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Current understanding of elephant reproduction

Dennis Schmitt,[a,b] Wendy Kiso[a]
[a]White Oak Conservation Foundation, Yulee, FL; [b]W.H. Darr College of Agriculture, Missouri State University, Springfield, MO

Abstract

Elephants have many unique reproductive characteristics, including a prolonged estrous cycle and pregnancy, possible embryonic diapause, musth in male elephants, and unique reproductive anatomy of both sexes. Most of what is currently known regarding elephant reproduction is from studies conducted from elephants under human care. Elephant reproduction continues to be explored and new information is vital for conservation of elephants under human care or in free-ranging habitats.

Keywords: Elephants, musth, estrous cycle, semen, pregnancy, fetal development

Introduction

There are distinct differences in elephant reproduction compared to other animals. Elephants have a long estrous cycle (~ 14 - 17 weeks) divided into luteal (8 - 10 weeks) and follicular (4 - 7 weeks) phases. Follicular phase has 2 luteinizing hormone (LH) surges. First LH surge (nonovulatory) occurs ~ 3 weeks after progesterone (P_4) reaches baseline concentrations, and the dominant follicle becomes the primary corpus luteum (CL) after the second LH surge (ovulatory) that occurs 19 - 22 days after first surge.[1] Some of the nonovulatory follicles from the first LH surge are recruited to become secondary CLs. Primary progestins in elephants are 5.α-reduced pregnanes, whereas in most other mammals it is progesterone (4-pregnene-3, 20-dione). Although P_4 is a minor progestin in elephants, it is present at lower concentrations that are measurable by enzyme-linked immunosorbent assays (ELISAs), useful for monitoring cyclicity and pregnancy. Normal serum P_4 concentrations during estrous cycle are in ng/ml and during pregnancy rise substantially over estrous cycle concentrations,[1] although the values reported vary among laboratories and with the antibody used.

Bulls have intrabdominal testis near the kidneys, with no pampiniform plexus; however, normal body temperature is 35 - 36 °C with normal sperm production. Bulls have all 4 secondary sex glands. Bulls exhibit musth (peripheral testosterone concentrations increase 8 - 100 times) and challenge bigger, older bulls for access to females in estrus. Musth in elephants may occur any time of the year, but poor body condition may delay or prevent musth.[2] Semen from bulls for artificial insemination (AI) or frozen storage may be collected in a sedated/anesthetized bull via electroejaculation, or in a nonsedated bull by manual rectal massage of the ampullae glands.[3]

Female reproductive anatomy

The vulvar opening is not located just beneath the anus, as in other mammals, but is between the back legs, ~ 1 meter below the anus, in an adult elephant. This location facilitates the calf to be delivered horizontally just above the ground below the abdomen. Nulliparous cows have a strong hymen present between the vagina and the urogenital tract with 2 or 3 openings; however, usually only 1 is patent, and the others might be remnants of Wolffian ducts,[2] and are present until the birth of the first calf. The opening is ~ 0.3 cm in diameter. The vagina in multiparous females may be ~ 50 cm long and the cervix has longitudinal folds that resemble equids. The uterus has a small uterine body with long horns 0.5 - 0.7 meter. The oviduct is relatively short, and the ovary is present close to the uterine tip.[2]

Although the penis does not reach into the vagina, the mobility of the penis and flexure of the glans penis allows penetration of the vertical urogenital canal. Ejaculation is into the cranial urogenital canal just anterior to the caudal pelvic brim. A substantial volume of ejaculate is usually expelled at dismount. The hymen remains intact in nulliparous cows after breeding and can rendering artificial insemination challenging. The ejaculate must travel through the opening in the hymen to enter the vagina, and then navigate through the vagina to the cervical os, through the cervix and into the uterus. For sperm to reach the oocyte, they travel over 1 meter from deposition in a nulliparous cow to the site of fertilization. Although ovulation is ~ 12 - 24 hours after second LH surge, the state of meiosis for the oocyte is unknown at ovulation or the interval from ovulation until it is ready for fertilization. Additionally, the lifespan of sperm in the reproductive tract of elephants after natural breeding or artificial insemination is unknown.[2] The minimum insemination dose is unknown as not enough inseminations have been performed with limited numbers of sperm. Fresh inseminations are performed using semen from 1- 3 bulls with shipped, cooled semen and utilizing all samples deemed suitable for

insemination at the receiving elephant facility.

