The Hunt for a Ruminant Pregnancy Test

Melanie Richter
mrichter@memphiszoo.org
Research Scientist, Memphis Zoo

Why is a pregnancy test difficult?

- Current methods:
  - Fecal progesterone
    - Non-invasive
    - Species specific patterns?
    - Longitudinal: need many samples over time
    - Susceptible to false positive/pseudopregnancy

Giraffe: A *well-studied* species

- High levels of variation
- Obvious elevation occurs late in pregnancy
- Variation in what elevated levels are (80,000ng/g vs 100,000ng/g)

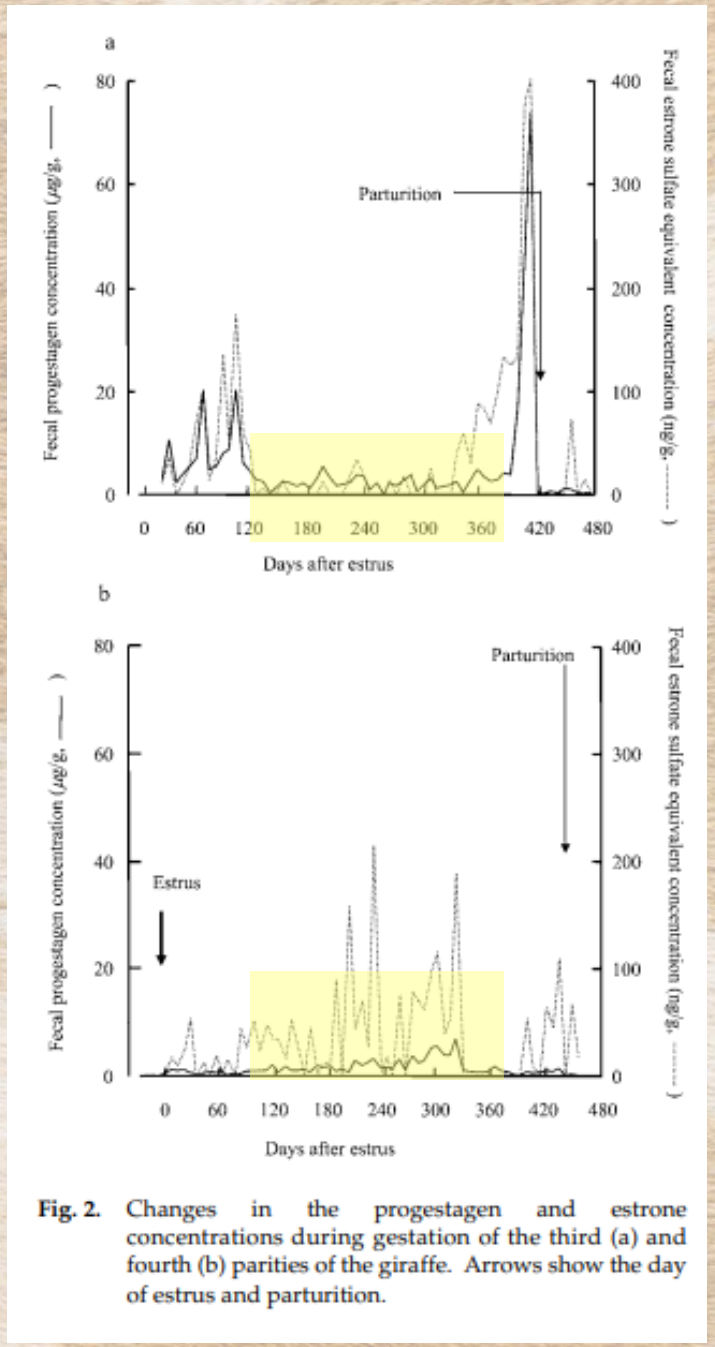


Fig. 2. Changes in the progestagen and estrone concentrations during gestation of the third (a) and fourth (b) parities of the giraffe. Arrows show the day of estrus and parturition.

