

## Practical guide for the collection of urine samples in elephant cows for monitoring reproductive status

Ann-Kathrin Oerke, European Elephant Service, Endocrinology Laboratory, German Primate Centre  
Leibniz Institute for Primate Research, Kellnerweg 4, D-37077 Goettingen, Germany ([akoerke@dpz.eu](mailto:akoerke@dpz.eu))

### Background

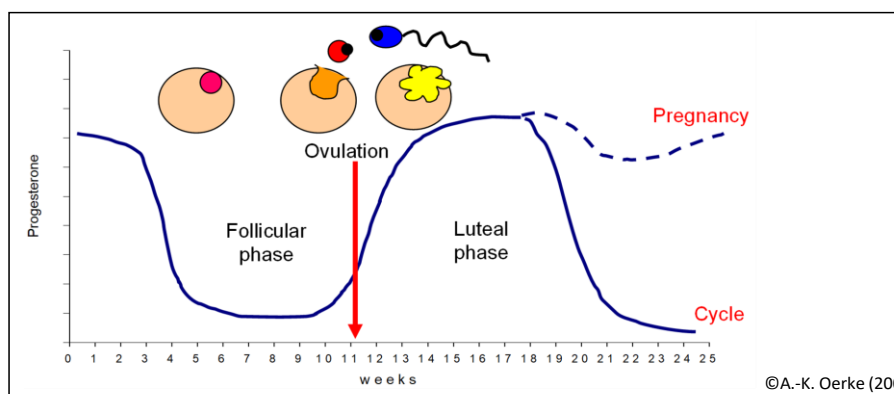
Monitoring of reproductive status in female elephants is best performed via longitudinal measurement of hormones. Hormone analysis provides an indirect method to determine the function of the ovaries and to detect the time of ovulation. Whilst all hormones are present in blood, their metabolites can be measured in urine and feces. The steroid hormone progesterone is most suited for the monitoring of cycle and pregnancy. This hormone is produced after ovulation by the corpus luteum.

#### *What is a corpus luteum?*

The cycle of the elephant, as in each mammal, consists of a follicular and a luteal phase. The follicular phase is characterized by the development of several follicles of which only one, the dominant follicle, grows until ovulation. At ovulation this follicle ruptures and releases the oocyte which, in case of matings, may be fertilized by a sperm, forming an early embryo. **After ovulation, the luteal phase starts.** On the ovary the follicle cavity transforms into an active endocrine gland, the corpus luteum. This structure is producing increasing amounts of progesterone in order to support a possible pregnancy. The ovarian changes described here occur in each cycle and each cycle is destined to result in a pregnancy! Elephant females show ovarian cycles between puberty and death, so they might still be cyclic when they are already too old to reproduce!

#### *What is the hormonal difference between cycle and pregnancy?*

If an elephant cow has conceived, the corpus luteum will persist and continue to secrete progesterone. This results in continuously elevated hormone levels in blood, urine and feces. If the cow was not mated successfully or not at all (due to absence of a male or separation of the bull), the corpus luteum will regress, progesterone levels drop and a new follicle phase starts, marking the onset of a new cycle. The changes on the ovary and the difference in the pattern of progesterone between cycle and pregnancy are schematically shown in the following figure:



## Reproductive stages

Longitudinal measurement of progesterone or its metabolites will reveal 3 different reproductive stages:

- *Cyclic = regular changes of low (follicular phase) and high (luteal phase) values*
- *Pregnant = constantly high values*
- *Not cyclic = constantly low values*

Females without cycles are either too young, in a post partum anestrus or truly not cyclic for different reasons.

It is important to remember that young Asian elephant females can start to show ovarian cycles with only 4 years of age, African elephant females with 7 years of age and are thus able to reproduce quite early! **It is NOT possible to predict the onset of ovarian cycle activity!** However, behavioural changes in the female, changes in colour and swelling of the vulva and discharge of mucus are signs of beginning sexual function. Sudden interest of the male in the young female might also indicate impending estrus. In all cases it is advised to start with hormone monitoring in that cow!

The time of post partum ovulation in both species depends on whether the calf is born alive and reared by its mother! In general cows stay in lactational anestrus for one year! **In case of abortion, stillbirth and handrearing of the calf, however, cyclic activity occurs significantly earlier** and cows can conceive again after just a slightly prolonged follicular phase! **It is NOT possible to predict the post partum ovulation!**

**NOTE:** whilst it is possible to predict ovulation in **cyclic** elephants dependent of previous duration of cycle phases, it is **NOT** possible to predict onset of cycles in young females or resumption of cycle in cows that have given birth!

