REPRODUCTIVE STRATEGIES AND BEHAVIOR OF THE ASIAN ELEPHANT (*ELEPHAS MAXIMUS*)

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CHAPTER I

INTRODUCTION AND BACKGROUND OF ELEPHANT REPRODUCTION IN NORTH AMERICAN ZOOS

INTRODUCTION

Asian and African elephants

The living elephant species, the African (*Loxodonta africana*) and Asian elephants (*Elephas maximus*), are the result of more than 50 million years of evolution (Shoshani, 1992). African and Asian elephants evolved from *Primelephas* beginning in the Pliocene on the African continent, but only the Asian elephant migrated beyond Africa (Shoshani, 1992; Palmer, 1999). The current range of Asian elephants includes the Indian subcontinent, continental Southeast Asia, Sumatra, Sri Lanka, Borneo and the Andaman Islands (Keele and Dimeo-Ediger, 1999). The current range of African elephants includes Sub-Saharan Africa with most in the savanna woodlands of Central and East Africa and the forests of Zaire (Keele and Dimeo-Ediger, 1999).

The two living species share many general morphological traits including similar trunks, tusks, large bodies, inguinal position of genitalia and mammary glands between the forelegs (Shoshani, 1992). Differences between the species include larger ears of the African, surfaces of their teeth (Africans have lozenge shapes, whereas Asians have narrow loops), concave back of the African versus the convex Asian back, domes on the head of Asians, more wrinkled skin of Africans and two "fingers" Africans have (instead of one) on the tip of their trunk (Shoshani, 1992). Some internal similarities include abdominal testes, lungs that are attached to the diaphragm and a well-protected brain in a thick "honeycomb" skull (Shoshani, 1992). African elephants are typically larger, weighing up to 7,000 kg and shoulder heights of 4 m, while Asian elephants reach 6,000 kg and 3.5 m (Shoshani, 1992).

Hormone profiles between the two species are very similar in females (Hodges, 1998). Both species exhibit an estrous cycle length of 13-16 weeks with one pre-ovulatory elevation in luteinizing hormone (LH) concentration occurring 3 weeks prior to a second elevation at ovulation (Figure 1; Hodges, 1998). Males of both species typically exhibit a musth period with increased serum testosterone concentrations and aggression (Rasmussen and Schulte, 1998). These animals also share a common social structure, with mature males living solitary lives while females live in small groups of matriarchal societies (Shoshani, 1992).

Population dynamics and reproductive problems

Asian elephants (*Elephas maximus*) are listed on Appendix I of the Convention for International Trade in Endangered Species (CITES), with their current numbers in the wild estimated between 37,530-48,180 (Keele, 2001). Asian elephant populations in the wild have been steadily declining, largely due to habitat destruction and population fragmentation (Keele, 2001). With this fragmentation and loss of habitat, it is now believed that the wild populations are incapable of avoiding inbreeding over time due to the isolation of herds (Keele, 2001).

The captive populations are also declining, with the North American Species Survival Plan (SSP) population numbering 24 males and 131 females (Keele, 2001). This Asian elephant population is managed by a group of elephant specialists within the American Zoo and Aquarium Association, (AZA), a self-regulating association of North American zoos and aquariums that formulates the Species Survival Plan (Hutchins and Smith, 2001). The mission of the elephant SSP is "to lead North American efforts in the management and conservation of elephants" (Keele and Dimeo-Ediger, 1999). This committee of elephant specialists examines the genetic makeup, age, health and transportation considerations of the captive population so that they can develop annual breeding recommendations for the AZA member institutions. These recommendations are either for temporary breeding loans of females to institutions with genetically suitable males or for artificial insemination of these animals. Adult males are not typically transported for temporary breeding loans due to logistical problems associated with their size and aggressive nature.

Since 1962, > 100 (exact figure not given) Asian elephant calves have been produced in the North American captive population (Keele and Dimeo-Ediger, 1999). Of the calves produced, there has been > 30 percent mortality due to stillbirth or death of the infant before they reached 30 days of age (Kurt and Mar, 1996; Keele and Dimeo-Ediger, 1999).

Within the SSP population there has not only been a serious population decline but also a decline in the number of participating institutions holding Asian elephants (Keele and Dimeo-Ediger, 1999; Schulte, 2000; Wiese, 2000). This situation leads to predictions of a crash of the captive population within 30 years unless institutions further their cooperation so the founder population can rapidly contribute offspring (Keele and Dimeo-Ediger, 1999).

