特集：ゾウの繁殖学国際シンポジウム

第 17 回日本野生動物医学会大会 大会実行委員会企画シンポジウム 2011 年 10 月 1 日（土）

Anatomical, Physiological, Behavioral and Pathological Aspects of the Estrous Cycle in Elephants

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ゾウ類の発情周期に関する解剖学的、生理学的、行動学および病理学的特徴

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ABSTRACT. The combination of a few factors, including poor captive reproduction, secession of importation from the wild, advances in hormone detection and ultrasonography, and, regrettably, elephant culling in Africa, has contributed to the current knowledge on the elephant cycle. Several reproductive features in elephants differ markedly from other mammals. These include the urogenital tract anatomy, length and structure of the cycle, the formation of multiple corpora lutea and the type and secretion pattern of reproductive hormones. Being 12 to 18 weeks in length, the elephant estrous cycle is the longest amongst all studied non-seasonal mammals to date. Progesterone rises one to three days after ovulation, indicating the start of the luteal phase, which lasts six to 12 weeks. This is followed by a four to six weeks follicular phase that is concluded by two, precisely spaced and timed, LH surges. In general, the first, anovulatory LH surge occurs exactly 19 to 21 days before the second, ovulatory surge. Normally, a single follicle is ovulated. However, beside a corpus luteum (CL) forming on the site of ovulation, multiple accessory CLs can be found on the ovaries. Unlike many other species, the predominant progestagen secreted by luteal tissues is not progesterone, but rather its 5- α -reduced metabolites. The currently known aspects of the unique estrous cycle in Asian and African elephants, covering estrous behavior, circulating hormones, ultrasonography and anatomy of the reproductive organs and its pathology as well as hormonal manipulation treatment possibilities, will be reviewed here.

Key words : female elephant, estrous cycle, hormonal pattern, follicular development, accessory corpora lutea

Jpn. J. Zoo. Wildl. Med. 17 (3) :97-109, 2012

INTRODUCTION

The order proboscidea contains only one family of living animals—the elephantidae, which includes the genera *Loxodonta* with two species—African savannah elephant (*Loxodonta africana*) and African forest elephant (*Loxodonta cyclotis*), and *Elephas* with a single species—Asian elephant (*Elephas maximus*). Survival of all three elephant species in their native habitats is threatened [1], in particular that of the Asian elephant, which is classified in the IUCN red list as an endangered species [2]. Nearly 150 years ago, Charles Darwin wrote the following prediction: “The slowest breeder of all known animals, namely the elephant, would in a few thousand years stock the whole world.” [3]. Unfortunately, primarily due to human activities, this prediction will probably not come true.

In the past, before importation of wild-captured elephants was brought to a halt, particularly for Asian elephants, few zoos saw the need to go into major investments related to keeping bulls and enhancing reproduction. It was easier and cheaper to replenish their group with new elephants from the wild when needed. Once the elephant was added to Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) in 1976, it was no longer possible to import new Asian elephants and importation of African elephants was greatly restricted. The inability to sustain captive populations through new imports from the wild, insufficient captive offspring production, and the decline of elephant numbers in their natural habitats, have prompted research on the reproductive physiology of these magnificent creatures. These efforts were coupled by advances in hormone detection techniques in serum, urine and fecal samples and

the utilization of ultrasonography as a non-invasive tool to help understand reproductive events such as the onset of puberty, ovarian cycle activity and reproductive senescence in elephants.

The elephants' separate taxon position makes comparison of their reproduction physiology with models of related animals difficult. Thus, research on these animals, primarily in their natural habitat, provides us with knowledge of new, and apparently successful, reproductive strategies within the animal kingdom [4-6]. In general, the reproductive processes within the family elephantidae are believed to be similar [7], however, when compared, slight differences in the hormone profiles, especially during pregnancy, are apparent [8, 9]. Elephants' reproduction, as our largest and most charismatic land mammal, has interested many researchers around the world. During the large culls in Africa from the 1950's to the 1980's, the understanding of reproductive anatomy and physiology increased considerably based to post mortem investigations [10-12]. Advances in the use of ultrasonography and radio- and enzyme immunoassays in wildlife medicine played a decisive role in the progression of reproductive understanding and management in both elephant genera.

The growing database on reproduction biology of elephants has led to the development of assisted reproduction technologies, including sperm cryopreservation and artificial insemination [13-15] and enhanced birth rates in captivity [16]. However, some gaps in our knowledge still remain.