Isobe et al., 2007

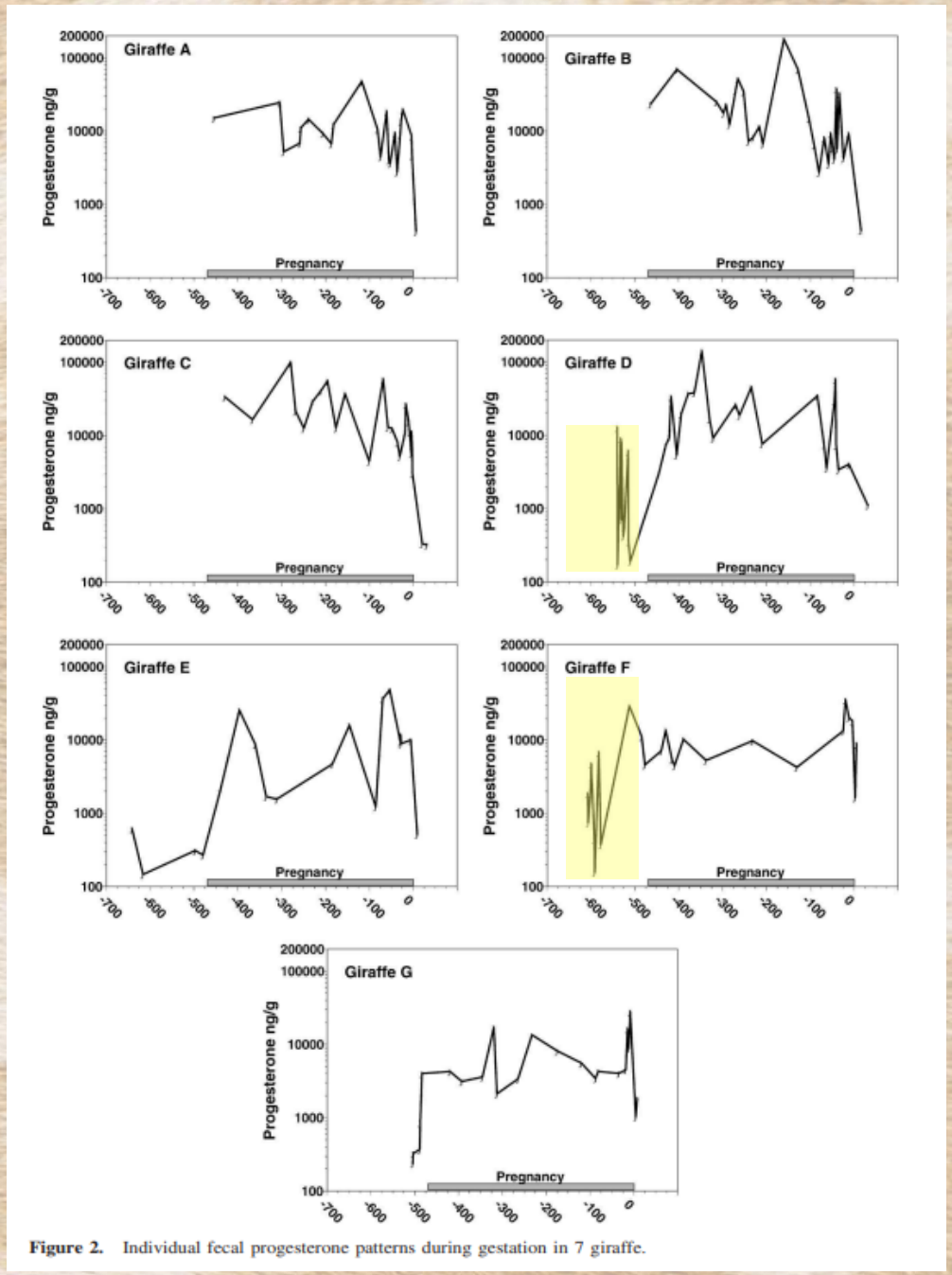


Figure 2. Individual fecal progesterone patterns during gestation in 7 giraffe.

