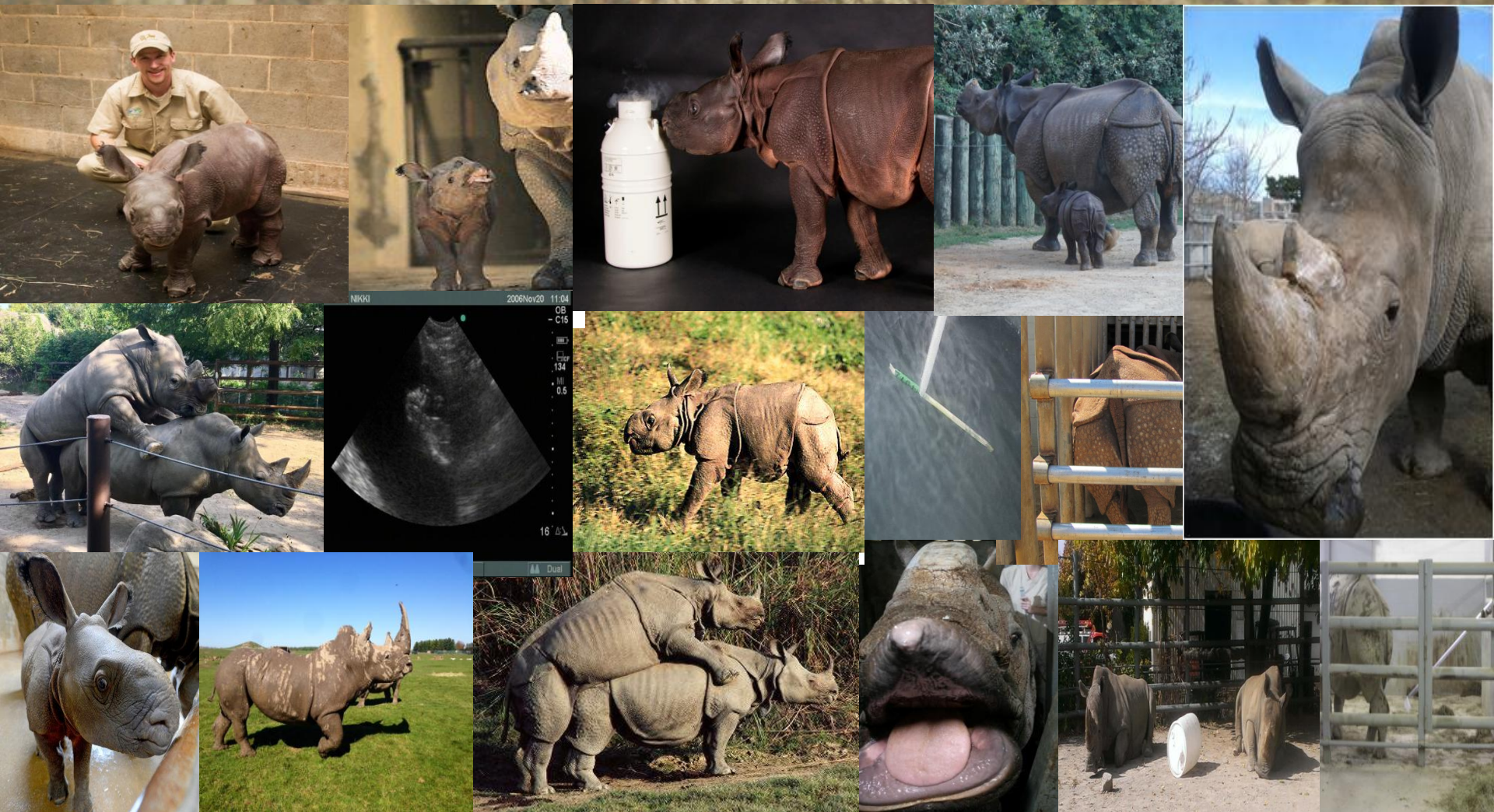


State of ART in Rhinoceroses

Greater One-Horned/Indian Rhinoceros

African White Rhinoceros



NIKKI
2006Nov20 11:04
OB
- C16
16' Δ

AA Dual



Cincinnati Zoo & Botanical Garden
Center for Conservation and Research of Endangered Wildlife
Saving Species With Science®



Rhinoceros Taxon Advisory Group Rhino Research Council

Reproduction

Five-Year Research Priorities

❖ Investigate major factors affecting rhino reproduction in captivity

- Acyclicity
- Anovulation
- Embryo loss
- Stillbirth
- Pathology
- Phyto-Estrogens

Assisted Reproductive Techniques (ART)

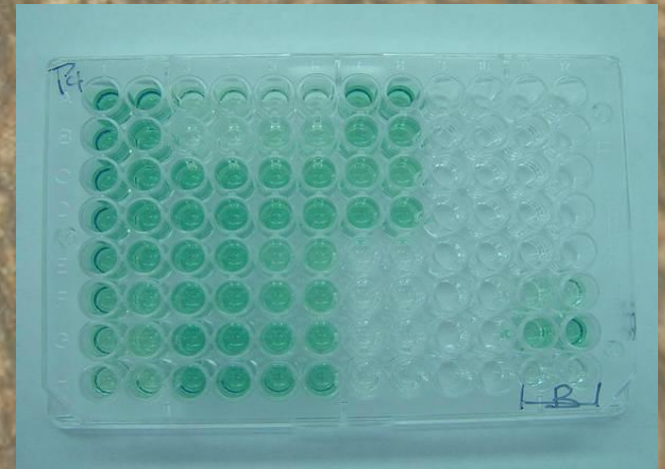
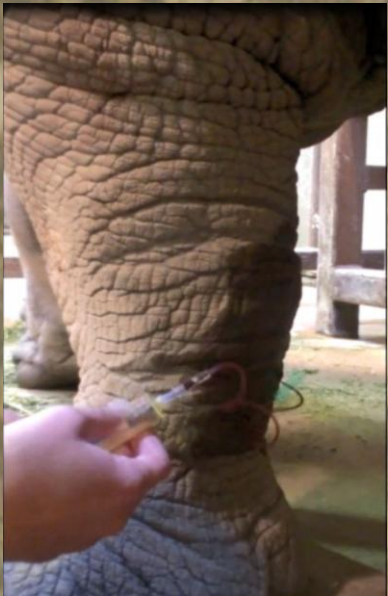
- Endocrine analysis
- Ultrasonography
- Semen collection/cryopreservation
- Artificial insemination (AI)
- Exogenous hormone administration