ANATOMY OF THE FEMALE REPRODUCTIVE TRACT

Formerly, most of the knowledge on the reproductive organs in elephants was derived from large culls of African elephants [10] and single specimens of Asian elephants [17]. Thus, historically, basic information has mostly relied on findings in African savannah elephants and from them applied to the other species. These pioneers provided the groundwork on our understanding of elephant reproduction. Possibly the most striking characteristic of the female elephant reproductive tract is its size. Being about 3 meters in total length, it is the longest amongst all land mammals [17, 18]. The vestibule (canalis urogenitalis) has an average length of 1.3 m and it opens ventrally, at the vulva, between the hind legs. This tube-like structure runs caudo-vertical from between the hind legs towards the tail, where it curves cranially around the pelvic bone to form a 20 to 40 cm long horizontal sac. At its cranial end, the hymeneal membrane is found, leaving only a tiny opening of about 4×2 mm to the vagina. This vaginal os is guarded left and right by two blind pouches of similar

size. The hymen will rupture during the first delivery and not during copulation, as is the case in other species. The vagina extends to a length of up to 50 cm and its internal surface is folded into longitudinal folds. The lumen of the vagina is filled with mucous which increases markedly during the transition from the luteal to the follicular phase. The mucous gradually dissolves prior to ovulation and is excreted during urination. The mixture of urine and mucous contain specific pheromones that act to attract breeding bulls to the female in estrous [19]. The folded cervix is about 15 cm long with a prominent cone-shaped portio cervicalis. The bi-corneal uterus includes a corpus uteri (about 15 cm long) that splits into two long uterine horns. The endometrium lining the uterine body and horns show longitudinal rugae. Each pregnancy leaves permanent damage on the endometrium that is repaired by scar tissue so that the number of pregnancies can be determined during autopsy by counting these scars [20]. The comparably small ovaries, averaging 55 to 65 mm [11, 21], of adult females are completely enveloped in a serosal pouch, which forms the oviductal infundibulum and is incorporated into the two ovarian bursae [22]. Physiologically, the ovaries always contain functional structures such as small follicles during the follicular phase, in addition to a preovulatory follicle at estrous, and multiple corpora lutea at all times, however the progesterone concentration remains at baseline during the follicular phase [23, 24]. Between 6 and 42 corpora lutea have been recorded on both ovaries [25], with larger numbers recorded in younger, pregnant cows [12].

ENDOCRINOLOGY OF THE CYCLE

A schematic representation of changes in reproductive hormones during the estrous cycle in elephants is presented in Fig. 1. Important reviews and summaries of the reproductive endocrinology of the elephant include those by Hodges [7], Niemuller et al. [26], Brown [27] and Brown et al. [8]. Routine endocrine monitoring of the estrous cycle has been achieved through sampling of faeces, blood and urine by many elephant keeping facilities. It is an indispensable tool for the successful management of elephant breeding groups. Recent improvements in the analysis techniques allow measurement of progestagens [28-31], but not LH or prolactin [32], non-invasively, from feces or urine, and even under field conditions. In the following text, a brief overview of major circulating hormones is presented.

Progestagens

Until the early 1980's it was believed that the Asian elephant has a cycle length of about three weeks [33, 34]. This assumption was due to the difficulties in measuring

Fig.1 Schematic of the elephant estrous cycle, hormone secretion and ovarian activity
I. Secretion of Progesterone, FSH and inhibin in relation to the LH peaks, and II. Follicular waves, estradiol surges, luteinizing follicles (LUF), accessory corpora lutea (acCLs) and the single corpus luteum derived from ovulation (ovCL). Note that multiple acCLs form after the 1st LH peak from luteinizing follicles. Luteinization and inhibin secretion occur in close time relation and most likely represent a precondition for the formation of an ovulatory follicle at the 2nd LH peak (Modified from [6, 91]).

progesterone at that time. In early attempts, no progesterone could be measured in serum samples [12]. Plotka et al. [35] demonstrated for the first time differences in serum concentrations of progesterone between immature, cyclic and pregnant African elephants, but no correlation with the stage of the ovarian cycle was detected. It was Hess et al. [36], who succeeded in measuring transient elevation in concentrations of progesterone in six non-pregnant Asian elephants in a typical biphasic cycle fashion. They reported that the mean concentrations of progesterone increased from 30-70 pg/mL during the follicular phase to a peak of 400-700 pg/mL during the luteal phase. Based on these fluctuations in 15 ovarian cycles, they determined the length of the cycle to be 16.3 ± 0.4 wk (mean $\pm$ SEM). The luteal phase was 10.5 ± 0.3 wk, with 5.1 ± 0.4 wk separating subsequent luteal periods. This was later confirmed by other scientists and was found to be true for African elephants as well. More than a decade later it was shown that it was not progesterone, but rather its 5- α -reduced metabolites (5- α -Pregnan-3,20-dione and 5- α -Pregane-3-ol-20-one) that are secreted by the CLs into peripheral circulation, with 5- α -Pregnan-3,20-dione predominating [37, 38]. Previous differences in the results on progesterone were therefore most probably related to cross-reactivity of the α -reduced pregnanes with the antibodies originally used to detect progesterone. In Asian elephants, 17- α -hydroxyprogesterone is another metabolite present in significant quantities in serum [39]. This is strikingly different from other mammals, where circulating progesterone is quantitatively the major progestagen.