## Sample collection

Given the long cycle of 14 - 15 weeks in elephants, only one sample per week for an initial period of 6 months is required to obtain reliable results on whether a female is cyclic or not. If a cow is diagnosed to be cyclic during this period, it can then be decided if sampling needs to be continued.

Samples should be collected on the same day of the week and if possible, roughly at the same time of the day, so either morning, noon or afternoon. In general only 2 ml urine are needed for the analysis. It is advised to use plastic tubes that close well, best with skrew lid.

Proper labelling of the sample is essential! Labels must be waterproof and show:

1. **name of animal** and
2. **date of collection.**

If the samples are not sent straight, they need to be frozen soon after collection.

In order to account for the quality of the urine sample, the hormone content is related to the amount of creatinine, a marker of urine concentration! It is therefore important to collect urine that has a yellow colour. If the urine looks like water, it is likely too diluted! In this case the calculated hormone value will be overestimated and is not reliable!

## Methods of sample collection

Urine can be collected in many different ways depending on keeping and housing system. In fact, hormone analysis in urine samples was originally invented as a **non-invasive method** that needs **no** contact to the elephant. It is therefore possible to collect urine samples in all keeping systems: full, protected or no contact! In general urine collection (as well as drawing of blood) can be integrated as part of the weekly working routine and training sessions!

*How do you get your elephant to pee?*

The event of urination must be initially connected with a special **command** and followed by a **reward**. This reward is a combination of a positive, laudatory voice of the keeper and a food item. The next step is to train the elephant to urinate on this command in anticipation of a reward. Depending on the housing system the difficulty is then to get access to the sample!

*How do you get access to the urine sample?*

In direct contact elephant females are usually trained with special commands to urinate. They are rewarded once they provide a sample and the urine can be collected straight from the cow! The same can be done in protected contact using positive reinforcement. Zoos have invented all kind of methods:

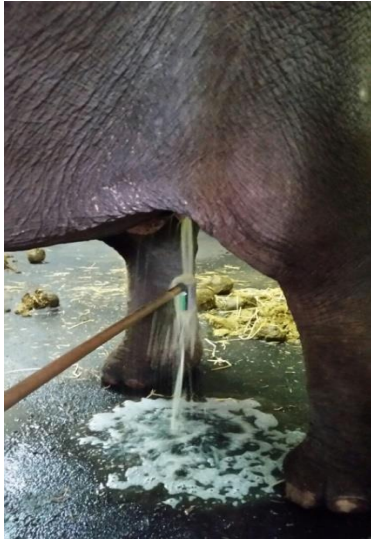
- females might stand parallel to the training wall and in reach for the urine sample through the training bars
- alternatively telescope sticks with little pots can be used to hold under the animal once the urine flows
- in order to prevent the urine from being soaked from the ground or run away out of reach, it is possible to use paper towel to absorb the urine which is then held over the tube to let the urine dribble in

In case of no contact, the urine can also be collected from the ground. In this scenario it is important that the sample is not diluted with water from either washing the elephant or cleaning the stable. Contamination with feces should be avoided. A cow can, for example, be locked in her box until she urinates. Ideally the elephant is then rewarded for providing urine and allowed to leave the stable so that the sample can be taken safely from the ground. If the floor has a gradient and the urine comes running to you, you might be able to collect it even from outside the stable!

In all cases it is important to let the animal see the collection of urine. Since elephants are clever, they quickly learn that providing urine is rewarded. The cow might remember this next time a keeper appears with a pot or tube, and pees even quicker!

If females are housed in single boxes over night, one can also use the “Cabarceno-method” and drill little holes in the ground where the urine collects over night which can then be picked up on the next morning with a clean syringe once the elephant has left the stable!

The pictures below show examples of urine collection through the bars in protected contact (left) and collection of the sample from the ground after the elephant has left the stable (right).



### **Packing and sending of urine samples**

In Goettingen hormone analyses for **both elephant species** are performed each week on wednesdays, and if more samples arrive also on thursdays. Samples arriving on fridays will be frozen for the next week`s analyses because assays run over night!