The captive population is faced with several other serious problems contributing to declining numbers. Some of these problems are associated with low reproductive rates (Hutchins and Smith, 2001). The current fecundity rate is estimated at 1-2% and the projected need for a viable captive population is 7-8% (Hutchins and Smith, 2001). This low rate may be due to infertility, the predominance of institutions only housing females, failure or inability of either the male or female to copulate; or loss of the fetus once conception has taken place through fatal dystocias, resorption of the fetus, or the retention of the fetus in utero (Rasmussen et al., 1982; Sukumar et al., 1997; Hildebrandt et al., 1998; Hutchins and Smith, 2001). Some of these problems may have a fundamental explanation based in their social structure (Carlstead and Shepherdson, 1994; Lindburg and Fitch-Snyder, 1994).

Social behavior

Female Asian elephants are social animals that live in a hierarchical system (Gadgil and Nair, 1984; Garai, 1992; Schulte, 2000). They live in herds consisting of a matriarch, her sisters, and their offspring (Rasmussen and Schulte, 1998; Hutchins and Smith, 2001). These herds form long-term bonds that are maintained over space and time

(Garai, 1992). The matriarchs have a learned knowledge of their surroundings that they use to navigate the herd to food, water and safety (Nair, 1989; Hutchins and Smith, 2001).

Much elephant social and survival behavior appears to be learned (Eisenberg, et al., 1971; Gadgil and Nair, 1984; Poole et al., 1997). Young elephants learn what is appropriate to eat by sampling what their older relatives are eating, sometimes taking food right out of their mouths (Nair, 1989). These young animals also learn to stay near their mother to avoid dangers, such as predators and mud flats (Nair, 1989). Young females learn how to react to the amorous attention of the males by watching their older kin during mating (Eisenberg, et al., 1971). From the reactions of the older females, they quickly learn that attempts to mate by related young males are not acceptable (Eisenberg, et al., 1971). The attention of adult males toward immature females also results in negative responses from the older, dominant females (Eisenberg, et al., 1971). These young females also learn how to care for calves by "babysitting" their younger siblings and cousins (Gadgil and Nair, 1984).

Male elephants largely exist in a different social structure. Once young males reach sexual maturity, older females expel the males from the herd, theoretically to prevent inbreeding (Eisenberg et al., 1971; Rasmussen et al., 1993). These young males will often form bachelor herds that may last throughout their teens and early twenties (Eisenberg et al., 1971; Schulte, 2000; Keele, 2001). Once the males have achieved sufficient size and age, they periodically exhibit a phenomenon called musth (Cooper et al., 1990). This period is characterized by a loss of appetite, drainage from the temporal glands, urine dribbling, increased aggression and elevated serum testosterone concentrations (Cooper et al., 1990; Lincoln and Ratnasooriya, 1996; Dickerman et al., 1997; Rasmussen and Schulte, 1998). Musth is hypothesized to provide a period in which the males scent-mark territories and will fight other males for mating rights to the females within these territories (Cooper et al., 1990; Rasmussen and Schulte, 1998). Once males are mature, they are largely solitary animals except when pursuing an estrus female (Rasmussen et al., 1993, Rasmussen and Schulte, 1998; Keele, 2001).

Elephants use chemical communication to signal their reproductive or physiological state (Rasmussen et al., 1982; Rasmussen et al., 1993; Rasmussen, 1998; Rasmussen and Schulte, 1998; Riddle et al., 2000; Lamps et al., 2001;

Rasmussen and Wittemyer, 2002). These chemicals are most often associated with secretions from the breath, urine, feces, urogenital openings, temporal glands, inter-digital glands and ears (Rasmussen and Krishnamurthy, 2000). As females reach the pre-ovulatory state, they release pheromones that signal the males of their impending ovulation (Rasmussen, 2001). Male elephants assess the female's reproductive state by means of a modified flehmen response to urine, feces and the urogenital opening utilizing their vomeronasal gland (Diephuis, 1993). The males also release pheromones signaling their musth status. There is a high correlation between the number of matings and incidence of musth in natural settings (Perrin et al., 1996; Rasmussen et al., 1996; Rasmussen, 1999; Rasmussen and Perrin, 1999; Schulte and Rasmussen, 1999).