Estrogens

Early attempts to measure fluctuations of estrogens during the estrous cycle in elephants failed to show a clear pattern [36]. Later studies showed that serum estrone and 17 β -estradiol seem to be mostly conjugated in the elephant serum so the remaining, unconjugated, estrone is low - less than 20 to 50 pg/mL [40, 41]. The assumption in the early days, based on measurements of both serum and urine estrogen contents, was that estrogen fluctuations occurred at 18 to 21 days intervals and reflected waves of follicular growth. Through the use of a more sensitive radioimmunoassay (RIA), Taya et al. [40] found baseline estradiol values during luteal phase and elevations in the follicular phase, with only one distinct peak (Asian elephant), whereas other scientists later found slight, but significant estrogen elevations in serum samples prior to each of the two LH surges (see point on LH herein under) in African elephants [42]. This was confirmed for the Asian species through urinary estradiol, where E2 start increasing five days prior to and reaching its peak a day prior to each

LH peak [43]. This estradiol pattern supports the suggestion regarding the occurrence of two follicular waves.

Inhibin

It is generally accepted, that inhibins are secreted from the granulosa cells of ovarian follicles in females and, through suppression of FSH secretion, play a key role in dominant follicle selection and determination of species-specific ovulation rates. As in many other mammals, in elephants, concentrations of FSH and inhibin are negatively correlated during the cycle [27]. Serum concentrations of inhibin remain low during early inter-luteal phase; begin increasing in the late part of this phase and the early luteal phase and reach maximum values during the mid-luteal phase. They then gradually decline to reach nadir concentrations approximately three weeks prior to the decline of progesterone that leads to the follicular phase of the following cycle [44, 45]. Concentrations of inhibin are therefore phase-shifted and positively correlated with concentrations of progesterone and during the late non-luteal and early luteal they are also negatively correlated with FSH [46]. This, together with immunohistochemical staining on luteal cells indicates that in elephants the CLs, in addition to the granulosa cells of ovarian follicles, secrete bioactive inhibins [44]. In Asian elephants, high level of bioactivity and immunoreactivity of inhibin in the follicular fluid has been demonstrated with inhibin α , β A and β B localized to the granulosa cells of antral follicles. In African elephants, the immunoreactivity of the α subunit of inhibin was found in the lutein cells, indicating its secretion from the CLs during the luteal phase [44]. Based on the secretion pattern of inhibin, it was suggested that the luteinization of the larger follicles from the first follicular wave enables the secretion of inhibin from the luteinized granulosa cells. Elevated inhibin in turn facilitates the selection of the dominant follicle from the second follicular wave, which ovulates following the 2nd LH peak. The level of immunoreactive inhibin was found to be positively correlated with the diameter of the accessory CL, with a lag-shift of two weeks ($R^2=0.82$, $P<0.001$) [6].

Luteinizing Hormone

Luteinizing Hormone (LH) is secreted by the anterior pituitary gland and known to peak shortly before ovulation in most mammals. This preovulatory LH surge induces maturation and ovulation of the dominant follicle(s) and initiation of granulosa cells' luteinization to finally form the corpus luteum. In elephants, however, it was noted that the LH activity is different. Hess et al. [36] already observed that "Peaks of immunoreactive LH were observed, although more than 50% of such 'surges' were unrelated to increments in progesterone (P4) secretion, and no evidence of bioactivity was observed

in any serum sample.” More than a decade later it was determined that not one but two distinct LH peaks occur in an estrous cycle (African: Kapustin et al. [42] ; Asian: Brown et al. [47]). These peaks occur consistently 19 to 21 days apart. No qualitative or quantitative differences between the two surges or between the two genera of elephants are detectable. Only the second LH peak induces ovulation and the subsequent progesterone rise. The time, however, from commencement of the follicular phase to the first LH peak may vary according to season, being significantly longer during the rainy season (33.4 ± 1.8 days) than during the summer (18.9 ± 2.6 days) or winter (22.2 ± 4.5 days) in semi-captive elephants in Thailand [48] . Several explanations were proposed for the function of the first, anovulatory LH peak. One theory suggests that it may be part of the elephants mating strategy [43] . Chemical signals in urine sampled near the first and second LH surges are similar. It was therefore hypothesized that the first LH peak produces the necessary chemical signal required to attract the usually solitary-roaming bulls early enough prior to ovulation. Another hypothesis suggests that the function of the first LH peak is to serve as a filter for follicular development so as to avoid multiple ovulations, which could lead to fatal twin conceptions occurrence [5, 24] . And still another explanation for anovulatory LH peak may be to prepare the uterus for ovulation and conception. After the long luteal phase, the first LH peak may have some sort of organizing function on the endometrium, and it was suggested to be a short cycle within the long cycle [42] . It can also be suggested that the purpose of the first LH surge is to reset the system so that proper and synchronized endocrine and follicular development will take place towards the second LH surge and the eventual ovulation. Based on correlations between ultrasound and endocrine measurements, we think that the most probable explanation is that the first LH peak is there to luteinize the larger follicles from the first follicular wave. In a study on 5 Asian elephants we found that larger follicles from the first follicular wave (10 to 19 mm in diameter) luteinize 7.0 ± 2.4 days prior to the 2nd (ovulatory) LH peak. In parallel to their luteinization, an increase in serum immunoreactive inhibin concentration is detected. We thus suggest that the first LH peak serves at least two purposes: it initiates the luteinization of follicles to form accessory CLs [24] and it helps create a source for inhibin which is important for the selection of the dominant follicle during the second wave [6] .

