

Veterinary guidelines for reproduction-related management in captive elephants

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The breeding process in elephants needs monitoring of several parameters in both males and females. The main ones are for the determination of the estrous cycle through progesterone and perhaps LH assay, the evaluation of the genital tract in both sexes, the determination of the number of fetuses and finally the moment of parturition. This document consists of 4 parts. The first part of this document briefly mentions the monitoring tools that can be used in female elephants. The second part describes a protocol for veterinary intervention in elephant parturition. The third part describes the vaginal vestibulotomy procedure and is followed by some remarks regarding fetotomy. This is not a static document. Colleagues are strongly encouraged to report any experience that may add useful information to these guidelines.

Main topics for monitoring and assisting female reproduction in elephants

1. Determination of estrous cycle:

- * Blood (progesterone) or urine (pregnanetriol)
- * At least every 2 weeks during the luteal phase. If more accuracy for estrous prediction (c.q. breeding) is required: every week
- * Every week during the follicular phase

Note: when using the urine test, one must be aware that the pregnanetriol concentration in the urine is compared with creatinine. If the creatinine level is too low, a new sample should be submitted. Even when monitoring the cycle by urine-pregnanetriol, much effort should be made to train all elephants to allow blood sampling.

2. Breeding:

- * The EEP has provided the following breeding recommendations: *In order to prevent the occurrence of age-related alterations on the female genital tract like endometrial or ovarian cysts (frequently found in African and Asian elephants), benign myometrium tumors, cystic malformations (leiomyomas, exclusively found in older Asian females):*
 - *The inter-calving period should be restricted to max. 5 years.*
 - *Cows should be bred before the age of approximately 12 years*
- * The occurrence of mating is not an indicator for impending ovulation; some animals mate even during advanced pregnancy or outside the estrous period.
- * Fertilization can only result from mating during estrus preceded by the pre-ovulatory (=second) LH-peak. Monitoring LH needs daily blood sampling for at least 10 days (A test kit is available presently: LH ELISA KIT, which can be ordered from Lenora Bruce, University of California Davis, Central Storehouse Receiving, California and Larue, Davis CA 95616 USA, phone +1-530-752-0663)
- * The fertile period is restricted to the time around the second LH-surge, which occurs

immediately prior to the rise in progesterone. For the prediction of the next ovulation, follicular phase length has to be determined and therefore it is important to know the moment of the preceding fall of progesterone/pregnanetriol. For better accuracy, weekly samples throughout the cycles have optimal predictive value.

- * LH monitoring daily during the non-luteal phase is necessary for artificial insemination programs. Determination of the post-luteal phase (=first) LH-peak can help predict the first opportunity for (natural) breeding, as the interval between both LH-peaks is rather constant.

3. Pregnancy confirmation:

- * Immediate increase of progesterone/pregnanetriol around the time of mating is suggestive for the right timing for breeding.
- * Continuation of high progesterone/pregnanetriol level continuing for at least 16 weeks after mating is highly suggestive of pregnancy.
- * Transrectal ultrasonographic examination at 8 - 20 weeks after mating allows visualization of single or twin calves. Between 10 and 20 weeks, the larger mature animal may need to lay down on its side for reliable ultrasonographic examination of the uterus. Uterine vascularization can be visualized to determine viability of the fetus, to exclude embryo absorption and mummification.
- * Transcutaneous ultrasonographic examination (both flanks have a small "window" where visualization of fetal movements may be seen) may help to determine the status of the fetus in the last months of pregnancy.
- * At 6 months post-breeding pregnancy can be confirmed by elevated serum prolactin concentration (test only routinely available in the USA).

4. Behavioral observation at the end of gestation:

24 hours observation including the use of a (time-lapse) video recorder starting in week 85 may add to information about relevant events prior to parturition: night pacing, kneeling down, climbing, short periods of separation from the group, beating the vulva with the tail, frequent production of small-sized feces and small quantities of urine, loss of mucous plug, playing with mucous plug, rupture of the amniotic sac, labor waves.