Endocrine Analysis

GOH: Variable estrous cycle length 28-60 days

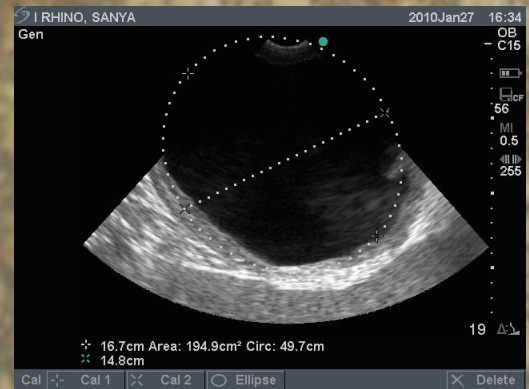


African White: Variable estrous cycle length of 30 or 70 days



Ultrasonography

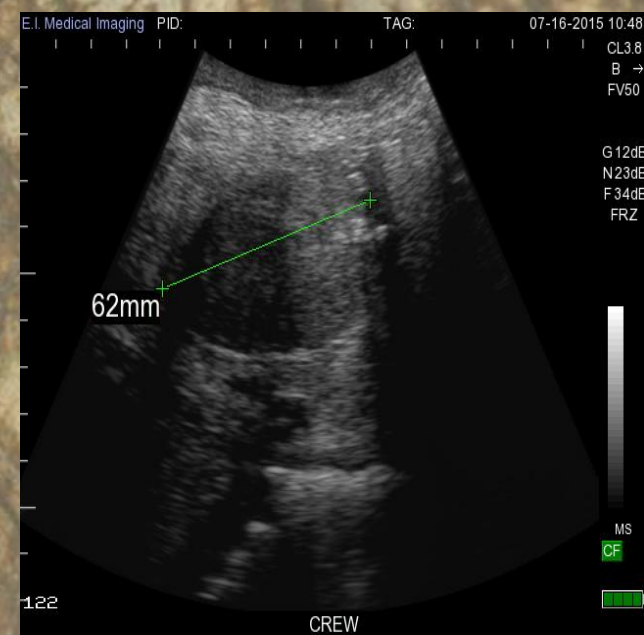
GOH: Growth of very large sustained pre-ovulatory follicles >10cm; 14 day follicular phase

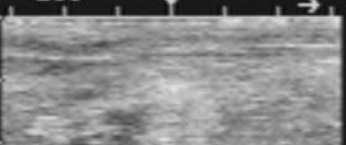


Development of reproductive pathology (leiomyomas)



African White: Follicular growth similar to domestic mare; 9 day follicular phase.





Gen

OB
- C15

134

MI
0.5

13

Invert

2009Sep23 09:57

OB
- C15

78

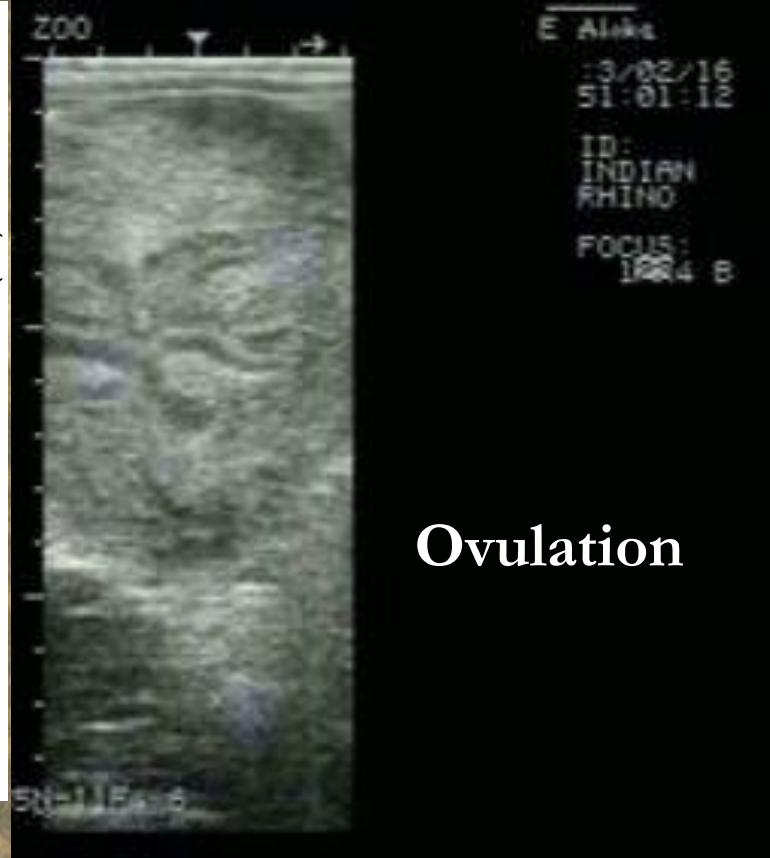
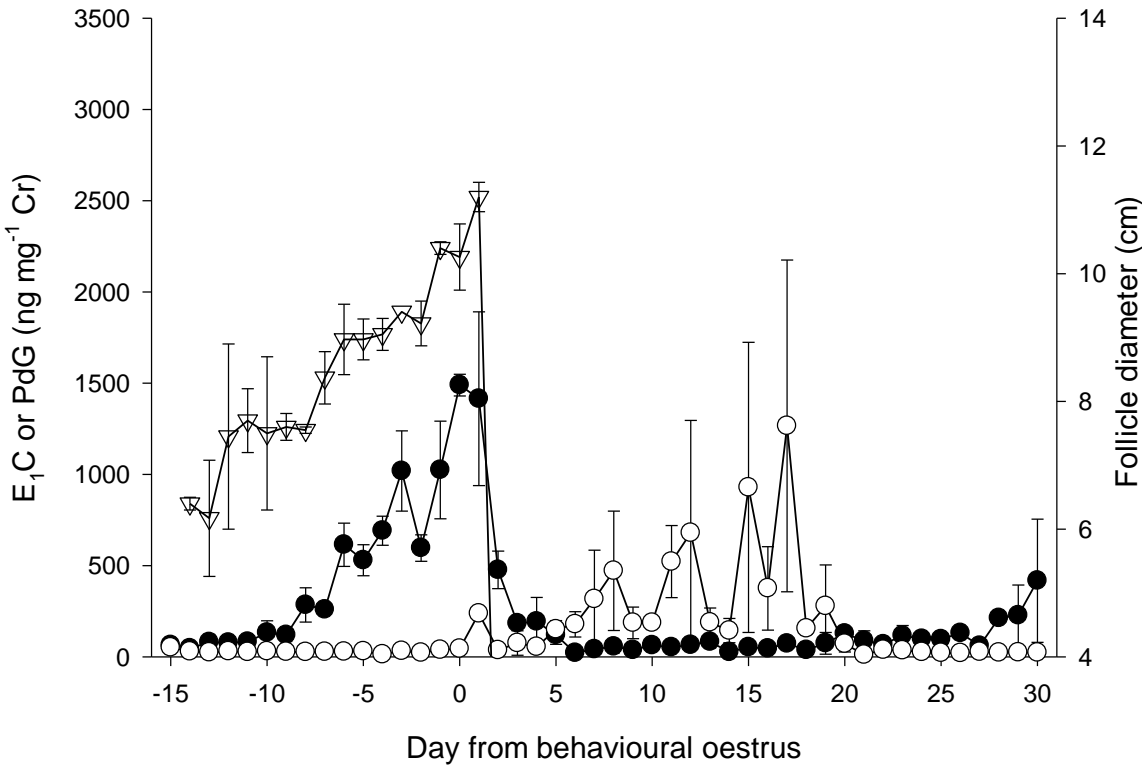
MI
0.5