Follicle stimulating hormone (FSH)

It is now known, that during the inter-luteal phase of the bovine estrous cycle, each follicular wave is preceded by an elevation in FSH secretion. Circulating concentrations of FSH

decline after the selection of dominant follicles in each wave and the secretion of inhibin and/or estradiol by them [49] . Yet, for elephants, FSH secretion seems to follow a different pattern. Its concentrations reach their maximum at the beginning of the non-luteal phase and then declined steadily to a minimum within four days of the ovulatory LH surge. Concentrations remain low until after the ovulatory LH surge and then increase during the early luteal phase followed by a relatively long period of elevated concentrations, lasting at least 7 to 8 weeks, that covers the late luteal and early follicular phases [8, 47] . Since FSH serves as the key hormone for follicle recruitment and growth [44] , these findings imply a single, prolonged, stimulation for follicular development of the two waves over most of the inter-luteal period. In a study on African elephants it was found that the most common follicles in the ovaries are the early primary follicles ($75.8 \pm 11.8\%$), followed by true primary follicles ($23.8\% \pm 11.8\%$) with primordial follicles constituting less than 2% [50] . The distribution of these follicles did not differ between ovaries [51] . It was also noted in this study that the ovarian early, pre-antral, follicular pool in is relatively small, being about 10% or less that of similar aged women. It is usually assumed that once a follicle advances from primordial to early primary stage, it is committed to complete development and either reach ovulation or progress to atresia. If this is the case in elephants, then the majority of follicles are committed to complete their development. During the luteal phase no follicular activity was recorded [24, 52] . However, there is one study, in which two peaks of bioactive FSH were detected within the 5-6 week follicular phase, when daily urine samples of an Asian elephant were analyzed [53] .

Prolactin

The species differences between Asian and African elephant reproduction are most apparent in the secretion of prolactin (PRL). Whereas in African elephants, concentration of PRL is highest in the follicular phase, in Asian elephants no clear pattern of PRL during the cycle has been detected [54, 8] . Elevated PRL to pathological concentrations (hyperprolactinemia) or even the mere lack of cyclic secretion pattern in African elephants were linked to acyclicity [54, 55] . In pregnant elephants, PRL rises after four to six months of gestation in both species [56, 57] , it peaks at around the 11th to 14th month of gestation and a second peak is observed around the 18th to 20th month of pregnancy [58] . Prolactin shows a sharp decline shortly after parturition. Using immunohistochemistry, these researchers showed that the placenta is the source for the immunoreactive PRL during pregnancy in both Asian and African elephants. PRL can have

luteotrophic, luteolytic or luteostatic effects depending on the species and reproductive status. It may therefore play a role in the control of the CL lifespan and function, at least during pregnancy [59] .

THE OVARIAN CYCLE

Elephants are considered to be non-seasonal, polyestrous breeders although the issue of seasonality has been challenged (see section on sociosexual behavior). In the wild, females reach maturity at an age of 10 to 12 years [10, 60] , whereas in captivity pregnancy was reported to occur at an age as young as four and seven years for Asian and African elephants, respectively. Analysis of the European and North American Asian elephant studbooks show, for example, that the earliest age at calving in both populations was at about five years and nine months, which translates to conception at an age of four years.