Dumonceaux et al., 2006

OTHER SPECIES DATA:

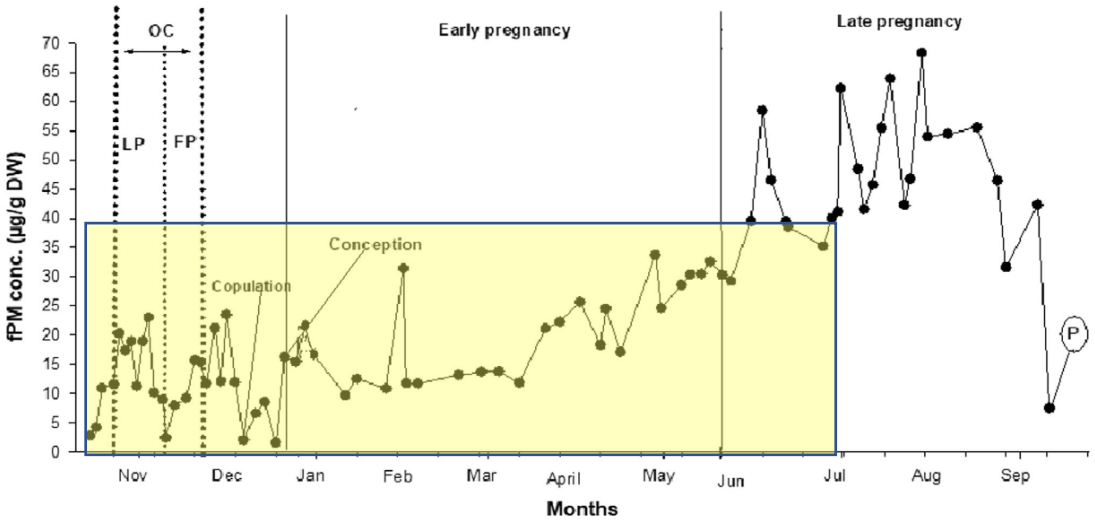
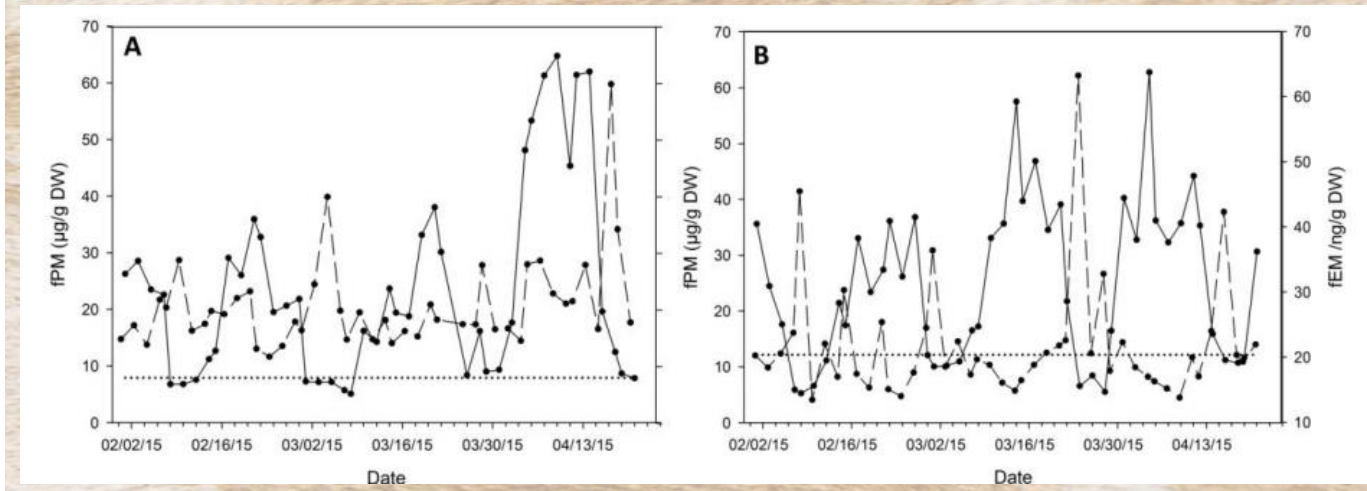


Figure 3 Longitudinal profile of faecal progestogen metabolite concentrations during oestrous cycle and pregnancy in female roan antelope. FP, follicular phase; LP, luteal phase; OC, oestrous cycle; P, parturition.



Single individual longitudinally sampled. Roan antelope (*Hippotragus equinus*). Kamgang et al 2023.

Fecal estrogen (dashed line) and progestogen (solid line) for 2 non-pregnant female Beira antelope (*Dorcatragus meglaotis*) Wolf et al., 2019.

