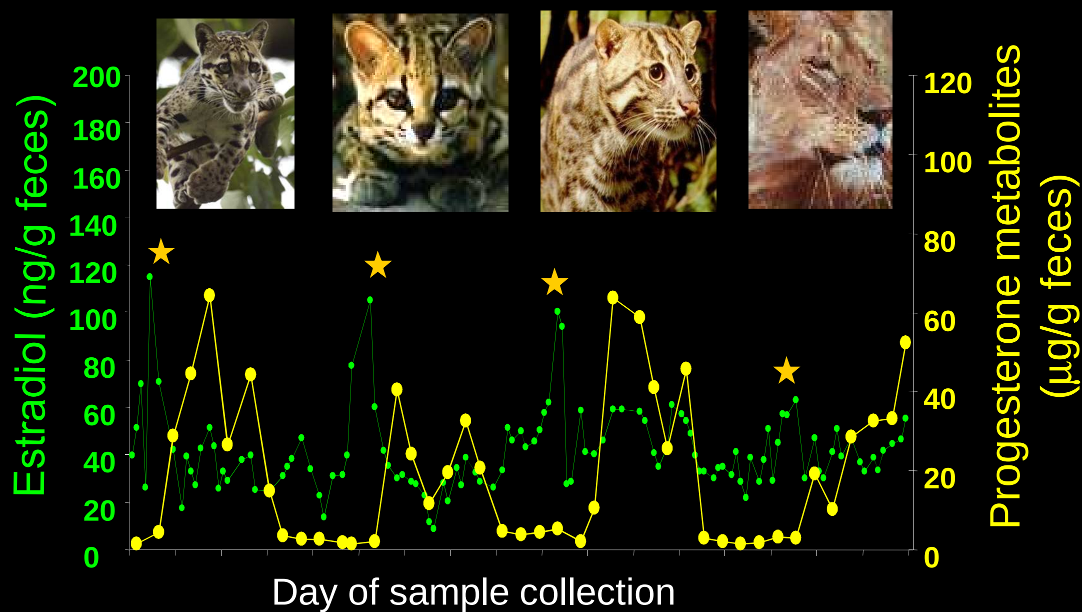
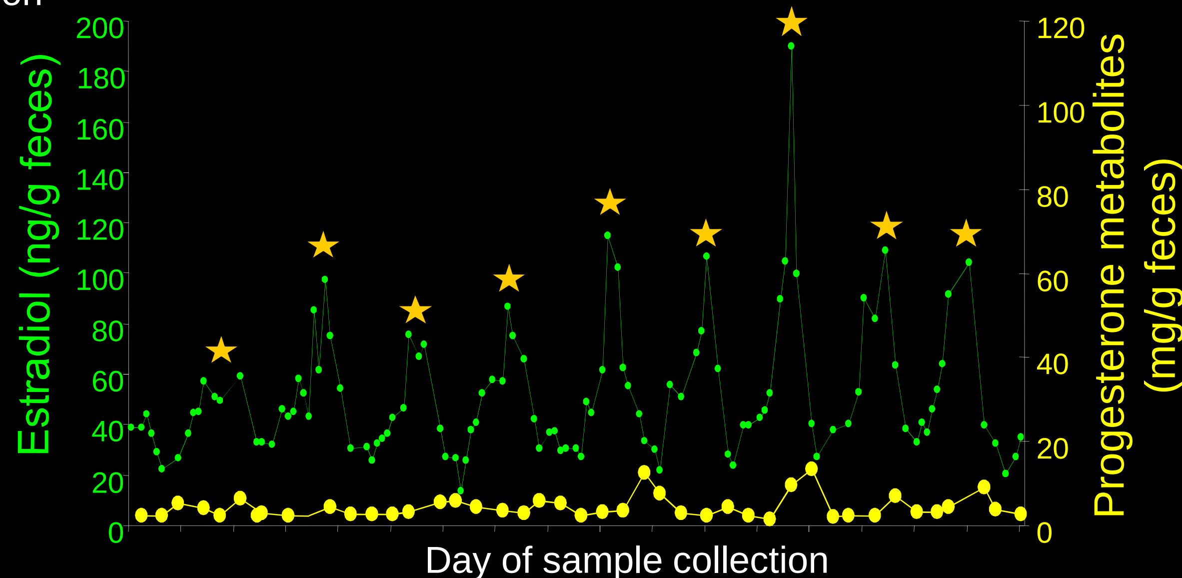
Ovulation Mechanisms



Spontaneous

Induced



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Sensitivity of Felids to Exogenous Gonadotropins

	Weight (kg)	eCG (iu)	hCG (iu)
Leopard cat	3	100	75
Domestic cat	4	100	75
Ocelot	9	500	225
Clouded leopard	15	75	50
Snow leopard	30	600	300
Puma	35	200	100
Cheetah	35	200	100
Tiger	250	1,000	750

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Diversity in Optimal Time of Insemination

After hCG

- Leopard cat (36-38 h)
- Ocelot (39-43 h)
- Cheetah (44-48 h)

Follicle Diameter +/- GnRH

- White rhinoceros (>26 mm)
- Indian rhinoceros (>130 mm)