The elephant exhibits the longest spontaneous estrous cycle of any mammal studied to date: 12 to 18 weeks in duration, with a six to 12 week luteal phase and four to six week follicular phase (First reports, Asian: Hess et al. [36] ; African: Plotka et al. [61]). This suggests that females may express only three to four fertile cycles per year. Free-ranging animals however are either pregnant or in lactational anestrus, thus multiple non-conceptional cycles seem to be rare in the wild [5] . The fact that elephants are primarily uniparous (twins occur in approximately 1 % of the births, [26]) and the finding of only one fresh ovulation in an African elephant female shot shortly after copulation [11] , led to the assumption that elephants must be a monovulatory species. It was not until the introduction of ultrasound technologies for reproduction monitoring in elephants [24, 62, 63] that it was possible to prove this theory to be correct. Today, elephants are known to be a monovular species, however multiple CL are present in every cycle and pregnancy. The origin of the accessory CL stayed unresolved for a long time, however, through ultrasonography it was possible to show that accessory CLs form from non-ovulatory, luteinizing follicles during each cycle [52, 64] .

Changes throughout the cycle were studied in African elephants by Hermes et al. [24] . The study demonstrated two follicular waves within the inter-luteal phase. Only the second wave bore a dominant follicle that ovulated. For an animal of such a body size, the ovulatory follicle ruptures at a comparatively small diameter of about 21 ± 0.5 mm, 12 to 24 hours after the ovulatory LH peak. This was later confirmed for the Asian elephant as well [6, 52] . Luteinization of non-ovulatory follicles can be regularly seen after the 1st LH

peak, prior to estrous. In African elephants, the ovulatory CL reaches a diameter of 22.5 ± 0.8 mm shortly after ovulation, and it can no longer be distinguished from the other CLs after that [24] . A different situation was found in the Asian elephant. In a study on 33 Asian elephants, it was observed that accessory CLs were derived from luteinization of larger, subordinate follicles after the 1st, anovulatory LH peak. The dominant follicle produced the largest CL after the 2nd (ovulatory) LH peak. The first luteal tissue formation became visible approximately 10 days after the respective LH peak. After ovulation, it took 29.8 ± 5.0 days for the accessory CLs to reach their maximum diameter (25.7 ± 3.3 mm), whereas the ovulatory CL reached a significantly larger size (33.2 ± 2.3 mm, $P < 0.0001$) about 10 to 15 days later. All CLs were visible throughout the new follicular phase, with some of the larger ones still present in the subsequent luteal period. The ovulatory CL started regression later than the accessory CLs [52] . In that study it was also found that ovulation occurred more in the right ovary (59.9%) and that more accessory CLs were observed on the ovary carrying the CL derived from ovulation. Follicles start forming, or at least can be discerned by ultrasound, as soon as concentrations of progesterone have reached baseline [52] .

The endocrine status has distinctive influence on the uterine texture. Under estrogen dominance, during the late follicular phase, the cross-section of the endometrium becomes enlarged (35.4 ± 0.2 mm) and the horns become more convoluted than during the luteal phase [14, 24] . Towards the end of the luteal phase, the endometrial diameter decreases (23.7 ± 0.2 mm) and reaches the smallest measured thickness (17 ± 1.5 mm) during the early follicular phase. The anovulatory LH peak in the mid-follicular phase has little to no effect on the thickness of the endometrium.

SOCIOSEXUAL BEHAVIOR

Under good conditions, elephants breed throughout the year, with some evidence of distinct seasonality or estrous synchrony. In wild African elephants, conception rates and births were reported to mainly occur in the rainy season, when feed resources and water are abundant [65] . Similar trend may appear in captive breeding herds, where irregular cycles may occur during winter time, when time spent outdoor and daylight duration are short [66] . In captive Asian elephants, living under their natural climatic conditions, seasonal differences in the length of the follicular and luteal phases were reported [48] and births peak during the cool season (December-March) [67] .

Because elephant estrous is such a rare event and

considering the fact that males are wandering between family groups over a large area in search of females in estrous, females within each family group will benefit from the ability to synchronize their estrous timing so that males will be available for mating for all when the time comes. Several researchers studying both wild [59, 68] and captive [69] elephants have suspected synchronization. Like bulls, females can also detect the reproductive cycle state of other females through pheromones excreted in their urine, so they might be using these cues for cycle synchronization [70]. The physiological function of such synchronization is not clear and might be the result of captive conditions since elephants in the wild are usually either pregnant or in lactational anestrus. Under natural and wild conditions, series of non-conceiving estrous cycles, which are frequently observed in captive elephants, most likely do not occur.

Based on behavioral studies, estrous lasts about four to five days [33, 71]. In captivity, a receptive period of two to three days was recorded and multiple copulations are usually observed during these days [5]. As a polygynous mammal, mating with more than one male during a single estrous does occur [11, 72]. However, mating with multiple males is a rare event due to the presence of the dominant breeding bull.