19

MEASURE
B-1 CLEAR

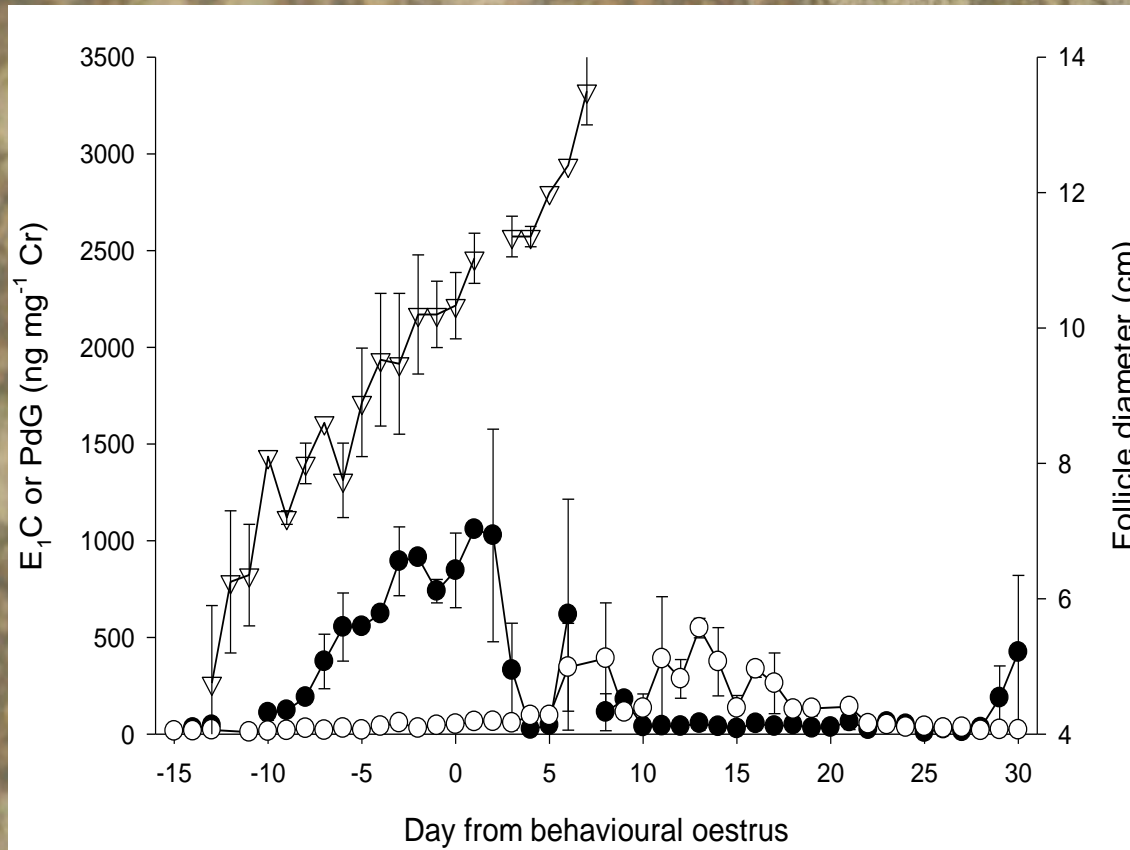
GOH RHINO
Gen

Ovulatory Estrous Cycles



- Oestrogen conjugates (E₁C)
- Progesterone metabolites (PdG)
- ▽ Follicle diameter (cm)

Anovulatory Estrous Cycles



- Oestrogen conjugates (E₁C)
- Progesterone metabolites (PdG)
- ▽ Follicle diameter (cm)

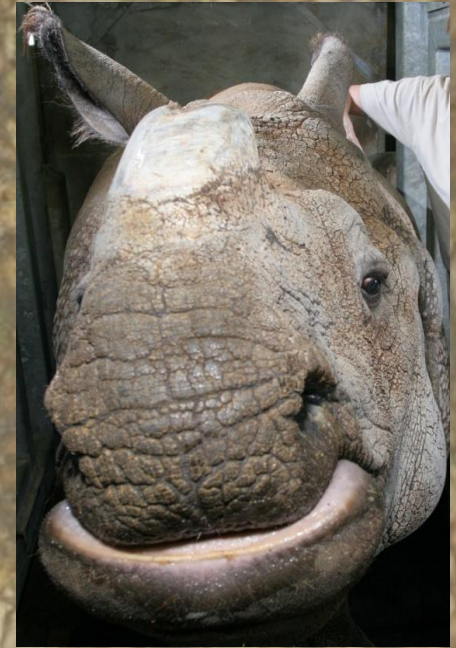


Male Ultrasound



Rectal/Penile Massage...