White rhinoceros
(Hermes et al., 2009)

Scimitar horned oryx
(Morrow et al., 2000)

After 2nd $PGF_{2\alpha}$

- Mhorr gazelle (48-60 h)
- Scimitar-horned oryx (56-72 h)
- Gerenuk (66-96 h)
- Blackbuck (72-96 h)

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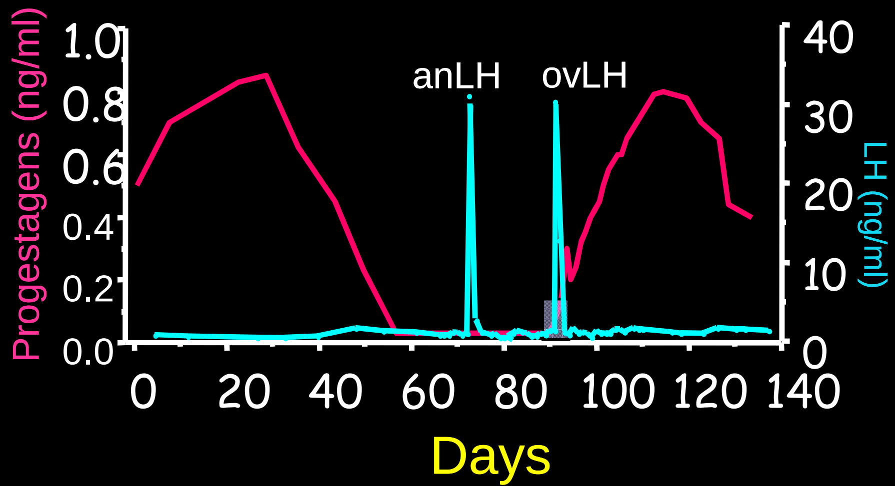
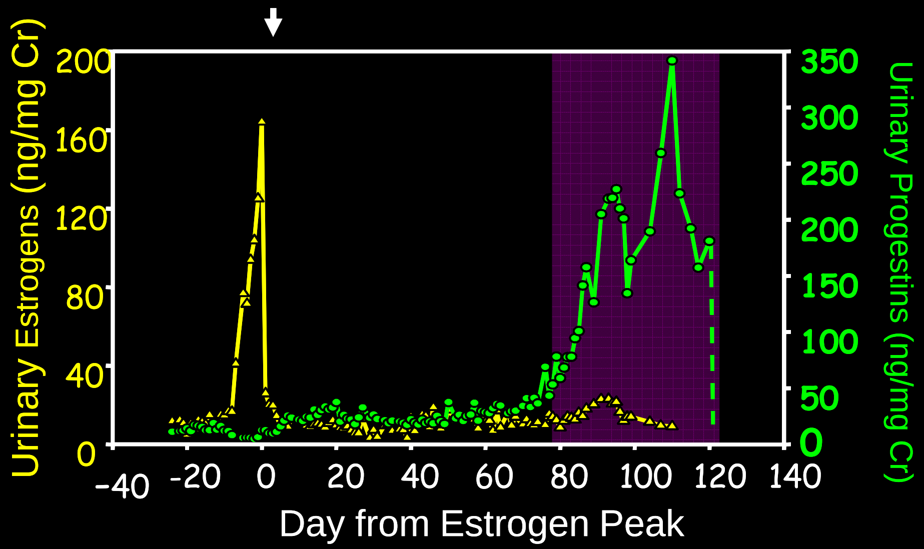
Hormone Profile as Cue for Insemination Timing





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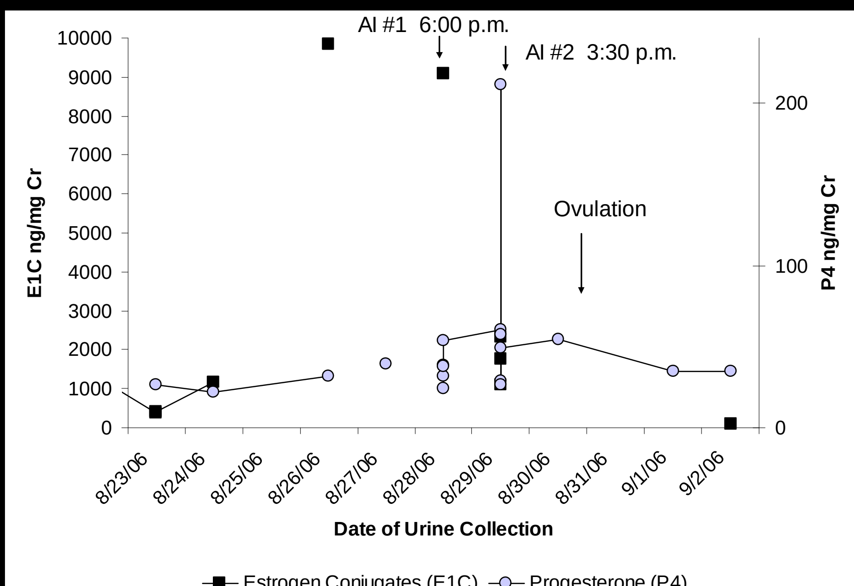
Integration of Hormone Monitoring/ Ultrasonography and AI