Both African and Asian elephants also communicate through a complex system of infrasound that can be utilized by females to alert males to their reproductive receptivity (Payne et al., 1986; Ben-Ari, 1999; Langbauer, 2000; Payne et al., 2003). In African elephants, a wide range of temporal patterns have been associated with reproductive vocalizations, from a pre-ovulatory estrus call by the females to chorusing between males and females at estrus (Leong et al., 2003 a,b). This infrasonic communication can also fulfill normal social functions within the female herd such as spatial distribution of the herd or alarm calls (Garstang et al., 1995; Larom et al., 1997; O'Connell et al., 1997; Payne, 1997; Byron et al., 1998; O'Connell-Rodwell, 2000). Male elephants have been known to signal their musth status with a "musth rumble". In playback experiments, musth males would approach this call, while non-musth males would leave the area (Poole, 1999). Elephants are even known to produce seismic signals that can travel great distances through vocalizations, foot-stomping and locomotion (O'Connell et al., 1997; Arnason et al., 1998; O'Connell-Rodwell, 2000). This seismic communication is often associated with infrasonic calls (O'Connell et al., 1997; Arnason et al., 1998; O'Connell-Rodwell, 2000).

General management

Due to their aggressive tendencies and solitary nature, male elephants are more difficult and expensive to house in captivity (Taya, 1993). These larger animals require fortified structures to contain them in captivity safely (Cooper et al., 1990; Lincoln and Ratnasooriya, 1996). They also require specialized handling techniques because their aggressive nature and large size prohibits close contact with humans (Cooper et al., 1990). The current method of handling adult male Asian elephants includes hydraulically operated doors and chutes to move them either to solitary enclosures or enclosures housing only females and subadult males (Hutchins and Smith, 2001). Many institutions also have constructed elephant restraint devices, a variation of cattle restraint chutes, to safely care for these dangerous animals (Hutchins and Smith, 2001). Due to the greater risk and expense of housing adult male elephants, few institutions in North America currently house males (Czekala et al., 1992; Hutchins and Smith, 2001).

Fortunately, adult females are generally less dangerous and expensive to maintain in captivity. Because females are naturally adapted to a social environment, they are much more accepting of human and conspecific interactions (Hutchins and Smith, 2001). This behavior allows many institutions to house herds of females in one enclosure, providing a more cost-effective method for displaying them than is possible for displaying males (Hutchins and Smith, 2001). Although most females are more docile than adult males, there are exceptions to this rule. Some females may not integrate well into a herd environment or may not accept new individuals, whether human or elephant. The history of the captive elephant's immigration to North America is largely the culprit in creating this problem. Most captive Asian elephants in North America today were wild-caught in Asia and transported here at a very early age and put into unrelated herds, of all females (Keele and Dimeo-Ediger, 1999; Schulte, 2000). The result was a great influx of very young animals with no older females to supervise and teach these immature animals the necessary life skills. They were not instructed in what was appropriate to eat, or how to develop herd structures, reproductive behavior or nurturing skills for rearing offspring (Nair, 1989; Fernando and Lande, 2000; Hutchins and Smith, 2001). Due to this development of unrelated, uneducated herds in North America, many of these now adult animals have difficulties with herd integration, reproduction and nurturing of their own offspring and of other infants born into the group (Poole et al., 1997; Sukumar et al., 1997).

Although natural breeding is the most productive means of propagating this species, there are problems associated with this method. The inability of many institutions to house male elephants places an excessive burden on the few institutions that have developed the facilities and staff to manage males (Balke, et al., 1988; Hutchins and Smith, 2001). To sustain the captive population, those few institutions that house the adult males must maximize their breeding outputs and genetic variability based on SSP recommendations. The population of elephants in captivity is aging rapidly, creating a need to breed many of these females soon to preserve their individual genetic diversity and to stabilize the age structure of the declining captive population (Keele and Dimeo-Ediger, 1999).

Current AZA guidelines require that male and female elephants undergo a reproductive assessment before transportation for breeding because of the associated stress and expense of transporting females to the bull-holding institution is high (Brown, 2000). Males and females may be given physical examinations to discern their fitness for reproduction. Females are monitored for reproductive hormone concentrations and informal behavioral evaluations may be conducted to discern the best candidates for reproductive success (Brown, 2000; Hutchinson and Smith, 2001).