Semen Collection



- Electroejaculation
- Urethral catheterization
- Cryopreservation



Gamete Rescue

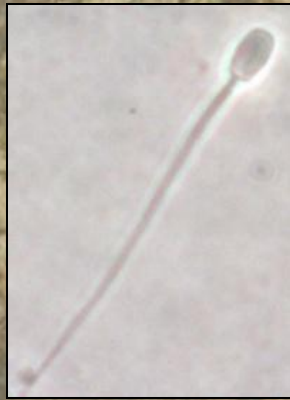
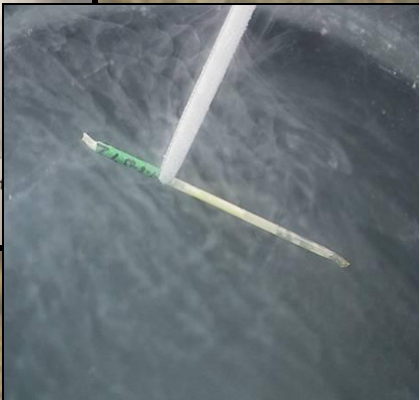


Table 1. Reproductive characteristics of male Indian rhinoceros ($n = 6$) whole ejaculates ($n = 10$) obtained by electroejaculation.

Parameter	Mean \pm s.e.m	High	Low
Semen volume (mL)	158.6 \pm 32.86	337.5	13.0
Sperm motility (%)	47.97 \pm 9.42	83	0
Sperm progressive motility status (0-5)	2.7 \pm 0.36	3.5	0
pH	8.74 \pm 0.05	9.0	8.5
Normal sperm morphology (%)	38.8 \pm 7.79	83	19
Primary defects (%)	0.83 \pm 0.18	1.0	0.3
Secondary defects (%)	60.38 \pm 7.9	81	17
Sperm concentration ($\times 10^8$ mL ⁻¹)	10.78 \pm 4.22 ^a 10.11 \pm 3.54 ^b	34.96 ^a 34.96 ^b	1.54 ^a .005 ^b
Total sperm per ejaculate ($\times 10^8$)	304.28 \pm 40.9 ^a 273.9 \pm 47.56 ^b	532 ^a 532 ^b	171.22 ^a 46.5 ^b
Motile sperm per ejaculate ($\times 10^8$)	207.51 \pm 45.45 ^a 166.01 \pm 45.29 ^b	478.8 ^a 478.8 ^b	98.61 ^a 0 ^b
Viability (%)	89.5 \pm 1.99	95	84
Osmolality (mmol kg ⁻¹)	308.04 \pm 4.793	410	272
Testosterone (ng mL ⁻¹)	2.08 \pm .355	4.0	0.9
Age at collection (yrs)	17.5 \pm 2.97	34	7.5
Number of stimuli	120.3 \pm 8.6	150	70
Number of series	3.9 \pm .277	5	2
Number of semen fractions	10.9 \pm 1.21	18	6
Time of procedure (min)	36.9 \pm 3.57	60	14

^a values represent the exclusion of $n = 2$ ejaculates that did not meet criteria for cryopreservation.

^b values represent the inclusion of $n = 2$ ejaculates that did not meet criteria for cryopreservation.



Table 2. Characteristics of male Indian rhinoceros ($n = 6$) fresh ejaculate fractions ($n = 8$) obtained by electroejaculation and subsequently processed for cryopreservation.

Parameter	Mean \pm s.e.m	High	Low
Semen fraction volume (mL)	25.69 \pm 4.05	55	4
Sperm motility (%)	78.80 \pm 3.31	90	50
Sperm progressive motility status (0-5)	3.45 \pm 0.13	4.0	2.5
pH	8.71 \pm 0.08	9.0	8.5
Normal sperm morphology (%)	56.2 \pm 7.56	88	19
Primary defects (%)	0.3 \pm 0.14	1	0
Secondary defects (%)	43.5 \pm 7.57	81	11
Sperm concentration ($\times 10^8$ mL ⁻¹)	5.16 \pm 1.61	20.16	1.25
Total spermatozoa per ejaculate fraction ($\times 10^8$)	84.02 \pm 13.46	194.72	36.8
Motile sperm per ejaculate fraction ($\times 10^8$)	66.49 \pm 1.02	138.22	18.4
Sperm viability (%)	90.4 \pm 2.03	97	80
Osmolality mmol/kg	309.75 \pm 9.13	347	273

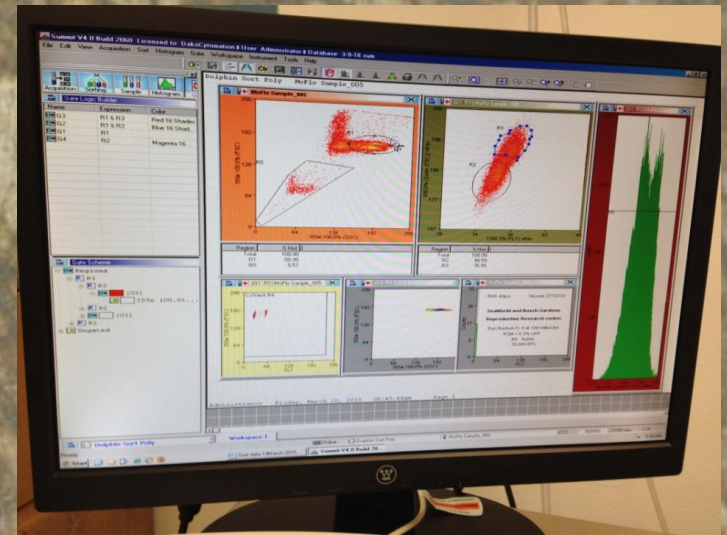
SEAWORLD PARKS & ENTERTAINMENT®



O'Brien et al., _____

•Sperm Sorting and Directional Freezing

34-160 x 10⁶ X-sperm sorted/frozen per ejaculate



Artificial Insemination

General Anesthesia

3.5-3.8mg etorphine + 12-14mg detomidine + 300-400mg ketamine

n = 4 post-ovulatory (SB238)

n = 5 pre-ovulatory (n=4 SB238; n=1 SB189)