- Elephant
- Marine mammals
- Rhinoceros
- Equids
- Wolves



(Stoops et al., 2007)

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Insemination Techniques

Urogenital Sinus Insemination

(Allen et al., 2008)

Endoscopic Cervical/Intrauterine Insemination

(Hildebrandt et al., 1998)

Laparoscopic Intrauterine Insemination

(Howard et al., 1992)

Intramagnal-Intraovarian Pocket Insemination

(Blanco et al., 2009)

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Other 'Consistent' Species

Cheetah

(Howard et al., 1997)

Ocelot

(Swanson et al., 2009)

Giant panda

(Howard et al., 2009)

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Other ‘Consistent’ Species

Eld’s deer

(Monfort et al., 1993)

Scimitar-horned oryx

(Morrow et al., 2000)

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Other 'Consistent' Species

Koala

(Allen et al., 2008)

White rhinoceros

(Hermes et al., 2009)

Elephant

(Saragusty et al., 2009)

25 calves produced by AI

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Repeated AI successes

Killer whale

Bottlenose dolphin

Pacific white-sided dolphin

Robeck, O'Brien, Steinman
Busch Entertainment Corporation's SeaWorld and Busch Gardens

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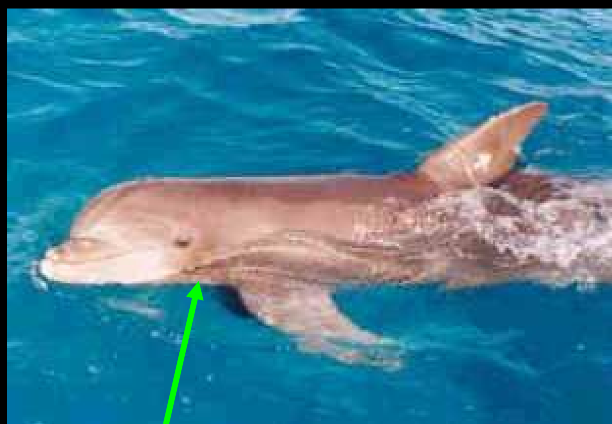
Repeated AI successes

Killer whale



Bottlenose dolphin

Calves from sex-sorted sperm

Pacific white-sided dolphin

Robeck, O'Brien, Steinman
Busch Entertainment Corporation's SeaWorld and Busch Gardens

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AI Successes in Marine Mammals

Species	Live Offspring Produced
Killer Whale (*Orcinus orca*)	4
Bottlenose Dolphin (*Tursiops truncatus*)	11*
Pacific White-Sided Dolphin (*Lagenorhynchus obliquidens*)	5
Beluga Whale (*Delphinapterus leucas*)	3

* Six calves with sex sorted sperm

Robeck, O'Brien, Steinman
Busch Entertainment Corporation's SeaWorld and Busch Gardens

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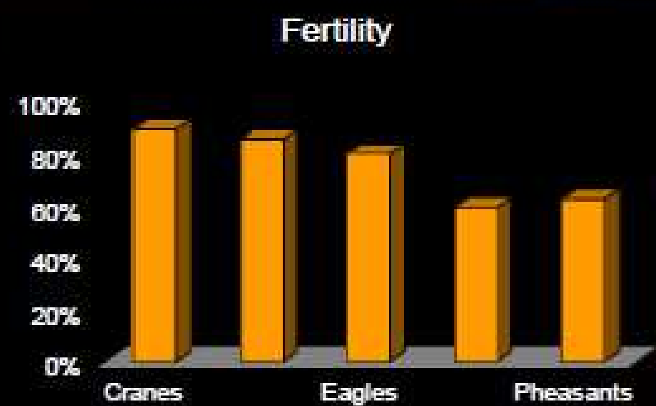
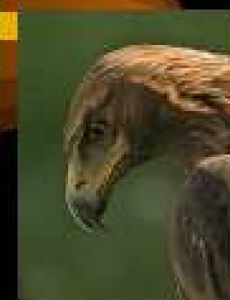
AI Success in Birds

- *Using FRESH SPERM, fertility can be as high as in natural mating, and even higher*

Gee and Mirande, 1996





Grier et al, 1995; Blanco, 2007

Bird et al, 1976

Blanco 2007

Saint Jalme et al, 1996

Durrant et al, 1995

Consistently Benefiting from Reproductive Science

Whooping crane
(G. Gee, G. Archibald)

White-naped crane
(W. Lynch)

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Artificial Insemination in Birds
(Species Managed with Artificial Insemination)

- Houbara bustard
- Whooping crane
- Hispaniola parrot
- Philippine eagle
- Spanish imperial eagle
- Bonelli's eagle
- Peregrine falcon
- Kiwi (free-ranging)

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Challenges/Opportunities

- Low numbers of animals
- Optimize methods to control ovarian function
- Improve sperm collection and cryopreservation technologies
- Develop novel tools for insemination
- Optimize conditions for maintaining pregnancy
- Pregnancy diagnosis

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