The physical exam may include both external and internal exams (Hildebrandt et al., 1998; Brown et al., 1999; Hildebrandt et al., 2000; Hutchinson and Smith, 2001). The physical fitness of the males and females to endure the rigors of mating, particularly associated with their mobility, may be assessed. Mating behavior may be strenuous and their ability to move freely and mount or be mounted is essential (Eisenberg, 1971). Particular attention is paid to the limbs and back of the animals, with a lesser emphasis being placed on the general weight and physical fitness of the animals.

An internal examination of the reproductive tracts may be performed with transrectal ultrasonography (Hildebrandt et al., 1998; Hildebrandt et al., 2000; Hutchinson and Smith, 2001). This exam is designed to determine if the male or female has any abnormalities that may preclude them from reproducing. Many breeding-age females have been assessed in the past five years and have been diagnosed with leiomyomas in their reproductive tract, which renders them incapable of reproducing (Keele and Dimeo-Ediger, 1999; Hutchinson and Smith, 2001). Several males also have been identified as infertile through transrectal ultrasonography in the past few years (Hildebrandt et al., 1998). Enlarged or underdeveloped accessory sex glands have been the primary cause of infertility in the males (Hildebrandt et al., 1998).

A reproductive evaluation of the females based on hormonal monitoring is also essential to the success of a breeding program (Brown, 2000). Most commonly, the female elephant's progesterone concentrations are monitored in an effort to assess her reproductive status and potential for breeding success (McNeilly et al., 1983; Plotka et al., 1988; Mainka et al., 1990; Olsen et al., 1994; Magunna, 1995; Wasser et al., 1996; Schwarzenberger et al., 1997; Carden et al., 1998; Hodges, 1998; Brown et al., 1999; Brown, 2000).

The elephant manager conducts an informal behavioral analysis of the animal in an effort to assess the individual's ability to cope with the stress of mating (Keele and Dimeo-Ediger, 1999). The ability of the females to cope with transportation to a bull-holding facility is weighed primarily based upon records of past experience and resultant behaviors associated with transportation, herd integration and acceptance of new handlers. In some cases, the stress associated with the act of transportation to a new facility has caused females to become acyclic (Hutchinson and Smith, 2001). Also necessary is an assessment of the female's ability to leave her herd and then successfully re-integrated into this herd once returned to her home facility. In many cases, a new female may not be capable of integrating into another herd at a new location for extended periods and may be forced to remain with the male as her only companion, which often stresses the female. Further study of these potentially stressful situations would be useful and may be possible utilizing measurements of cortisol concentrations in saliva (Foley, et al., 2001).

Some females have more aggressive tendencies than others and this information also must be carefully evaluated to insure the safety of the elephant handlers at the breeding institution. There are two basic recognized types of elephant management systems in captivity, free contact and protected contact (Hutchinson and Smith, 2001). Free contact allows the handlers to enter the enclosure without barriers separating them from the animals for their caregiving, whereas protected contact requires that the handlers maintain a barrier or have a restraint between themselves and the animals. Each system has its own advantages, balancing animal care and human safety. These systems are not interchangeable and it is very difficult to move an animal accustomed to one management style to another without prolonged training periods for the adjustment. Adult males are virtually all managed in some form of protected contact for the safety of the males and their human handlers. Although the SSP is trying to coordinate the elephant population so that females will not have to alternate management styles, they recognize that with the limited population, they may not be capable of totally secluding these two populations (Hutchinson and Smith, 1992).

Reproductive diagnostic methods

Hormonal fitness of the females has largely been determined by the analysis of serum progesterone concentrations. Progesterone has been a useful indicator of the female Asian elephant's reproductive status in many capacities including profiling normal estrous patterns, monitoring pregnancy and predicting impending births (Hess et al., 1983; Plotka et al., 1988; de Villiers et al., 1989; Taya et al., 1991; Wasser et al., 1996; Schwarzenberger et al., 1997; Carden et al., 1998; Brown, 2000). Although studies on luteinizing hormone have proven useful for timed breeding, other studies conducted on estrogen compounds have not yielded consistent results and are not commonly utilized in reproductive assessments (Brown et al., 1991; Brown et al., 1999; Czekala et al., 1992; Taya et al., 1991; Wasser et al., 1996).