In the wild, female elephants rarely show any signs of being in estrous unless a male is present. When there are males around, especially when large adult bulls are present, the estrous female will show one or more of the following behaviors [73]: (1) Wariness—the female will be alert and wary of males, it will carry its head high so the top of the head is higher than the shoulder bones (usually it is at the same level or lower), it will open its eyes wide and will move away quickly when a male approaches her to test her vulva. (2) Estrous walk—the female walks in a large arch away from her group with the male(s) following at a 10 to 75 m distance. It is walking with the head held high or slightly turned to the side so she can keep the males in view at all times. The position of the head makes her twist a bit during her walk. At times the tail is kept high and away from the body and the stride looks as if it is faster and of greater length than normal walk. When either the male(s) or the female stops, the other will stop as well and when one of the concerned starts walking, the other will do the same. (3) The chase—the walk may escalate to a chase during which the bull seems to be trying to catch the female while the female is running away from it. At this stage the bull usually shows an erection. If the male manages to touch the female with his trunk, the female will usually stop and the male will then attempt to mount her. Young males usually do not attempt to chase females, and when they do try they will

most likely fail to touch the female, suggesting that the female is exercising her choice or that some experience is needed to succeed. (4) Mounting—occurs after a chase or a brief walk with the male closely behind the female. Once the male has touched the female and she stops, he will place his trunk along the female's back and his head and tusks on its rump and use these as leverage to help himself stand on his hind legs. The front legs are then placed at the sides of the female's spine while the trunk touches her head or face. Intromission may then ensue if the female is cooperative and does not walk away. (5) Consort behavior—formation of a temporary bonding between the male and the female, possibly to guard the female from other suitors. Males usually do not exhibit this behavior and the larger adults rarely do. When a large bull does form such a bond, it will usually spend his time close to a specific female while other males will be around the periphery of the group and show agonistic interactions between themselves. They will avoid the larger bull and when this chases away one of them, the others will immediately approach the female. The latter, however, will avoid them, often seeking the proximity of the larger bull.

Estrous is also signaled through infrasonic sounds and olfactory chemicals, which can be transmitted over greater distances (Asian: Payne et al. [74]; African: Poole et al. [75, 76]). This method of communication seems to be less affected by reflection, refraction and absorption [60] and might help in attracting bulls over long distances (African savannah elephant) or in dense forest areas (African forest and Asian elephants). Pheromones in the urine seem to play an important role in signaling an approaching estrous, inducing flehmen response in bulls, using their vomeronasal organ [4, 68]. The pheromone, (Z)-7-dodecenyl acetate (Z7-12:Ac), is excreted in the pre-ovulatory urine [77]. It increases during the follicular phase and was reported to induce sexual behavior. Other indications of estrous include mucus droppings and the reddening of the clitoris, which may appear through the vestibular opening when the female is urinating or simply relaxed.

When a bull approaches a herd of females, it will inspect the anal and vestibular region and taste out the urine of its members, showing great interest in females in heat [33]. Relying on this behavior, bulls are used today for estrous detection through urine tasting [78]. Behaviorally, if the male engages in wrestling with intertwined trunks, reaching over and chin resting, driving, neck biting and attempt to mount, it can be assumed that the female is in estrous [33, 79]. The bull is dependent on the female's cooperation when breeding, as she needs to stand perfectly still to allow intromission of the

S-shaped penis into the vulva, which is positioned ventrally, between her hind legs. In the wild, large males, over 35 years of age and in breeding excitement [5] are preferred partners for mating by mid-estrous females and are frequently observed to guard them [76]. Since most signs of estrous relate to social interactions, they might be difficult to discern in captive situations, where the matriarchal social group structure is not preserved [80] and bulls are often absent. As part of social interactions, authors of a study on wild African elephants speculated that older, parous females display estrous behavior at times when they are not in estrous but when other, young group members come into estrous for the first time [81]. They assumed that the older and more experienced females show the younger ones towards whom they should direct their estrous behavior and how they should do it.

CYCLING PROBLEMS

Many females, especially in the older age groups and primarily in African elephants, stop cycling or have irregular cycles [82, 83]. A study on captive African elephants found that females higher on the hierarchical ladder (dominant females) were more likely to be non-cycling, with 9/17 noncycling females in the study being ranked as dominant whereas only 10/58 cycling females were ranked as dominant [83]. Yet, in a later study by the same group, only a tendency for a relationship between social rank and cyclicity was found ($F_{1,31}=2.97$, $P=0.09$) [84]. Still in another paper by this group, they report on a survey in which 19/21 institutions in which there are both cycling and noncycling females, the noncycling female is the dominant one [85]. In this last study, the elephants' BMI was found to be correlated to acyclicity. Dominant females generally tend to be fatter in zoos because they get the priority within the group in access to food and because they also take food from subordinates. With currently 30.5% noncycling and 15.8% with irregular cycles, close to half of the North American African elephant population have cycling problems [86]. A study on 81 African elephant females in North American zoos (45 cycling, 36 noncycling) evaluated the relationship between cyclicity, dominance and the level of cortisol [87]. However, no correlation between the level of dominance or cyclicity and cortisol was found, suggesting that stress is not involved in either. In the most recent study from this group, correlation between circulating testosterone levels, dominance and cyclicity status was evaluated in 49 captive African elephants (24 cycling and 25 noncycling) but no such correlation was found [88]. Three surveys of the North American elephant population (both Asian and African) were conducted over the past decade (2002, 2005