For each new species of interest you would need to start by doing a longitudinal study of multiple individuals to build up typical ranges for pregnant and non-pregnant individuals.

WHY IS A PREGNANCY TEST DIFFICULT?

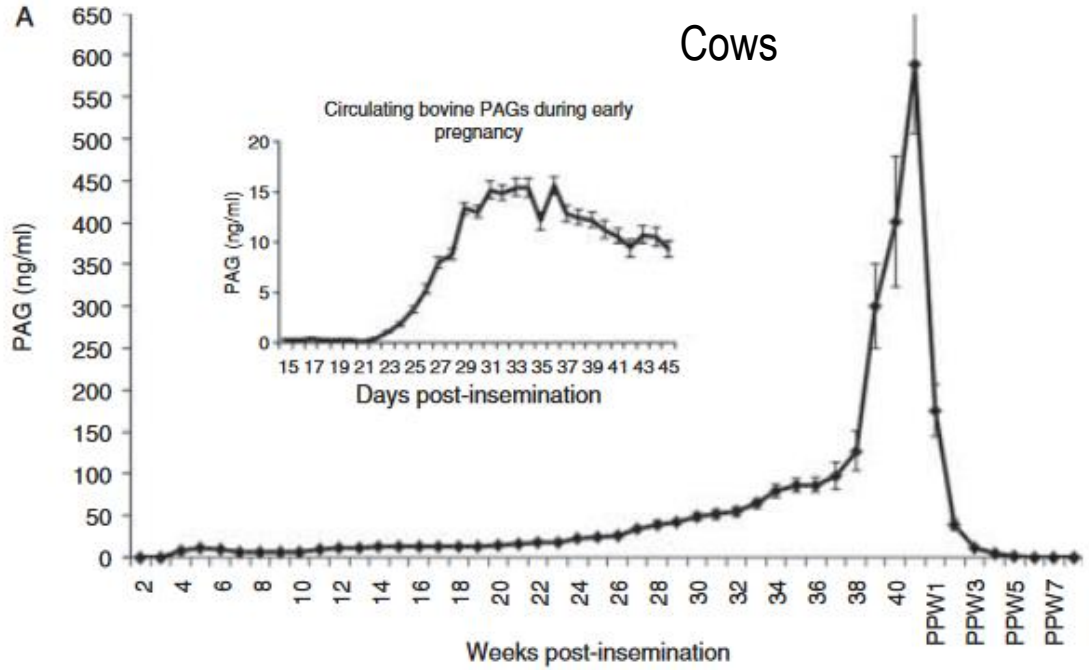
- Current methods:
  - Fecal progesterone
    - ✚ Non-invasive
    - ▬ Species specific patterns?
    - ▬ Longitudinal: need many samples over time
    - ▬ Susceptible to false positive/pseudopregnancy
  - Ultrasound
    - ✚ Clear, definitive result
    - ✚ Non-invasive (?)
    - ▬ Requires behavioral training and cooperation or knock-down
    - ▬ Requires a trained vet/staff

Punk the giraffe from Abilene Zoo, 2020.

MY SOLUTION: PAG

- Pregnancy-associated glycoprotein (PAG)
  - Family of glycoproteins produced by mononucleate and binucleate trophoblastic cells
- In cattle – detect pregnancy <30 days after artificial insemination
  - <40days for sheep and goats
- Commercial ELISA assays are available
  - Designed for use in blood/serum samples or milk samples