Asian elephants have an average estrous cycle lasting sixteen weeks (Mainka and Lothrop Jr., 1990). Within this estrous cycle is an extended luteal phase typically lasting eight to ten weeks and a shorter follicular phase lasting four to six weeks (Brown et al., 1991; Magunna, 1995; Hodges, 1998; Bechert et al., 1999). Within the estrous cycle is estrus, or the heat period, which averages 3 days (range: 1-7 days) in Asian elephants, during which the male exhibits interest in mating with the female (Carden et al., 1998). In African elephants, studies have correlated a similar period of estrus with the initial rise in progesterone, an elevation in LH, and ovulation confirmed by ultrasonography (Hermes et al., 2000). Although male elephants are capable of detecting when the cow is in estrus, researchers must rely on the use of serum progesterone or LH analysis for this information (Brown et al., 1991; Carden et al., 1998). Daily monitoring of either serum progesterone for a sustained increase above basal follicular concentrations (above 100 pg/ml), or the pre-ovulatory and ovulatory elevation of LH (mean of 3.1 and 3.6, respectively) are both useful tools for planned breeding and artificial insemination (Brown, et al., 1991; Brown et al., 1999; Carden, et al., 1998; Hodges, 1998; Mainka, et al., 1990).

Weekly serum analysis for progesterone content can be used to determine if a female is cycling properly or is acyclic (Brown et al., 1997; Magunna, 1995; Heistermann et al., 1997). In a study of captive elephants in India, females produced calves from their mid-teens to their mid-sixties, with the prime breeding years between 20 to 40 (Sukumar et al., 1997). In the North American captive population, the youngest female successfully to give birth to a live offspring was 5 1/2 years old and the oldest was in her mid-thirties (Keele and Dimeo-Ediger, 1999; Rasmussen and Schulte, 1998).

Several researchers are studying the growing number of anestrus females of prime breeding age (teens through twenties) in the North American population (Brown and Lehnhardt, 1997; Brown et al., 1999; Schulte et al., 2000; Hutchinson and Smith, 2001). Although some females experience temporary acyclic periods, others remain acyclic indefinitely (Brown and Lehnhardt, 1997; Schulte et al., 2000). Ultrasonography of the reproductive tract has shown uterine tumors, endometrial cysts and ovarian cysts resulting in acyclicity (Hildebrandt et al., 2000). Other researchers have investigated hormonal patterns of these acyclic females and found progesterone, LH and estradiol to be suppressed to basal concentrations (Brown and Lehnhardt, 1997). Due to the limited gene pool in North America, the development of many young acyclic elephants has become a major concern for managers (Schulte et al., 2000; Hutchinson and Smith, 2001).

Inconsistencies in the reports of estrous synchronization in elephants have come from small sample sizes: mixed results have been found. Some researchers have speculated that female elephants housed together may develop synchronous estrous cycles; others have not found this pattern (Bechert et al., 1999; Leong et al., 2003b; Rasmussen and Schulte, 1998). Researchers have reported that the female Asian elephant signifies her mating receptivity to the male elephant with a pre-ovulatory pheromone (Rasmussen, 2001). This pheromone also could be detected by other female herd members and provide a source for natural estrous synchronization among herd members (Rasmussen and Schulte, 1998). If synchronicity is established within a herd, it may be practical for managers to transport two or more reproductive females to the male's breeding institution at one time or to plan multiple artificial inseminations to maximize the breeding output of the male (Rasmussen and Schulte, 1998).

Reproductive management

The more dangerous temperament of the adult male elephant often causes managers to avoid housing these aggressive animals (Hutchins and Smith, 2001). Unfortunately, many successful breeding institutions have now suspended their breeding programs due to a shortage of suitable housing for male elephant calves. This cessation of reproduction in viable animals only exacerbates the problem of a declining captive population (Hutchins and Smith, 2001). Recently, researchers have reported that the gender of an elephant calf in utero may be determined by analysis of total testosterone concentrations in maternal serum (Duer et al., 2002). This information has assisted managers in making decisions for future breeding based on their institution's current gender ratio, including male calves in utero (Duer et al., 2002).

The shortage of suitable housing for the new males has become one of the leading problems in captive elephant management (Keele and Dimeo-Ediger, 1999; Hutchins and Smith, 2001). The use of sorted elephant sperm in artificial insemination to determine the gender of the calves before conception is a logical alternative to the natural 50:50 gender ratio produced, but this technique has not been developed.