and 2008) and the general picture seems to have stayed the same over the years [82, 86, 89]. Ovarian acyclicity ranged between 10.9 and 13.3% for Asian elephants and significantly higher for African elephants (range between 22.1 and 31.2%). To these numbers one should add those females with irregular cycle activity, which was similar between 2008 and 2005 for both Asian (14.3% and 7.6%, respectively) and African (15.8% and 11.8%, respectively), but was higher than the values in 2002 (2.6% and 5.2% for Asian and African elephants, respectively). There were more reproductive tract pathologies in non-cycling females but such pathologies did not account for most cases of acyclicity. Ovarian acyclicity increased with age but decreased in the presence of a bull in the facility or when the elephants were managed in free contact compared to protected contact [86]. At least some of the females that were not cycling in the 2002 survey were cycling during the 2005 survey, suggesting that this condition is reversible, at least in some of the cases [89]. This was demonstrated in a study that followed the cycling activity of three African elephants [90]. All three elephants were cycling normally but then experienced an extended non-luteal phase of 16 to 27 weeks with serum progesterone concentration, on average, of 30.6 ± 2.6 pg/mL (well within the levels during the follicular phase). These elephants spent less time outdoors during these acyclic events than otherwise but the events took place during the winter so the time spent outdoors may be related to the winter rather than changes in the cycling activity. While this progesterone level is within the normal values for a non-luteal period, the duration is much longer than the normal duration of 5.1 ± 0.4 weeks [91]. In another study on four African elephants, one cycling and three non-cycling, it was found that prolactin followed estradiol's pattern with high levels in the follicular phase and low in the luteal phase. The non-cycling females lacked this cyclicity in prolactin [55]. In wild African elephants, in one study, it was found that 90.6% of the females were reproductively active (pregnant or lactating) and the balance 9.4% were mostly in the young (<15 years) or old (>50 years) age groups [92]. Thus, even if some do not cycle, the rate is far below that seen in North American zoos.

HORMONAL MANIPULATION OF THE CYCLE

Luteal regression

Knowledge on hormonal manipulation in the normal estrous cycle remains very limited. Prostaglandin F₂- α (PGF₂ α), which is used in domestic animals to interrupt the luteal phase and induce the initiation of a new cycle, failed to achieve a similar effect in elephants. In a study on two cycling African elephants, PGF₂ α was injected i.m. at a dose of 80 to 125 mg per

elephant on days 25, 30, 45 and 60 post estrous. However, only in one female treated on day 60, did serum concentrations of progestagens decline four days after the injection [93]. This, however, is also the natural timing of the end of the luteal phase. In an anecdotal report, the administration of $\text{PGF}_2\alpha$ to a young, 5-month pregnant Asian elephant, on three consecutive days failed to induce abortion. Although urinary progesterone metabolites concentrations dropped to baseline during treatment, they returned to normal pregnancy concentrations 10 days after the last injection. A healthy calf was delivered by this dam (Stephanie Sanderson, Chester Zoo, UK, personal communication). Based on this report, it can be suggested that $\text{PGF}_2\alpha$ is capable of inhibiting progesterone secretion from the CLs but not to induce lyses of luteal cells. Further studies on the role of prostaglandins as luteolytic agents are warranted.

Ovulation induction

When applying an assisted reproductive technology such as artificial insemination (AI), appropriate timing of insemination is determined by detecting of the anovulatory LH peak and then timing the procedure for 19 to 21 days later. To ensure ovulation at the time of AI, human choriongonadotrophin (hCG) has been used (unpublished data). A dosage of 5000 IU, injected at the time of the last AI, induced rupture of the follicle in six cases. However, coincidental occurrence of the natural second LH peak cannot be ruled out in these observations. Furthermore, in one case of an Asian elephant female, hCG failed to induce rupture of the dominant follicle. The follicle, which had a diameter of 18.9 mm 19 days after the anovulatory LH surge, eventually ovulated naturally. A regular ovulatory LH peak and subsequent ovulation were detected two days later (21 days after the first LH peak) when the follicular size was 21.0 mm. Nevertheless, this female became pregnant. This case indicates that the follicle needs to reach a certain size or stage of maturation to be receptive for ovulation induction. In another personal observation on an African elephant female, hCG injection led to follicular stimulation. The ovaries showed more than 15 follicles each, with diameters ranging between 7.0 and 16.0 mm.