MY SOLUTION: PAG



Wallace et al., 2015

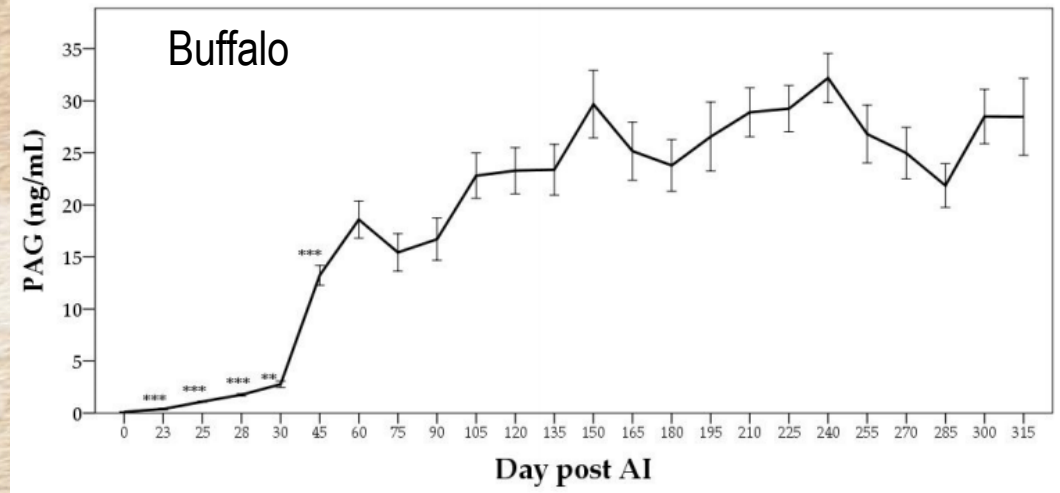
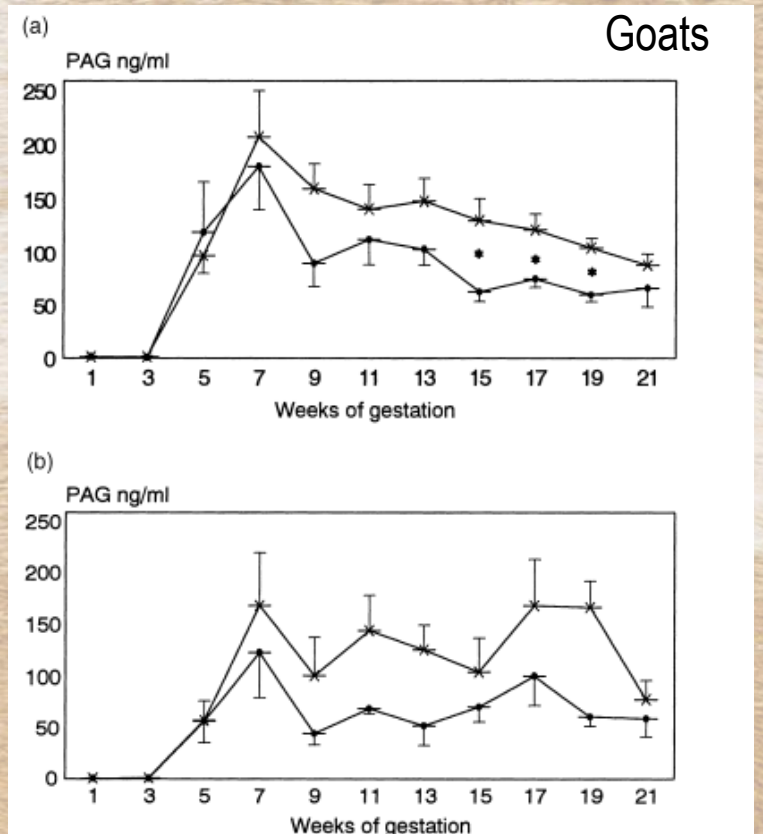


Figure 5. Pregnancy-associated glycoprotein (PAG) plasma profile during pregnancy in buffalo cows. *** $p < 0.001$, ** $p < 0.01$ versus previous day. Adapted from Barbato et al. [116].