No Anesthesia; Operant Conditioning

endoscopic insertion
 $n = 9$ ($n=6$ SB238; $n=3$ SB189)



manual I
 $n = 12$ (



Standing Sedation Manual Insertion

$n=4$ ($n=3$ SB274; $n=1$ SB241)
Butorphanol + Azaperone

Female	AI Procedure	Butorphanol (mg)	Azaperone (mg)
274- Montgomery	1	80	75
274- Montgomery	2	100/150	75/105
274- Montgomery	3*	140	75
241- Buffalo	1*	160	100



Summary of Indian rhinoceros (*n* = 4 females) AI procedures

Level of Sedation	Insemination Technique	Ovarian Response	Number of AI's	Conceptions	Term Pregnancies
General Anesthesia	Varied ^a	Ovulatory	8	0	0
		Anovulatory	1	n/a	n/a
No Sedation	Endoscope ^b	Ovulatory	9	2	0
		Anovulatory	0	n/a	n/a
	Manual ^c	Ovulatory	7	2	2
		Anovulatory	5	n/a	n/a
Standing Sedation	Manual ^d	Ovulatory	3	2	2
		Anovulatory	1	n/a	n/a

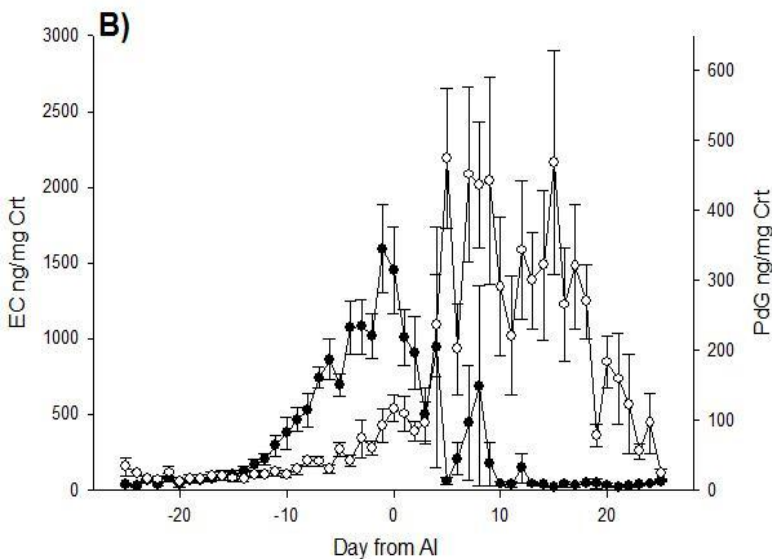
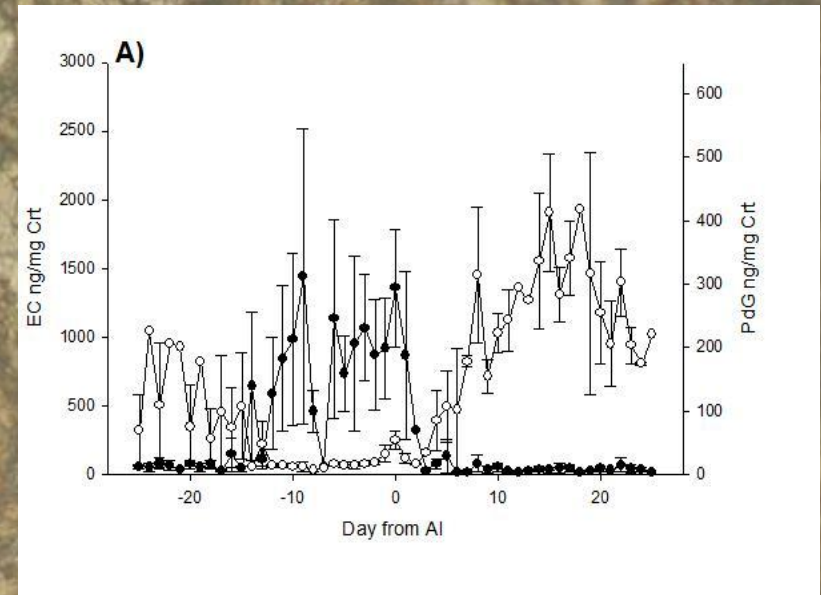
^aMethod 1; ^bMethod 2; ^cMethod 3; ^dMethod 4

Conceptive Procedures $n = 6$

46.5 ± 3.4 d (range 32-54)

26 ± 11.8 h (range 12-48)

$755.8 \pm 384.8 \times 10^6$ total motile (500-1533)



Non-Conceptive Procedures $n = 21$

46.2 ± 1.1 d (range 39-59)

66 ± 80.7 h (range 0-312)

$1356.6 \pm 927.6 \times 10^6$ total motile (564-2558)