Estrous cycle

Ovaries are approximately twice as large as in cattle. Ovulatory follicles and CLs are 1.5 - 3 cm in diameter. The follicular phase of the estrous cycle is prolonged with 2 LH surges during the 6 - 8 weeks follicular phase. There are 2 - 3 nonovulatory follicles on each ovary that partially luteinize following LH1 and remain dormant until LH2, when 1 dominant follicle ovulates and becomes a CL and the nonovulatory partially luteinized CLs are thought to activate with LH2.[1-7] Approximately 3 weeks after progesterone concentrations reach baseline, an LH1 surge occurs with several of nonovulatory follicles partially luteinizing. On average 21 (range 19 - 23) days after LH1, an LH2 surge stimulates ovulation 12 - 24 hours later. Progesterone concentrations in the US are usually monitored from weekly serum or fecal samples via ELISA assays. Serum P_4 concentrations often briefly increase during 2 - 3 days prior to LH2 surge, then decrease on the day of ovulation and subsequently increase dramatically within 1 - 2 days following the LH2 surge. Progesterone concentrations are elevated for ~ 10 - 14 weeks and then decrease to baseline concentrations.

Estrus begins at ~ 3 - 4 years in elephants in human care in Western countries, and at 6 - 7 years in range countries. Free-ranging Asian elephants in range countries begin cycling at 5 - 7 years and at 8 - 10 years in African elephants. They usually become pregnant after 2 or 3 cycles and have a pregnancy interval of 4 - 6 years.[5]

Uterine pathologies develop in many nulliparous cycling elephants starting during their mid to late 20's and early 30's. Asian elephants primarily develop leiomyomas and African elephants primarily develop endometrial cysts; both are identified throughout the uterus.[7] It is assumed that these are the result of repeated estrous cycles due to elevated estrogen concentrations, as they are not observed in multiparous elephants that first became pregnant at 6 - 10 years and have calves every 4 - 6 years.

Pregnancy

After a successful breeding with fertilization, P_4 concentrations remain similar to nonpregnant elephants in their luteal phase until they begin to decrease at 8 - 9 weeks before recovering at weeks 10 - 12 postbreeding equivalent to and higher than normal luteal phase concentrations.[3] P_4 concentrations during first two-thirds of pregnancy are often 2 - 3 times higher than nonpregnant estrous cycles. During the last third of pregnancy, most elephants' P_4 concentrations begin to decrease, but remain higher than nonpregnant estrous cycles.[8-12]

Prior to 8 - 9 weeks of pregnancy an embryonic vesicle is not visible via ultrasonography. At 8 - 9 weeks, the embryo is ~ 1 - 3 cm in diameter and reaching ~ 5 cm in diameter. Over the next 2 - 3 weeks the embryo grows dramatically until ~ week 12 - 14 when the head, leg buds, and trunk can be identified via transrectal ultrasonography. During this time, the zonary placenta and umbilical cord become evident. The placenta is endothelial zonary, similar to dogs.[2] The possibility that this is a fixed embryonic diapause has been proposed, but it could be slow development of the early embryo and embryonic vesicle rather than a true embryonic diapause. At week 16, the calf is well formed and independent movement of the fetus is observed. Crown rump length can be used to determine gestational age in a growing fetus after weeks 10 - 12 of pregnancy. Most detectable embryonic loss occurs after 9 weeks (when the embryo can be detected via transrectal ultrasonography) during the first 14 - 16 weeks of pregnancy.[8] However, fetal loss/abortion can occur throughout pregnancy.