5. Predicting the time of parturition, measures and observations:

- * Daily blood sampling from week 89: every other a day progesterone assay, until week 91 (637 days). From week 91 (637 days): daily assays and as soon as progesterone starts to decrease: sample twice daily.
Daily monitoring of progesterone is only possible if you have a nearby facility that runs these assays on a daily basis. Find out from your nearby hospital in the early stage of pregnancy! Many veterinary labs do not have sensitivity for levels of progesterone (P4) in elephants 1-3 days prior to delivery. Also check the availability during the weekend.
- * Loss of mucous plug (not seen in many facilities)
- * Pre-and post-parturition ventral edema may be noticed.
- * Group members may react differently (vocalizations, restlessness)
- * In most cases (75%) rupture of amniotic sac and loss of amniotic fluid (looks like urine) is seen within 2 hours prior to birth.
- * The size of fecal balls may get smaller towards the end of pregnancy.
- * Frequency of urination may increase around parturition, resulting in more "watery" consistency of the urine, resembling more like amniotic fluid .
- * Development of mammary gland and production of milk shortly before birth is often seen.

Milk accumulation can be visualized using transcutaneous ultrasonography several hours prior to parturition.

- * Softening of the pelvic ligaments (due to estrogen surge) may result in slightly abnormal locomotion of the hind legs.

6. Parturition:

Preparations for calving:

- * Training and/or desensitizing of the pregnant elephant for veterinary intervention, like blood sampling, injections, IV-infusions, milking and rectal manipulations
- * If possible, store some colostrum (freezer) or store plasma obtained from the dam in weeks prior to parturition.
- * Have artificial milk available (Salvana GmbH, Germany; hand raising has been done at Emmen Zoo and Berlin Zoo)
- * Check restraint chains and fixation points for the legs and one extra fixation point between the hind legs for pulling devices. Soft ropes for pulling the calf away if needed should be available. The use of a calf harness has been described.
- * Check the stable and place bars where a calf could possibly escape. Block all possible escaping routes for the calf (not for staff!!).
- * Take out all obstacles.
- * Be prepared for closing the elephant house for the public (sign post, etc.)
- * Make sure there is a good stock of commercial cat litter or saw dust to be used on a concrete floor as soon as the calf is born. This will absorb much of the amniotic fluids and prevent the animals from slipping on the wet floor.
- * 2 or 3 pairs of keeper-gloves (soccer) to get a better grip on the wet, slippery calf when needed
- * Plastic hose pipe (with pump, if necessary) for rectal cleaning with lukewarm water
- * 3 birth-chains with proper handles (2 for the legs, 1 for trunk or tail); find a way to avoid back sliding when manual extraction (vaginal vestibulotomy) is required.
- * Drugs to be kept in store: Ca-borogluconate, estrogens, oxytocin, lidocaine, xylazine, azaperone, atipamezole, doxapram, oxygen, (Betadine-)iodine solution for navel disinfection (umbilical infection is a major cause of perinatal complications), many liters of a lubricant.
- * To avoid dystocia, the normal calving process should take place within 2 hours after rupture of membranes (release of fetal fluids).
- * Normal calving should take place within 48 hours after decrease of the progesterone blood level to below base line concentration

Guidelines for veterinary assistance around the parturition time

To determine the right moment when calving starts, 2 parameters are essential: the progesterone blood level and the relaxation of the cervix, monitored by ultrasonographic examination.

Progesterone: the sensitivity of the equipment and the time needed to run the assay are the bottleneck for using the progesterone concentration as a reliable tool. Today many human hospitals use advanced equipment with a minimal detection level of 0.1 ng/ml that can be determined in less than 2 hours. Make sure that you have made arrangements with a lab long before you expect the parturition.

Ultrasonography: to use this technique as a reliable tool, it is indispensable for the veterinarian to gain experience *long before the elephant birth is expected*. This will enable the veterinarian to distinguish the relaxed cervix (figure 2a + b) from the normal cervix. A 3.5 or 5 MHz probe can be used transrectally.

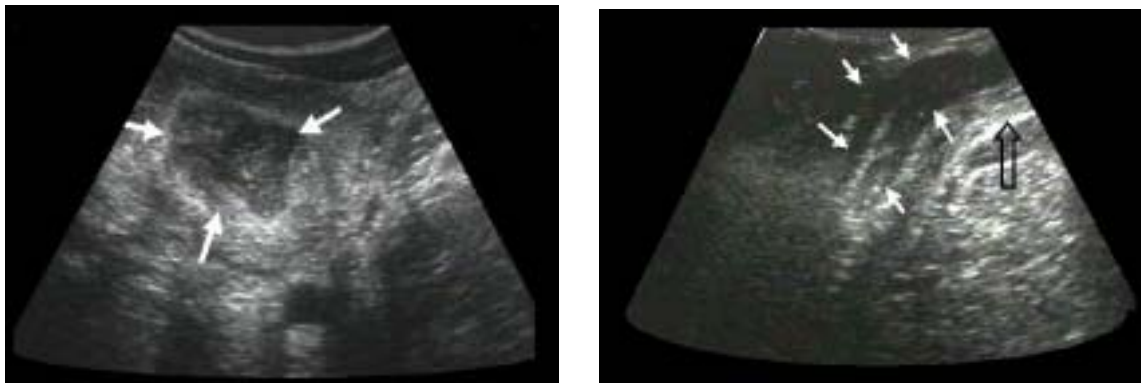


Figure 2a (transverse) and 2b (longitudinal): amniotic sac with cloudy amniotic fluid in the cervix of an Asian elephant 12 hours prior to delivery (white arrows: amniotic sac, open arrow: pelvic bone)