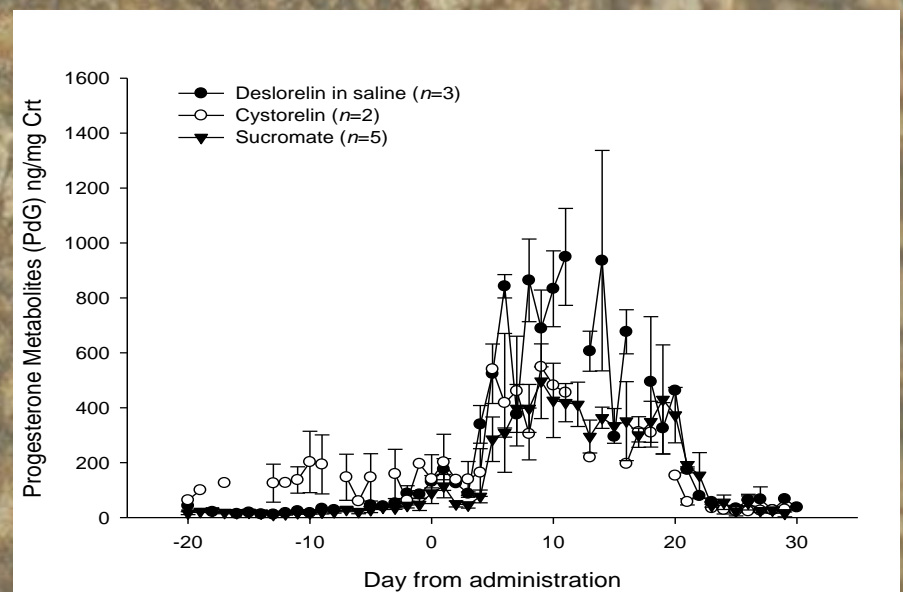
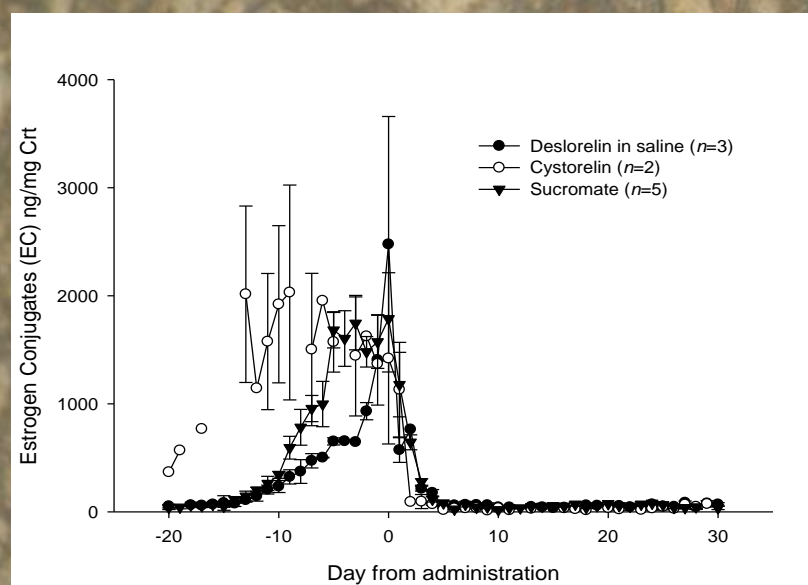
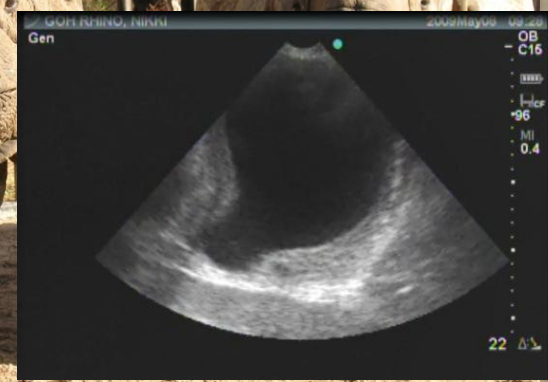


Improving assisted reproduction in the Indian rhino

- Indian rhino AI currently requires intensive monitoring
- Many females have anovulatory cycles
- GOAL: Improve chances for successful AI
 - Rhinos at institutions where intensive monitoring isn't possible
 - Help ensure that the rhinos will ovulate

Ovulation Induction

- Cystorelin
 - 500ug IM
- Deslorelin (in saline)



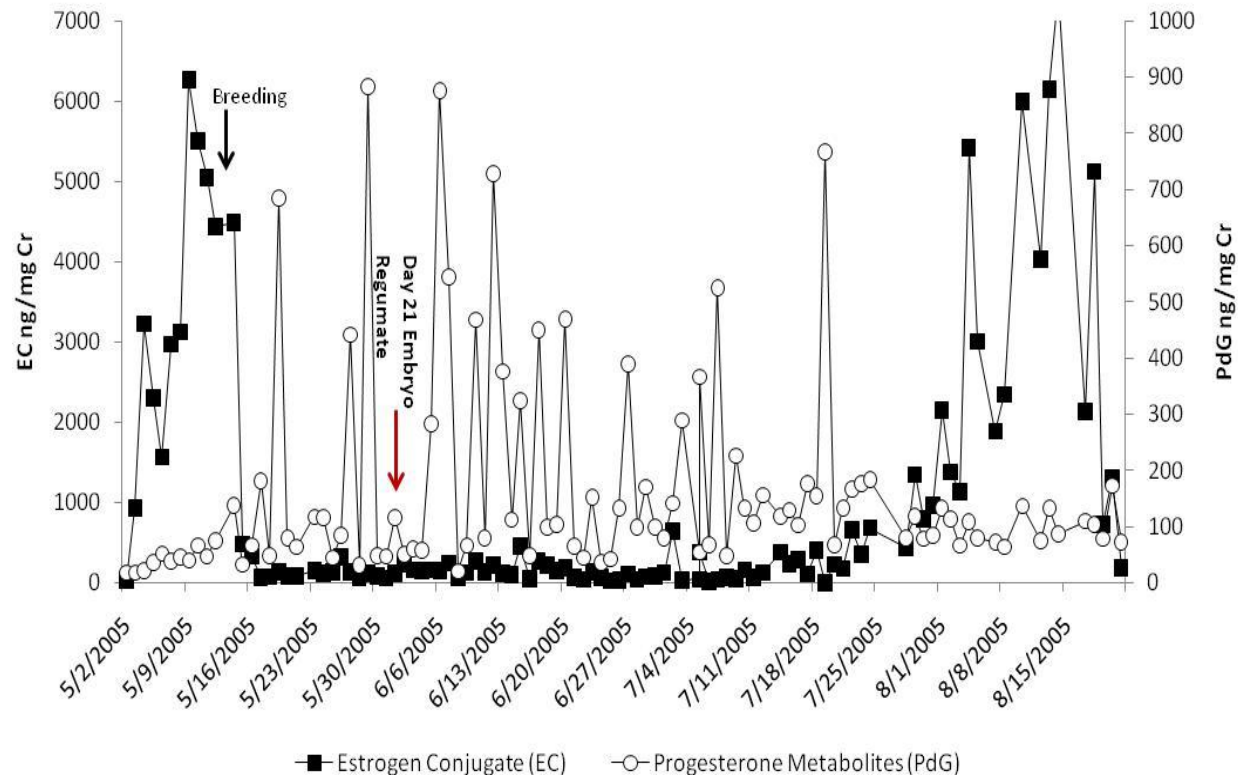
Supplemental Progesterone: Regu-Mate

Standard equine dosage = .044 mg/kg

GOH Rhino: mean gestation length = 480 days

Day 445 begin reduction (2mL/day)