In most pregnant cows, the ability to visualize the fetus transrectally is lost due to the pregnant uterus moving below the depth of ultrasound penetration in a standing elephant. However, the ability to visualize the growing fetus can be extended if the pregnant cow is examined in lateral recumbency. The pregnant uterus can often be visualized after ~ 40 weeks of pregnancy while standing as it is usually heavier than the gastrointestinal tract and descends to the floor of the abdomen. This allows transabdominal monitoring of the growing fetus; however, that may not be possible during all examinations.[13] The gender of an elephant fetus is not evident in ultrasonography due to similar locations of external genitalia, ovaries, and testes in both sexes. At 12 months the fetus is ~ 30 cm in length and height. The calf continues to grow until term at ~ 93 weeks (~ 660 days) ± 4 weeks. At term, the fetus is 90 cm in height and weighs ~ 100 - 150 kg in western countries and is 70 - 80 cm in height and weighs 50 - 75 kg in range countries.

Parturition

Pregnant elephants in the US are usually monitored weekly with P_4 ELISA assays. As parturition nears, the frequency of serum sampling increases. Usually, daily serum sampling begins by day 630 of a known breeding date and is followed closely until parturition. In most elephants, P_4 concentrations decrease to baseline 3 - 5 days prior to onset of phase 1 labor, although phase 2 labor can occur as early as day 2 of baseline or as long as 2 weeks following P_4 going to baseline. Phase 1 labor is noted by behaviors associated with impending parturition. They may include agitation, frequent urination and defecation, slapping vulva with tail, sudden freezing of stance, etc. The transition of phase 1 to phase 2 labor can be monitored via transrectal ultrasonography. Relaxation and dilation of the cervix is visible and the progression of the fetal membranes and fetus into the birth canal can be easily determined.[11] Calves are presented approximately equally cranially or caudally into the birth canal. Prior to the observation of active labor, passing of a cervical plug may be observed.

Assisted delivery of a calf is limited to manual stimulation of labor contractions by massage of the rectum over the vagina and cranial urogenital canal and/or exogenous oxytocin. Prior to treatment, the position of the calf in the birth canal with

both feet in position should be confirmed. Manual massage of the rectum is performed with a closed fist and substantial amounts of good-quality lubricant. Often 3 - 4 attendants are needed in rotation to initiate productive contractions by the cow. If contractions are not continued, the cow can be treated with low doses of oxytocin. Elephants are sensitive to oxytocin and it should be used with discretion. One of the authors uses 40 IU of oxytocin as an initial dose intramuscularly. If after 1 hour, contractions are not maintained, he considers giving 50 - 60 IU of oxytocin. If the cow has 1 or 2 contractions after either dose and stops immediately, no further stimulation is given to eliminate the risk of uterine rupture.

As the placenta and calf pass out of the pelvis it creates a bulge under the anus. Often the bulge will disappear from view as contractions ebb and flow. Next to appear are the feet of the calf. If the calf is presented caudally, the legs protrude much further compared to cranial presentations. Then, the calf turns downward into the vertical urogenital canal. It is during this turn that the chorioallantois ruptures or as often described, when the 'water breaks'. This occurrence can be difficult to differentiate visually from urination, as there is not much fluid in this compartment. As the umbilical cord is relatively short, time for delivery of the calf after turning downward is critical. It still has almost 2 meters to travel before being expelled. Delivery of the calf occurs as the calf is expelled from the vulvar opening, as the calf becomes located just caudal of the umbilicus of the cow due to extension of the vertical urogenital canal forward as the calf passes through.