During the last 2 weeks of gestation, the mucous that is present in the vagina during gestation will be discharged gradually. This is a clear indication for a pending parturition.

Recognizable onset of parturition occurs normally within 24-48 hours after progesterone has dropped to below base-line level. In these guidelines the absence of signs of parturition in the same time frame is defined as silent parturition or dystocia; **this status requires veterinary intervention.**

At this point 2 situations may occur: delivery has started without or with spontaneous rupturing of the amniotic sac.

1. No rupture of amniotic sac noticed:

If the calf is not born by natural way 24 hours after blood progesterone has dropped to baseline level, rectal palpation and ultrasonographic examination of the cervix is highly recommended. This will demonstrate the rate of relaxation of the cervix, the presence of the amniotic sac or parts of the fetus in the cervix or vagina and should be repeated at least every 8 hours.

* A blood sample should be taken to measure the calcium level. If below 2.5 Mmol/l, calcium should be administered as an IV-infusion (NB: when given in an ear vein, it should be given strictly IV in order to avoid damage to the vein) or orally (suggestion: calcium syrup)

concentrate for human use, enveloped in the carton core of toilet paper, covered and sealed with fresh tamarind paste has worked well; most elephants will eat it, including the carton material). The effect of the calcium should be confirmed by rectal palpation (increase of contractibility of the uterus) Store an EDTA and heparine sample for herpes virus diagnosis (both cells and plasma in freezer after separation).

Transrectal ultrasonographic examination at 48 hours:

A. No relaxation of the cervix at 48 hours: search for calf movements, blood flow in fetal vessels, nail position of the fetus.

Transcutaneous ultrasonographic examination (both flanks have a small "window" where visualization of fetal movements may be seen) may help to determine the status of the fetus. Apply estrogens rectally. Good results have been obtained by the rectal application of Oestrogel™, Pump pack, containing 17-beta-oestradiol (product of Hoechst Marion Roussel, Hoechst). This drug should show effects within 1 hour. At this time, rectal massage should be applied to test and stimulate the contractibility of the uterus. Technique: remove feces from rectum, flush out the rectum, use abundant lubrication, keep both **gloved** hands (NB: the rectal mucosa is vulnerable due to estrogens) with the fists joined in a firm grip and press with the wrists or the palmar sides of the hands against the pelvic ring to stimulate the pelvic receptors until strong labor waves appear or at least 10 minutes. When labor waves occur, continue this massage for 3 hours (if needed change operator). Check regularly ultrasound the condition of the cervix. If there is still no cervix relaxation, continue monitoring the viability of the calf. Consider the presence of pseudopregnancy (ovarian tumor, dysfunction of the pituitary gland, etc.).

B. Partial or complete relaxation of the cervix (figure 2) at 48 hours or later as a result of the situation described under 1A:

Apply rectal massage to test contractibility of the uterus. If limited or no reaction, the administration of oxytocin is contra-indicated. In this case, the administration of calcium is recommended (even when blood calcium level is within normal ranges). After 2 hours the use of estrogens as described under 1.A is advised.

Only if uterus contraction can be provoked by the rectal massage, the use of oxytocin may be considered using the following dosage: 50 IU oxytocin s.c. or i.m. (if needed use a blow dart).

Oxytocin should be used with care, as it may dramatically exhaust the contractibility of the uterus muscles as well as the general condition of the female. There might also be the risk of reduced blood circulation in the umbilical chord, due to the spasms in the myometrium.

Depending on the progress obtained, rectal massage and the administration of oxytocin are the 2 major treatments to follow from this point. In between these treatment events, the animal should be exercised to relieve the pain and stimulate position changes of the calf and preferably it should be kept in the group. Oxytocin should be given in intervals of at least 2 hours for a maximum of 12-24 hours under the guidance of ultrasound to evaluate the progress.