Barbato et al., 2017



Sousa et al., 1998

MY SOLUTION: PAG

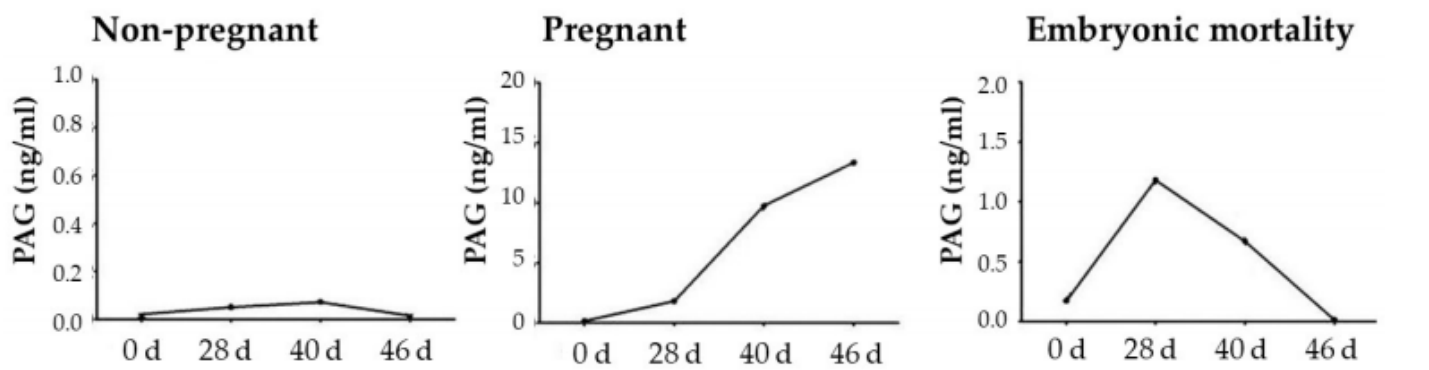


Figure 7. Pregnancy-associated glycoprotein (PAG) plasma concentrations in non-pregnant and pregnant buffalo cows, and those that experienced embryonic mortality. Adapted from Barbato et al. [119].

Barbato et al., 2016

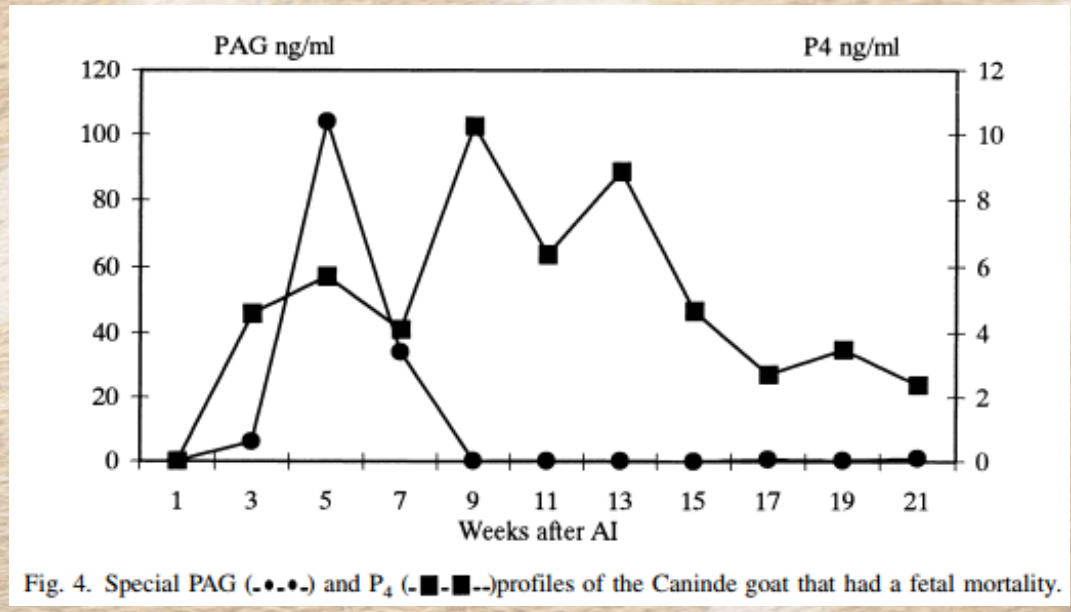


Fig. 4. Special PAG (-•-•-) and P_4 (-■-■--)profiles of the Caninde goat that had a fetal mortality.

Sousa et al., 1998

Table 2
Number of animals in each category (pregnant and non-pregnant) that tested positive or negative with each pregnancy diagnosis method

Days after breeding	Pregnant animals ($n = 79$)						Non-pregnant animals ($n = 64$)					
	US		P4		PAG		US		P4		PAG	
	+	−	+	−	+	−	+	−	+	−	+	−
20	9	70	–	–	42	37	0	64	–	–	0	64
22	35	44	79	0	75	4	0	64	22	42	0	64
24	62	17	–	–	77	2	0	64	–	–	0	64
26	78	1	–	–	79	0	0	64	–	–	0	64

US: Ultrasound; P4: progesterone; PAG: pregnancy-associated glycoprotein.