Dystocia in elephants occurs primarily because of obesity of the cow and oversized calves. However, occasionally dystocia occurs as the result of malformed calves, or malpositioned limbs. No cow has successfully survived a cesarian surgery.[2] However, if the allantochorial membrane has not ruptured and the feet have not passed through the pelvis, the cow will usually survive. The calf may be resorbed, become semi-mummified, or be passed many months later. The cow often begins cycling again in a year or 2 and may deliver the calf later. One of the authors observed a cow beginning to cycle again 2 years later and trying to deliver the calf 5.5 years after term. At term the calf was presented cranially, but when delivered 5.5 half years later, the calf was presented caudally. The calf was extracted via a vestulotomy and weighed > 450 pounds without head and 1 leg that apparently had liquified. There was no indication of an infection upon delivery. The calf was intact except for the parts that had liquified. A few cows have become pregnant subsequent to delivering a retained calf.[9]

Phase 3 of parturition involves expulsion of fetal membranes and usually occurs within a few hours after delivery of the calf, often subsequent to the calf's first nursing bout. Occasionally, elephants experience retained fetal membranes. Removal is complicated by the weight (15 - 20 kg) and the inability to reach it manually. Elephants transfer most of their antibodies transplacentally, and there is no true colostrum excreted by the cow. If it is necessary to supplement calves that are not effectively nursing, milk can be extracted from the cow's mammary glands either by hand milking or by using a human breast pump. Elephant nipples have 10 - 13 milk ducts on each breast. Mastitis is rarely noted in elephants.

Male reproductive anatomy

As mentioned earlier, the elephant penis is very mobile and has strong levator penis muscles. The bull has large seminal vesicles that produce most of the ejaculate. Ampullae store a substantial amount of sperm and the success of semen collection can be gauged on the size of seminal vesicles and ampullae. However, manual stimulation often results in urine contamination. Prostate glands vary in each species. African elephant prostate glands are larger (~ 5 cm) and the lobes are filled with irregular glandular tissue. The Asian elephant prostate gland is small (2 cm) and the glandular tissue is homogenous. All of these glands can be reached transrectally with a hand-held ultrasound transducer. The bulbourethral glands are located caudal to the root of the penis and dorso-laterally to the urethra; they can be examined transcutaneously on either side of the penis. Testes are located just caudal and medial to kidneys. They can be examined ultrasonographically by using a transducer holder, extending the operator's reach by 35 - 45 cm. Bulls with testis > 12 cm in diameter usually are considered capable of producing viable sperm.[14] Testes do not have a pampiniform plexus to cool them as the normal body temperature of elephants is in the range of scrotal testes in other species. Additionally, epididymides of the elephant are not adhered to the testes but are coiled ducts that extend to the vas deferens that connect to the ampullae.

Reproductive evaluation of the bull is hindered by the fact that most collections without an electroejaculator do not yield a complete ejaculate, although a few bulls will respond to manual massage with complete ejaculation and very high-quality semen samples. Attempted use of an electroejaculator without heavy sedation or anesthesia is not recommended.[3] If the ejaculate does not contain seminal vesicle fluid the sperm are usually immotile. Manual massage often results in urine contamination.

Semen storage

High quality semen can be used for fresh cooled insemination. African elephant semen is very tolerant to a range of extenders. A common extender used for fresh cooled insemination with short-term storage can be as simple as HEPES buffered saline or as complex as TEST or homemade egg yolk-based extender.[2] The complete ejaculate is extended depending on the sperm concentration 2 - 4 times and cooled, and put into an equine semen shipper to be sent to the cow's facility. Successful pregnancies occurred in the last few years using African elephant frozen semen. Asian elephant semen is not tolerant of simple extenders, and for fresh cooled semen TEST extender is recommended for cooling with the same protocol as noted for African semen. However, there were no successful Asian elephant pregnancies

from frozen semen to date, although a conception was reported with early embryonic loss.

Musth

Musth is a unique physiological state in elephants. Musth bulls become aggressive and their testosterone concentrations are highly elevated. Beyond behavioral and endocrine changes, it is manifested by drainage from temporal glands (a modified salivary gland) located between their eye and their ear, excessive urine dribbling, and loss of appetite. During musth, the smell is similar to that of cattle with ketosis, but much more potent. This communicates to other elephants their status, to discourage competition for estrous females. Bulls in this state of musth often begin to lose weight and go out of musth.[2] However, bulls in human care with high-quality feedstuffs can be obese and may stay in musth for several months, whereas bulls in free-ranging conditions have much shorter musth. It is thought that the dominant bull in free-ranging conditions will cause subordinate bulls to delay their musth around the dominant bull in the region.