Day 465 no longer receive supplement

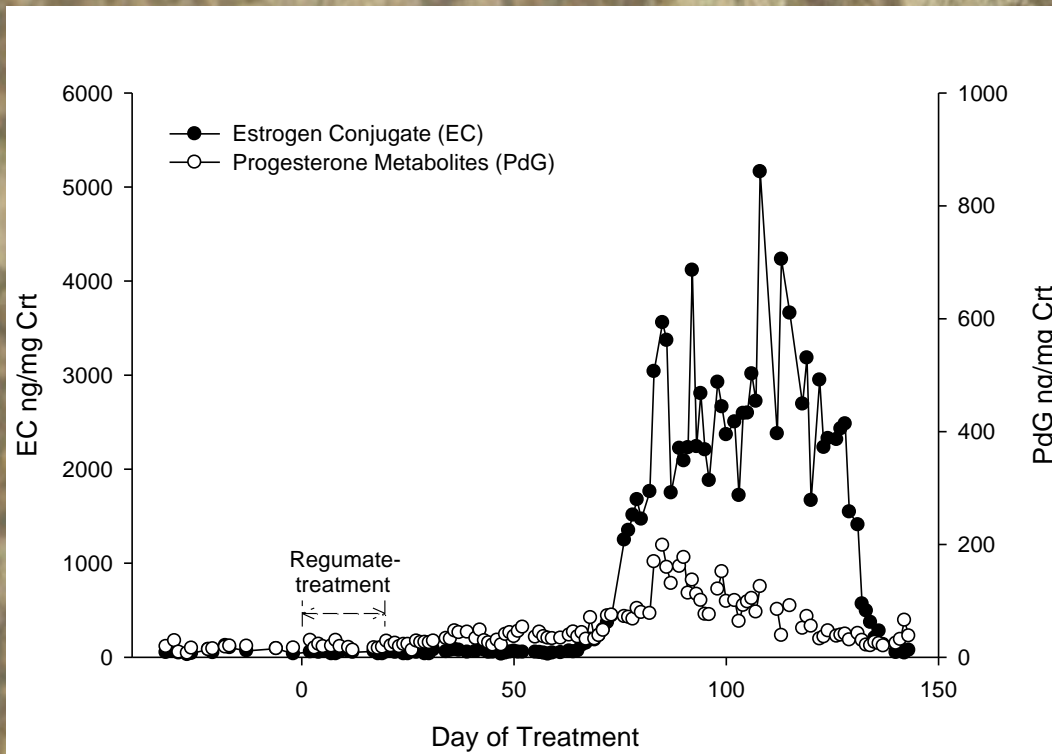


Oral Progesterone: Regu-Mate

Administration to stimulate reproductive cyclicity?

GOH Rhino: luteal phase 17-21 days

- standard equine dose 21 days

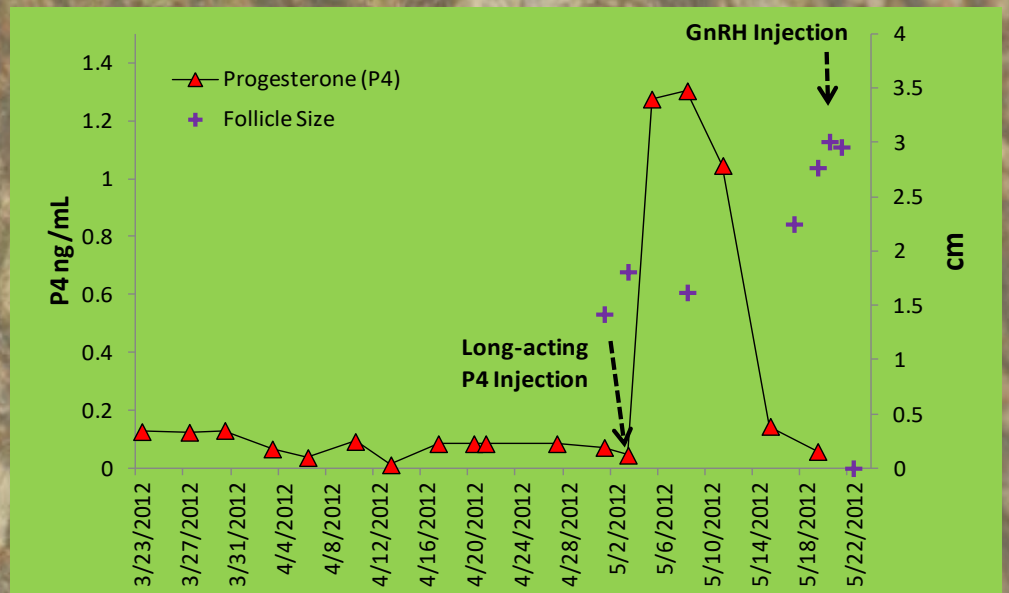
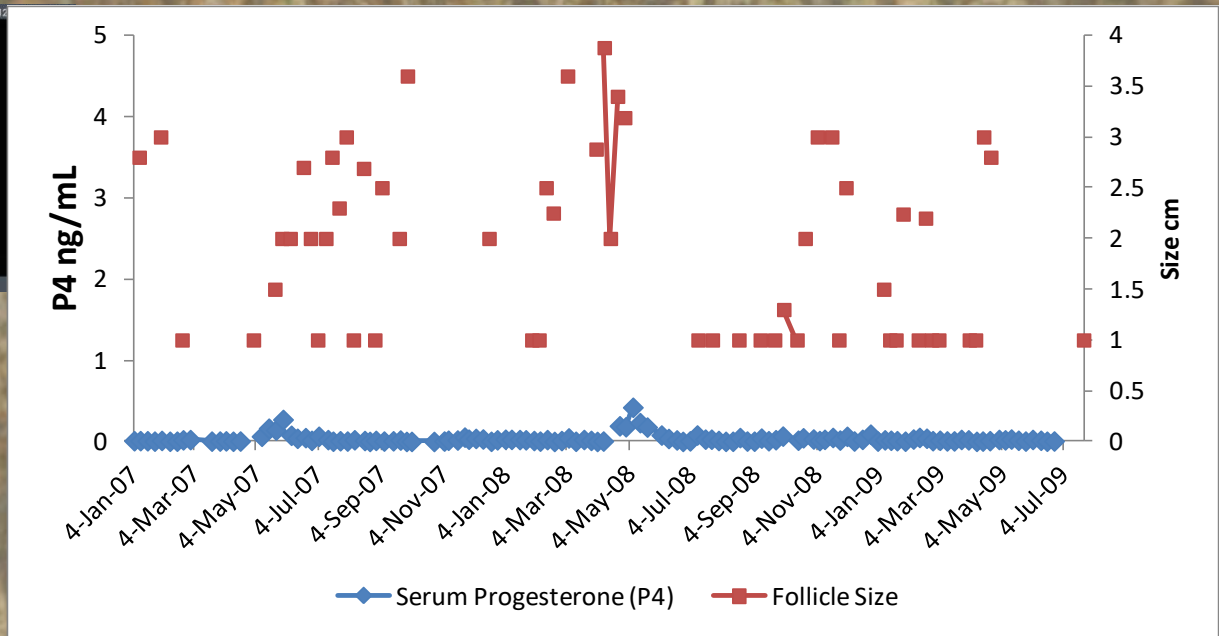


