

## The challenge of obtaining reference values for use in captive animals like elephants

In the past 3 years, I have been involved in 3 elephant parturitions in Asia. The animals were privately owned under relatively good conditions, but quite old for getting their first calf (39–40 years). During the night they were able to forage in the forest, while during day time they were used for tourism, until four months before the expected calving date. Their blood progesterone levels were monitored weekly throughout the major part of their pregnancy. During the last month of pregnancy, total plasma calcium was also monitored, fluctuating between 2.2 and 2.7 mmol/L. The birth process in these three old females was stagnated: several days after progesterone had dropped to baseline, there was still no sign of a beginning parturition as confirmed by transrectal ultrasonographic examination. I had the privilege to assist two of these calvings “hands-on” till the end. And similar to what I observed in other elephant parturitions in European zoos in the past, the intravenous administration of calcium-magnesium borogluconate explicitly increased the contractibility of the uterus. In the first animal, I found the calf “floating” in the vagina when I first examined her 10 days after the drop of progesterone. At that time the total serum calcium concentration was 2.2 mmol/L. Rectal stimulation of the pelvic wall to induce the Ferguson reflex had no effect. After slow IV infusion of 1.0 liter of calcium-borogluconate (110 g/L), the Ferguson reflex resulted in uterine contractions (Figure 1). Adding 50 IU of oxytocin during the last 20 minutes of the IV-drip resulted in labor activities and the birth of the calf 4 hours later.

I found the second elephant 3 days after progesterone had dropped completely (day 3). Total blood calcium level was 2.4 mmol/L and the cervix was still closed. The Ferguson reflex could not be induced by rectal massage of the pelvic wall. On day 4 the cervix started to dilate after transrectal application of 400 mg estradiol. Three hours later, the animal was given 55 g calcium-borogluconate in a slow IV-infusion. One hour later, a lot of clear vaginal mucous was discharged from the vulva, the cervix opened even more and one foot of the calf could now be visualized by transrectal ultrasonography. The elephant was repeatedly given IV-infusions of calcium-borogluconate. Each time both front feet of the calf appeared on the cranial side of the cervix. During the next 72 hours, however, 3 hours after each infusion, the calf “dropped back” into the abdomen. Unfortunately, there was no follow up of blood total calcium levels, but it was quite obvious that

the calcium infusions consequently resulted in uterine contractions, even in the absence of exogenous oxytocin administration.

For many years I have been intrigued by the calcium metabolism in Asian elephants. The first time was during a stagnating parturition in what later on appeared to be a severe hypocalcaemic elephant (total calcium 0.84 mmol/L, ionised calcium concentration 0.37 mmol/L in heparine-plasma). We set up a pilot study (study A) at Rotterdam zoo involving 4 Asian elephants (*Elephas maximus*) by supplementing their diet with calcium and we concluded that “captive Asian elephants might be subclinically hypocalcaemic. Based on the findings we suggested that the normal serum concentration of total calcium in Asian elephants should be around 3.6 mmol/L and the normal heparine-plasma concentration of ionised calcium around 1.25 mmol/L.” Another conclusion was that the calcium uptake in Asian elephants should be compared with horses (van der Kolk et al. 2008).

A purely descriptive paper (study B) recorded the serum parameters related to the calcium metabolism in captive African elephants (*Loxodonta africana*) in Florida, where the UVB-radiation is more favorable for tropical animals than in the Netherlands (Miller et al. 2009). The average serum total calcium level in these animals was  $2.7 \pm 0.90$  mmol/L, while 25(OH)D<sub>3</sub> levels did not fluctuate significantly over the 4 seasons.

Stimulated by the outcome of the first study, a larger research project (study C) was initiated, involving both elephant species in 4 different zoos in a temperate climate (van Sonsbeek et al. 2013). These herds were alternating supplemented with oral calcium or vitamin D<sub>3</sub>. The conclusion of this study was that Asian elephants depend predominantly on dietary calcium, while African elephants depend more on the vitamin D<sub>3</sub> supplementation and solar UVB-exposure. In contrast to the findings in Florida, the African elephants in study C showed a significant higher serum 25(OH)D<sub>3</sub> concentration in summer compared to winter. Based on the significant difference in length of the small intestines (in Asian elephants 2 times longer than in African elephants) (Shoshani et al. 1982), it was hypothesized that this might explain why Asian elephants profit more from dietary calcium intake than African elephants.

Recently, another paper on the calcium metabolism in elephants was published (study D) (Childs-Sanford et al. 2020). Relevant blood parameters (25-



**Figure 1.** A 39-year-old Asian elephant getting IV-infusion with calcium-borogluconate during parturition, accompanied by a 60-year-old “aunty” elephant.

hydroxyvitamin D, 24,25-dihydroxyvitamin D, 1,25-dihydroxyvitamin D, parathyroid hormone, total calcium, ionised calcium, phosphorus, and magnesium) in serum samples of 6 adult Asian elephants kept in the northern temperate climate were measured as well as several related parameters in their diet and exposure to UVB. Over the year no differences in serum/heparine-plasma parameters were noticed. Calcium (2.7 mmol/L) and ionised calcium (1.4 mmol/L) were within expected ranges (Species 360). The authors conclude that “... this study suggest that vitamin D metabolism in Asian elephants has fundamental differences from many other animal species but may be similar to domestic horses”. This conclusion is in line with the previous statements from study A and C. Interestingly, the serum values for ionised calcium (1.4 mmol/L) in study D were higher than the suggested reference value in study A (1.25 mmol/L), while total calcium (2.7 mmol/L) in study D was much lower than proposed as reference value (3.6 mmol/L) in study A. The proposed reference value in study A was based on the finding that oral calcium supplementation in Asian elephants resulted in an increase of serum ionised calcium from 0.93 to 1.25 mmol/L and total calcium from 2.9 to 3.6 mmol/L. As the calcium metabolism is aiming to stabilize the blood level of ionised calcium, it is interesting to observe the difference in total calcium concentrations between studies A and C and this recent study. Apparently captive Asian elephants are capable to maintain their ionised calcium concentrations at adequate levels for maintenance, while their serum total calcium buffer is in the low range or even too low. This brings me back to my experience with stagnating parturitions in elephants. The fact that in my own experience IV-infusions with calcium had a positive effect on the contractibility of the uterus, suggests that these females suffered of hypocalcemia at the time of calving.


There has been some debate amongst zoo veterinarians about the time span between progesterone drop to baseline value (the lowest value during the follicular phase) and the moment when the calf should be on the ground. The *Veterinary guidelines for reproduction-related management in captive female elephants* of the European elephant TAG, considers 72 hours as the period to be pursued, while sporadically calves have been born alive even after 10 days (Schaftenaar and Hildebrandt 2018). Unfortunately, in these anecdotal cases the influence of IV-infusions of calcium was not tested.

We can learn from these stories and research projects that there is still much more to investigate. So called reference values for zoo animals are based on common practice in zoos worldwide, even including animals kept under climatological circumstances that differ from their natural habitat and fed an artificial diet (Species 360). We should not always take these reference values for granted and try to collect more data (including ionised calcium) from free ranging animals. We need to share data (at least serum total calcium and ionised calcium) of captive elephants during parturition, as during this critical phase their calcium metabolism is heavily challenged. I encourage clinicians working with elephants to collect and share their data with researchers for the benefit of these unique animals.

## References

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