Canary dairy goats. González et al., 2004

EVIDENCE SUPPORTING PAG USE

Table 1. Number of individuals and respective samples that are represented in this study. Discrepancies between number of individuals and number of samples in the case of dama gazelle and eland represent nonpregnant sample unavailability and assay retesting in the case of the dama gazelle.

Species	Individuals	True positives (total pregnant samples)	True negatives (total nonpregnant samples)
Dama gazelle	$n = 15$	2 ($n = 22$)	11 ($n = 11$)
Eland	$n = 4$	5 ($n = 5$)	3 ($n = 3$)
Okapi	$n = 3$	0 ($n = 3$)	3 ($n = 3$)
Gerenuk	$n = 11$	11 ($n = 11$)	11 ($n = 11$)

Dvornicky-Raymond et al., 2020
IDEXX PAG Elisa using serum

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Dvornicky-Raymond et al., 2020
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SAMPLE TYPES OF INTEREST

- Serum
  - Difficulties in collection
- Milk
- **Fecal Extracts**
  - Easy to collect
  - Easy to extract
- **Saliva**
  - Relatively easy to collect
  - Needs animal cooperation
- Urine
  - Has proven impossible

SAMPLE TYPE: SERUM

Species	# Samples Assayed	# Positive	# Negative
Cattle	6	6/6	0/0
Sheep	3	3/3	0/0
Goat	3	3/3	0/0
Giraffe	2	2/1	0/1
Bongo	1	1/1	0/0
Grant's Gazelle	1	0* 108d postpartum	1*

Sample Type: Fecal Extract

Species	# Samples Assayed	# Positive	# Negative
Cattle	4	4/4	0/0
Sheep	3	1/2	1/1
Giraffe	2	0/2	0/0
Dik-Dik	8	4/8	4/0

Fecal Extracts:

- Both **Wet** and Dried Feces
- 40% and **80%** MeOH

Dik-Dik troubles

SAMPLE TYPE: SALIVA

Species	# Samples Assayed	# Positive	# Negative
Cattle	4	4/4	0/0
Sheep	2	2/2	0/0
Giraffe	30	12/1*	2/4*
Grant's Gazelle	1	0* 108d postpartum	*

Trouble with Giraffe numbers and samples

Case Study: Wendy Giraffe

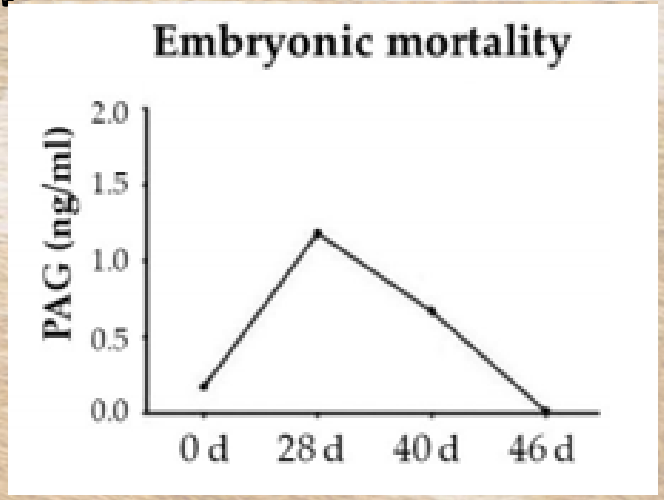
5 Aug 2022: keepers suspect Wendy is pregnant

- Ultrasound preformed: no fetus seen
- Saliva and serum collected and assayed for PAG: positive for PAG

Keepers continue to collect ~weekly saliva for potential longitudinal study

8 Feb 2023: email from keepers. Wendy suddenly is looking “not pregnant”

- Assay samples collected from August 2022 to February 2023
  - NONE test positive
  - Embryonic mortality?