Although detailed effects of GnRH on the pituitary-gonadal function in elephants are still unknown, several studies suggest that administration of GnRH can induce release of LH at levels lower than normal LH surges [27]. A recent study in Thailand presented more promising results for LH-peak induction [94]. A GnRH-agonist (Buserelin Acetate), given 13 to 43 days after progesterone had reached baseline concentrations (but not earlier), at a dose of 80 μg , resulted in an immediate anovulatory LH surge. This was followed by an ovulatory LH surge 15 to 22 days later. One female was bred and became

pregnant following this protocol. Induction of the first LH peak in proximity to the time it would naturally occur, as shown in this study, could help determine the right time for an AI.

Suppression of ovarian activity / contraception

In particular situations, it can be desirable to shut down cyclical estrous activity. For example, when pathologies of the reproductive tract occur and pregnancy may risk the female, to avoid inbreeding under captive settings, or if overpopulation in certain African regions needs to be prevented. Shutdown of estrous cycles can be accomplished by Gonadotropin-releasing hormone (GnRH) agonist implants [95] or the use of a GnRH vaccine. Other immunocontraceptives such as porcine zona pellucida (PZP) vaccine, prevent fertilization, yet do not suppress the ovarian activity. PZP stimulates antibodies against the zona pellucida and therefore can block ovum fertilization. It has been successfully applied to African elephants in South Africa, however long-term effects are not yet known [96]. Synthetic GnRH analogues (agonists or antagonists), when given at the right doses, suppress the secretion of FSH and LH through negative feedback or desensitization. Females treated with GnRH vaccines or analogues should be ultrasonographically examined on a regular base. In a controlled study, such a treatment was used to avoid musth and aggressive behavior in captivity and to induce infertility in free ranging African bulls [97]. Inhibition of LH and FSH secretion stops follicular growth and avert ovulation. This in turn abolishes estrogen stimulation and thus might have a therapeutic use to reverse or prevent the growth of uterine leiomyomas, a commonly found pathology in non-reproductive elephants.

CONCLUSION

The enhanced scientific interest in the reproduction biology of African and Asian elephants resulted in the accumulation of a vast database of information. However, many gaps in our knowledge on cycle activity still prevail and await elucidation. These include, for example, questions such as further elucidation of the two LH peaks expressed by elephants, why their estrous cycle needs to be, on average, 16 weeks long, what roles do the accessory CLs have, and what is the mechanism of luteolysis?

Elephant are certainly difficult to study. Due to their endangered status and restrictions imposed by international laws and conventions, sample collection is not without problems and mostly limited to populations in zoological facilities or occasionally from dead individuals in the wild. Their different physiology and, at times, use of different hormonal metabolites make many commercially available measurement

kits not sensitive enough or not suitable for use in elephants. The study of comparative reproductive physiology is also difficult. The elephants' closest relatives are the *sirenia* and *hyracoidea*. The reproduction aspects of both orders are not studied well enough to allow evolutionary related conclusions. Nevertheless, the information accumulated thus far has already helped to better manage captive elephant populations.

要 約

近年、ゾウは、飼育下での低い繁殖成功率や、野生からの新たな個体導入の停止により生息数が減少している。一方、ホルモン測定や超音波検査の発展、アフリカでの生息数調整などから得られた研究成果は、現在のゾウの生殖周期に関する知識の発展に貢献してきた。ゾウの繁殖特性は他の哺乳類と明らかに異なっており、泌尿生殖器の解剖学的構造、発情周期の長さや構成、副黄体の形成、生殖ホルモンの分泌パターンなどが特徴的である。雌ゾウの発情周期は 12 ～ 18 週であり、現在までに知られている周年繁殖動物の中で最長である。プロゲステロンが排卵後 1 ～ 3 日で上昇して黄体期が始まり、6 ～ 12 週続く。その後 4 ～ 6 週間ある卵胞期の終わりには、正確に時間差のある 2 回の LH サージが起こる。一般的に、1 回目の LH サージは排卵を誘起せず、その 19 ～ 21 日後の 2 回目の LH サージで排卵が誘起され、通常、1 個の卵胞が排卵する。しかし、排卵部位での黄体形成に加えて、卵巣には複数の黄体が観察される。他の多くの動物種と違い、黄体組織から分泌される主なプロゲスタジェンはプロゲステロンではなく、プロゲステロンから 5 α 減少した代謝物である。今回、アジアゾウ、アフリカゾウで現在分かっているユニークな発情周期について、発情行動、生殖関連ホルモン、生殖器官の超音波と形態学的構造、病理学的面を、ホルモン処置の可能性も含めて説明した。

キーワード：雌ゾウ、発情周期、黄体化卵胞、卵胞発育、副黄体

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