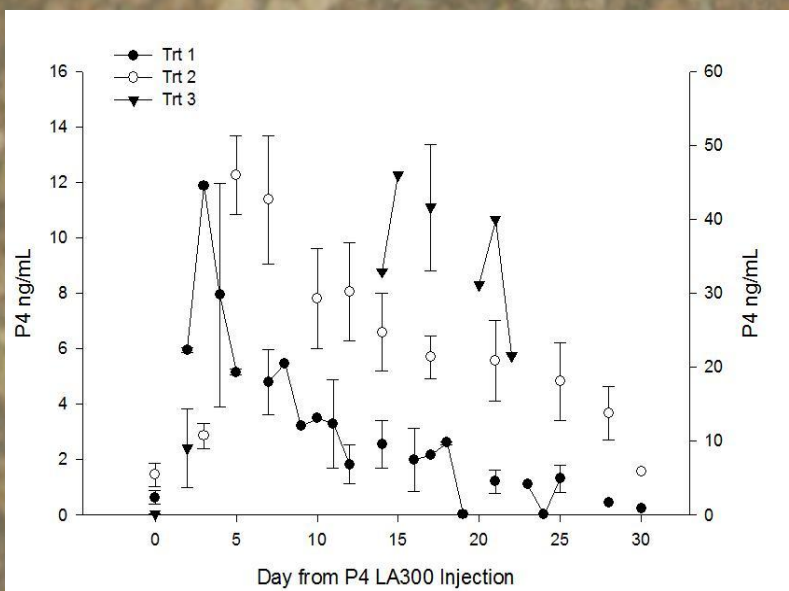
Day -4 from treatment



Day 10 of treatment







Animals

n= 5 anestrous white rhinos
 n=1-5 replicate treatments per female

Long-Acting Progesterone Administration

- Trt 1 3000mg + 1.5" needle
- Trt 2 4500mg + 1.5" needle
- Trt 3 3000mg + 3.5" needle

Ovulation Induction Agent GnRH (Cystorelin or Factrel)

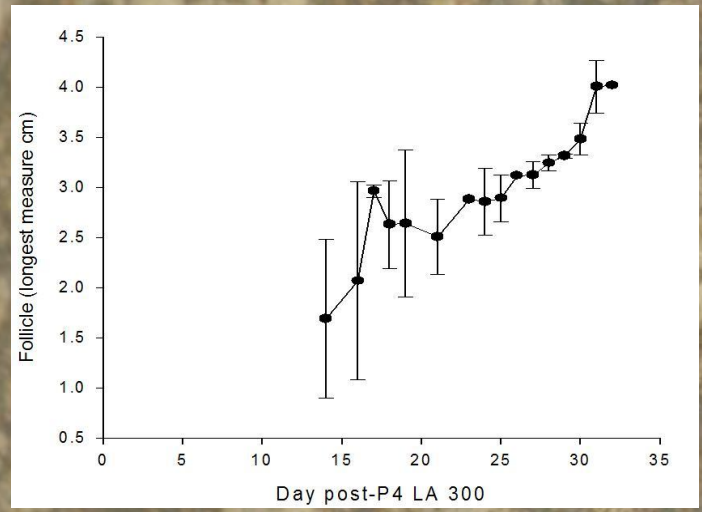
3.27 ± 0.2cm (range 3.01 – 3.6cm)

Subsequent estrous cycles occurred at 31.3 ± 4.0 (n=7)
 or 61.7 ± 10.8 (n=7) days post-GnRH.

OR

Short-Acting Estradiol

3.4cm



ID. No. : ■
Sex: Age:
D. O. Birth:

Name :

01/01/2000
00:14:36

CVP:
D. F:
Et: 1 Gr: N



Physician :
Comment :



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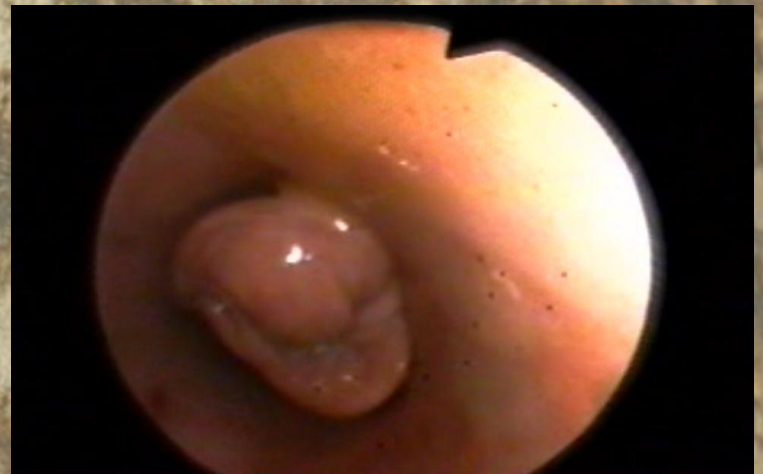
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01/01/2000
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CVP:
D. F:
Et: 1 Gr: N



Physician :
Comment :





Questions?