Continue this approach of treatment until parts of the calf have entered the pelvic cavity. If the efforts remain unrewarded and no access to fetal parts is possible, not much can be done. Continue monitoring the viability of the calf. If the calf has died, while the membranes are still intact, the risk of intoxication is limited, but immunosuppression could be a complication for the cow. To date, no proper data are available.

The dosage of oxytocin may only be increased to 100 I.U. after parts of the calf have entered the pelvic area and progress is clearly observed. At this time, a bulge containing parts of the

body under the tail of the dam should be visible. Progress of parturition must be monitored strictly at this stage. If this increased dose of oxytocin does not result in parturition a vaginal vestibulotomy should be performed soon to get better access to the calf. See next chapter.

Expulsion of the calf should follow soon after the bulge appears under the tail of the dam. The amniotic sac usually ruptures during the (induced) passage through the pelvic canal. One complication described at this stage, is reduced passage space as a result of edema in the urogenital canal resulting in a “catching effect” of the head and/or shoulder of the calf inside the soft part of the distal genital tract. Suffocation of the calf is a realistic complication. Quick interference is required, applying firm manual pressure from the outside on the calf in the sliding direction of the calf. Be aware of the risk of kicking by the mother.

2. Ruptured amniotic sac:

A significant event in the parturition process is the rupturing of the amniotic sac, which acts as a hydraulic dilatator for the cervix, a natural lubrication for the dam and a pressure protection for the calf.

NB: The allantois sac that covers the body directly, usually remains intact during the expulsion of the calf and ruptures during the final passage through the birth canal and is actively removed by the dam.

NB: a chained dam, may not be in the position to remove these membranes, possibly resulting in suffocation of the calf.

Differentiation between urine and amniotic fluid is extremely difficult; smell, creatinin test strips and possibly protein concentration could be helpful.

If no progress in parturition is observed, major complications should be considered, like a dead calf (herpes virus infection?), malposition of the calf (which is often dead), oversized calf, malformation and twin pregnancy. Because of the urgency of this situation, the calf should be born within 2 hours after rupture of the amniotic sac and loss of amniotic fluid. If not so, veterinary intervention has to take place. Two situations may occur:

No fetal parts positioned in the pelvic area: treatment should aim on the urgent relaxation of the cervix. Calcium status should be determined and treated accordingly (see above). The further approach is according to 1A, however the situation is more critical for both the dam and the calf.

Fetal parts have entered the pelvic area: Calcium status should be determined and treated accordingly (see above). Ultrasound is essential to determine which fetal parts have entered the birth canal, determine the position of the calf (visualization of the nails B proximal or distal position, number of nails, trunk) and viability. Malposition (e.g. only one leg in birth canal, no head in anterior position) is an indication for vaginal vestibulotomy or fetotomy.

If no abnormalities are found during ultrasound, 50-100 I.U. of oxytocin should be given i.v. or i.m. and rectal massage should be practiced. Birth should be completed within 1 hour.

Post-partum care

Disinfection of the navel with Betadine iodine

The afterbirth usually comes off within 12 hours. There are a few reports on retained (parts of) placenta for several weeks, without major complications for the dam. Hygienic measures should be applied to reduce the infectious burden for the calf.

Realize that a second calf can still be present in the dam. There are reports that second calves were born between several hours up to 3 months (the prevalence of twins in elephants is 1:3000).

The calf should drink (colostrum) as soon as possible, at least within 24 hours. If not, or when the calf makes a weak impression, the banked serum (or freshly taken serum) should be given to it orally. Try to find the reason why the calf is not drinking successfully: e.g. too small, weak, painful mammary glands, malbehaviour of the dam.

Vaginal vestibulotomy in elephants

Vaginal vestibulotomy is a surgical procedure, in which the vertical part of the uro-genital tract (vestibulum vaginae) is exposed by a percutaneous approach.

Indications for a vaginal vestibulotomy:

- * No progress in calving despite of treatment according to the guidelines for veterinary assistance around parturition.
- * Malposition

Contraindication for a vaginal vestibulotomy:

- * No fetal parts in the birth canal (confirmed by ultrasonographic examination and rectal palpation)

Preparations:

A. 1 extra ring in the floor between the hind legs should be present in any calving box for elephants. A pulley can be attached to it in order to provide optimal conditions for pulling the calf out (in a ventral direction). If not present, one should consider to use a steel bar fixed to the walls behind the cow. A pulley can be attached to this bar.