Dependent on how often you want or need results, you can either send fresh urine samples on a weekly basis or frozen samples on a monthly schedule. If you can make sure that the samples arrive within 2 days, no cooling is needed!

If you collect and send the samples directly after collection on a weekly schedule, you have to collect over the weekend or latest on Monday and sent them straight away to be sure that they arrive on Wednesday, latest on Thursday for the analysis.

If you send on a monthly schedule, you have to freeze the samples first and send them with the last collected fresh sample of that month, again on a Monday, so that they arrive on Wednesday, latest Thursday, for the weekly analysis.

Please pack the samples according to the regulation of biological material for analysis. You have to inform yourself how to send the urine beforehand because the rules for sending biological samples may differ between countries!

Most importantly you have to **make sure that no urine will run out** (check lids before packing!) and that the tubes are not broken during transport (no glas tubes please!). This means that you have to pack the tubes with the urine samples in a **plastic bag** and then in a **padded envelope**. Do NOT wrap the tubes in paper towel because in case urine runs out, it will be soaked and is lost! If samples are sealed in plastic bags we are still able to absorb the urine from there (if not more than one sample has run out).

If samples are not sent in regular intervals, it is advised to inform us beforehand. Please write a mail to: [akoerke@dpz.eu](mailto:akoerke@dpz.eu)

We are then aware that your samples are on their way and can plan the analysis accordingly. We will also let you know once the samples are here! In case of late arrival (fridays) you will be informed that results are only sent out a week later!

For sending samples please use the following address:

**For Asian elephants:**

Ann-Kathrin Oerke and Miriam Polten  
HORMONE LABORATORY  
German Primate Center  
Kellnerweg 4  
D-37077 Goettingen  
Germany

**For African elephants:**

Ann-Kathrin Oerke and Andrea Heistermann  
HORMONE LABORATORY  
German Primate Center  
Kellnerweg 4  
D-37077 Goettingen  
Germany

**Availability of results**

All samples that arrive on wednesday, latest thursday will be analysed straight away. Since the analysis runs over night, results will be sent out by mail 1 or 2 days later, on thursdays and fridays, in case of many samples also on saturdays and sundays!

**Interpretation of values**

Given the physiological background on cycle and pregnancy as described above, values will provide the following information:

- Low values: follicle phase of a cycle or no cycle
- High values: luteal phase of a cycle or pregnancy
- In case of weekly results it is possible to detect ovulation which occurs between the last low and the first high value!
- Usually the exact week of cycle is given
- Facilities are warned if an estrus is expected in order to adapt reproductive management accordingly!
- In case of weekly results the next ovulation can be predicted in order to either place the cow with the bull to be mated, or separate the bull to avoid pregnancy
- Once ovulation was confirmed, separated cows can be placed with the bull again, or, in case of mating, the correct time of mating can be determined!
- Pregnancy is diagnosed after 16 weeks of high values following ovulation (and mating, if known)
- After diagnosis of pregnancy, the time of birth can be predicted. Usually a gestation length of 630 days is calculated from the day of first mating (marking the first possible day of conception within the week of ovulation). Primiparous females usually have a shorter gestation, whilst older cows that gave birth before, tend to extend pregnancy up to 670 days and more!

**Costs**

At the moment (November 2020) the price for the analysis of a urine sample (in both elephant species) is 10 Euros. This analysis includes the measurement of the progesterone metabolite plus measurement of creatinine (as indicator of the concentration/dilution of the urine sample).

Low creatinine values (below 0,1 mg/ml urine) are an indicator of a less concentrated urine sample (for example when a lactating female is drinking a lot of water) or for dilution of urine with an unknown amount of water (for example if taken from a wet floor). Since the hormone value is related to the creatinine value in the sample, low creatinine might cause

overestimation of hormone values. These values need to be interpreted cautiously, however, usually zoos are informed about this with the results.

Please note that elephants with kidney failure also show persistent low levels of creatinine! If creatinine is below 0.1 mg/ml over a longer period of time, in special if samples are collected **directly** from the cow, it is advised to check the kidneys of this elephant. You can do this via different parameters in blood samples. In elephants with a bad body condition, that are showing symptoms of weakness, that shiver frequently or drink a lot, it is advised to examine the kidneys also by ultrasonography in order to prevent the animal from suffering seriously.

*Version 11/2020*