Future Directions and Collaborations

- Looking for collaborators to facilitate collections
  - Interested in feces, saliva, urine
- Looking to possibly expand to species other than giraffe

- Propose taking this to the field
  - Collect feces from free-ranging animals to determine how many females are pregnant
  - Determine pregnancy for animals being relocated
  - Potentially help determine success of artificial inseminations

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- Memphis Zoo Dept of Conservation and Research
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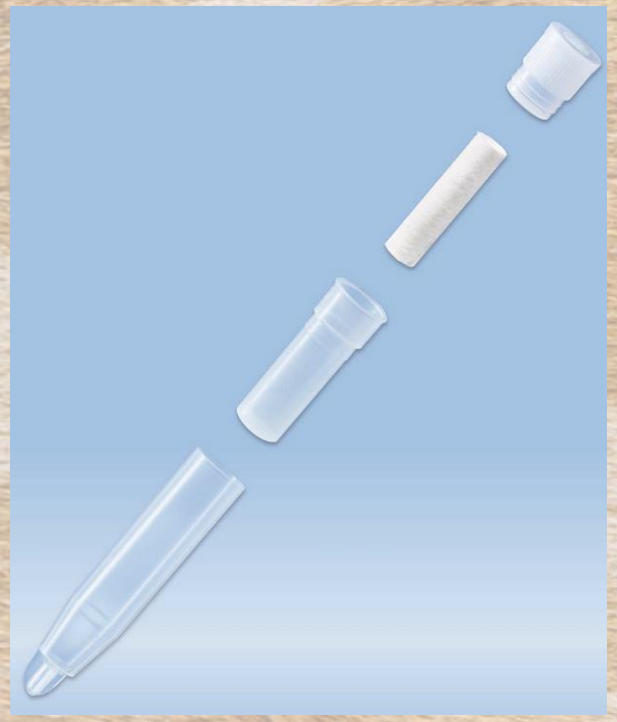
- Walkapony Goat Ranch
- Memphis Zoo Keepers
  - Giraffe: Jason Bankston and Katherine Driscoll
  - DikDik: Allison Bruenner
  - Farm

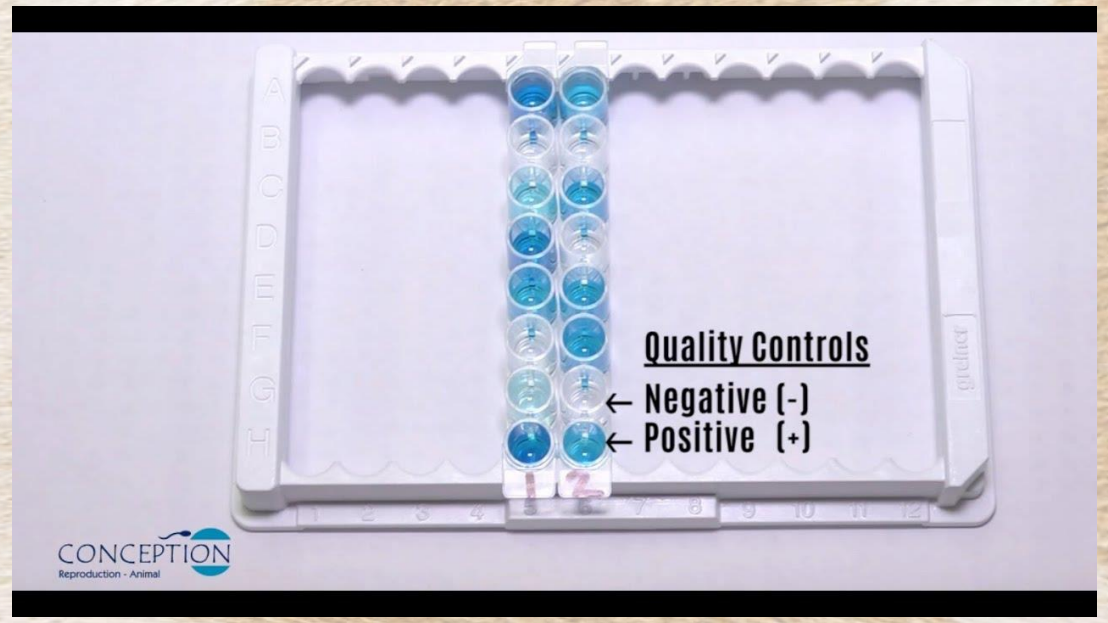
QUESTIONS!

MRichter@MemphisZoo.org

SUPPLY COST:



Salivette® Swabs:
$71/pack of 100



Conception PAG Assay:
$400/100 tests

Total cost per sample: $4.71

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