B. Don't use any sedation in the cow if not strictly needed, as you will need the straining support during the extraction. If the animal is completely hands off and restraint in a chute or otherwise is not an option, use a reversible sedation (xylazine/atipamezole) to chain the elephant on 4 legs. The sedation should be antagonized (if safety permits!) as soon as straining support from the elephant is needed. Remember that the behavior may have changed during this phase of the calving most likely in favor of the veterinary intervention: the animal is probably more interested in getting the calf out than in attacking the keepers around her. But there is not sufficient information about this point. The situation has to be evaluated for each animal separately.

C. Make sure you have a long balloon catheter to be placed into the urinary bladder at the end of the procedure (see under L).

D. Use local anesthesia only.

E. After cutting through the skin, place a flexible plastic tube (5-10 cm diameter or a rumen tube) retrograde into the vestibulum vaginae to locate the incision side. To facilitate orientation, a 10x2 cm window should be made in the tip of the tube, 3 cm from the end. This window can be palpated transcutaneously and facilitate a quick perforation incision.

F. Use normal calving chains. Don't use more than 3 people on each leg.

G. When the calf is in posterior position and can't pass through the pelvic girdle, **try to rotate the calf (90° longitudinal axis) during extraction.** In cattle, active rotation is the normal way of a live calf to pass its pelvis through the pelvic canal of the mother. Figure 3 (Rotterdam Zoo, 1998) shows the birth of an elephant calf after stagnation of the delivery caused by hypocalcemia, demonstrating clearly that the calf has rotated 90°. This had happened already at the time both hind legs could be palpated in the vagina. When the calf is

dead, the absence of this phenomenon might be responsible for stagnation of the calving process. In the experience of one of the authors (W.S. Rotterdam Zoo, 1993) it was only possible to remove a dead calf in posterior position by vaginal vestibulotomy after 90° rotation of the calf (figure 4).



Figure 3: Birth of an Asian elephant calf, showing the sideward rotation of the calf. (hypocalcemia, Rotterdam Zoo 1998)



Figure 4: Vaginal vestibulotomy in an Asian elephant. Extraction was only successful after 90° rotation of the dead calf. Note the umbilical chord twisted twice around the hind leg. (Rotterdam Zoo, 1993).

H. Don't hesitate to push the calf in the direction of the uterus; it might return in a better position. During this action, both legs and the trunk or tail should be well connected to the chains. Refill the genital tract with at least 5 l. of "artificial embryonic fluid" by using a pump (aquarium-type).

I. Don't pull at both legs in one direction at the same time. You must cross the chains and pull alternatively on each leg. It is more likely that each leg will pass the pelvic canal bit by bit, rather than that the two legs can pass in symmetry.

J. When the calf is out, flush the uterus with cold water, preferably until the placenta has come off. Give 50 IU of oxytocin i.v.

K. At this time (only if needed) the cow can be given a xylazine sedation (if sedated and reversed previously, use azaperone)

L. Place the balloon catheter into the urinary bladder. Cut it to the proper length, i.e. just below the wound. Use acrylic glue or some stitches to keep it in place.

M. Close the vaginal vestibulum wall in 2 layers with PDS or Vicryl.

N. Reverse the sedation, wash your hands, clean up the mess and have a beer. But **DON'T CLOSE THE SKIN**. Forget everything you learned about surgery. Whatever fancy suture technique you may use, the skin wound will open again. Don't think that your special suture will work, because every possible suture technique has already failed. Even when closing the skin, feces will still contaminate the wound, because the skin property does not allow a watertight wound closure; suturing the skin has proven to result in a permanent fistula. You may hope that by leaving the skin wound open, the vestibulum sutures may be exposed to a minimum of tension, giving the wound the chance to heal. By doing so, you might be the first

veterinarian performing this surgery without leaving the animal with a fistula!
Give antibiotics during at least 7 days.

O. If you were not able to remove the calf: **DON'T TRY TO PERFORM A CESAREAN UNLESS YOU ARE ABSOLUTELY SURE THAT THE COW WILL NOT SURVIVE CONSERVATIVE TREATMENT.** All cesareans performed till now have resulted in the death of the mother. If you see yourself facing the critical situation that the calf can not be removed through the wound and fetotomy is either no option or it has failed, the first advice is to leave the wound of the vestibulotomy open completely and see what happens during the days to come. Flush the uterus frequently with large amounts of water with a disinfectant like Betadine iodine to prevent the occurrence of Bandl's rings that may cause necrosis of the uterus. An antibiotic treatment is recommended.

Fetotomy

Only very few cases have required fetotomy. If you decide that a fetotomy is needed, you are strongly advised to contact Thomas Hildebrandt.

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