Control of musth of bulls in human care has been a concern for many years. Castration was performed in > 20 bulls in the 70's and early 80's. However, they were not castrated until they were 4 - 6 years. Several of them had already developed an aggressive attitude and castration did not alter their personalities. There are currently 3 castrated bulls in the US and all are of mild disposition. Recently, with the availability of a GnRH vaccine, a few bulls were vaccinated and with subsequent boosters have not displayed musth.[2] The success of this vaccine will depend on whether it can be reversed if needed for the bull to resume sperm production, or if vaccinated prior to puberty, whether it will be permanent, or can go through puberty at an older age.

Conflict of interest

There are no conflicts of interest to declare.

References

1. Brown JL: Reproductive endocrinology. In: Fowler ME, Mikota SK: editors. Biology, Medicine, and Surgery of Elephants. Ames; Blackwell: 2006. p. 377-388.

2. Schmitt D: Reproductive system. In: Fowler ME, Mikota SK: editors. Biology, Medicine, and Surgery of Elephants. Ames; Blackwell: 2006. p. 347-355.

3. Hildebrandt, T, Lueders, I, Hermes, R, et al: Reproductive cycle of the elephant. Anim Reprod Sci 2011;124:176-183.

4. Hermes R, Olson D, Goritz F, et al: Ultrasonography of the estrous cycle in female African elephants Loxodonta africana. Zoo Biol 2000;19:369-382.

5. Lueders I, Niemuller C, Rich P, et al: Gestating for 22 months: luteal development and pregnancy maintenance in elephants. Proc R Soc B 2012; doi:10.1098/rspb.1038

6. Allen WR: Ovulation, pregnancy, placentation and husbandry in the African elephant Loxodonta africana. Philos Trans R Soc Lond B Biol Sci 2006;29:361:821-834.

7. Thitaram, Chatchote and Brown, Janine L. Monitoring and controlling ovarian activity in elephants. Theriogenology 2018;109:42-47.

8. Brown, JL, Schmitt, DL, Bellem A, et al: Hormone secretion in the Asian elephant (Elephas maximus): characterization of ovulatory and anovulatory luteinizing hormone surges. Biol Reprod 1999;61:1294-1299.

9. Lueders I, Niemuller C, Gray C, et al: Luteogenesis during the estrous cycle in Asian elephants (Elephas maximus). Reproduction 2010;140:777-786.

10. Stansfield FJ, Allen WR: Luteal maintenance of pregnancy in the African elephant Loxodonta africana. Reproduction 2012;143:845-854.

11. Hermes R, Hildebrandt TB, Goritz F: Reproductive problems directly attributable to long-term captivity – Assymetric reproductive aging. Anim Reprod Sci 2004;82-83:49-60.

12. Howard J, Bush M, de Vos V, et al: Electroejaculation, semen characteristics and serum testosterone concentrations of free-ranging African elephants Loxodonta africana. J Reprod Fertil 1984;72:187-195

13. Hildebrandt TB: Reproductive and diagnostic ultrasonography. In: Fowler ME, Mikota SK: editors. Biology, Medicine, and Surgery of Elephants. Ames Blackwell: 2006. p. 357-355.

14. Drews B, Hermes R. Goritz F, et al: Early embryo development in the elephant assessed by serial ultrasound examinations. Theriogenology 2008;69:1120-1128.

15. Hildebrandt T, Hermes R, Pratt N, et al: Ultrasonography of the urogenital tract in elephants Loxodonta africana and Elephas maximus: an important tool for assessing female reproduction function. Zoo Biol 2000;19:321-332.

16. Hildebrandt T, Hermes R, Pratt N, et al: Ultrasonography of the urogenital tract in elephants Loxodonta africans and Elephas maximus: an important tool for assessing male reproduction function. Zoo Biol 2000;19:333-245.