Although artificial insemination has been recently developed in both the African and Asian elephant (1998 and 1999, respectively), the conception rate in African elephants has been much higher (Hutchinson and Smith, 2001). Artificial insemination techniques in both species require intensive training and cooperation of the humans and elephants involved (Hutchinson and Smith, 2001; Schwammer et al., 2001; Duer et al., 2002). These methods require very stringent protocols and laboratory staffing which most institutions do not have (Hutchins and Smith, 2001). Couple this lack of resources with low conception rates, (partially due to problematic semen storage and difficulties in accurate semen deposition), and artificial insemination is an unlikely solution for the declining population of captive Asian elephants (Keele and Dimeo-Ediger, 1999; Kitiyanant et al., 2000; Swain and Miller, 2000). Currently, natural breeding is the most productive method for propagating this species (Keele and Dimeo-Ediger, 1999; Hutchins and Smith, 2001).

Reproductive behavior of the Asian elephant

Research describing reproductive behavior in the wild has been anecdotal or consisted of small sample sizes (Eisenberg et al., 1971). There has been little research on captive animals, with most of the literature citing incidences of mating or factors relative to olfaction or vocalizations, lacking descriptive analysis of behavior (Jainudeen et al., 1971, Rasmussen et al., 1982; Rasmussen et al., 1993; Carden, et al., 1998). Although natural breeding has been achieved in captivity, it has been at the limited number of institutions that house the male elephants (Hutchinson and Smith, 2001). The information gained by these institutions has not been documented or well dispersed among the elephant community. For this reason, many institutions that are acquiring males to start their own breeding programs are not prepared for managing a breeding herd of elephants. Long-term data collection on the reproductive behavior of the males and females needs to be analyzed to describe typical behaviors. This information could prove useful to new breeding program managers that are unfamiliar with the earlier onset of sexual maturity in the captive animals and the intense nature of social interactions of mature Asian elephants (Hutchinson and Smith, 2001). Knowledge of the elephant's reproductive behavior could be useful in determining herd size, designing facilities, and making decisions concerning the welfare of females in aggressive encounters with the much larger males.

Lactational anestrus

Post-partum suckling is known to regulate endocrine function in some mammals, causing them to experience lactational anestrus (Williams et al., 1998). In many species, suckling of the young triggers prolactin secretion that causes the lactational anestrus period to be maintained (McNeilly, 1980). Because elephant calves typically begin eating solid foods at three to six months old, cessation or reduction of suckling may influence the duration of the lactational anestrus period (Nair, 1989). Previous studies have shown mean calving intervals ranging from three to nine years, indicating that the earliest conception noted was approximately 14 to 16 months postpartum (Laws, 1967; Rasmussen and Schulte, 1998). Other authors noted that a cow with a calf experienced only a twelve-week lactational anestrus period (Brown and Lehnhardt, 1995). Another study indicated that two Asian cows that did not nurse their calves (one calf died, the other was bottle-fed at her mother's side) resumed cycling at 8.5 and 10.5 weeks, respectively (Olsen et al., 1994). Yet another study revealed a cessation of cyclicity that had still not resumed at twelve weeks post-partum (Mainka and Lothrop Jr., 1990). Others report an average range of 45 to 46 weeks of anestrus (Olsen et al., 1994). Clarification of lactational anestrus ranges may assist managers in determining natural breeding schedules for cows. This information also may demonstrate that the common practice of early calf weaning is unnecessary to bring about the end of anestrus in the lactating cow.

Study design

This dissertation is organized into three individual manuscripts and written in the format for submission to Zoo Biology. This study addressed three problems associated with the decline of the captive population of Asian elephants (*Elephas maximus*) in North America. One reproductive issue examined in this study was to determine whether females that are housed together in stable social groups develop synchronized estrous cycles over time. It was hypothesized that females that were brought together would develop synchronous estrous cycles (based on weekly serum progesterone analysis). Currently, only a few North American institutions house adult, breeding-age male Asian elephants. To maximize their breeding potential, it would be advantageous to transport stable, synchronized herds of females to the males. This strategy would not only make maximum use of the males over time, but would be a shorter duration of stay at the bull-holding facilities thus allowing the larger number of females to be serviced by the small number of males presently housed in North American zoos. This accelerated reproduction is considered a high priority by the Species Survival Plan managers to prevent a population crash within 30 years due to the aging population of the captive Asian elephants in North America (Keele and Dimeo-Ediger, 1999).

In this study, progesterone was used as a foundation for the reproductive assessment of female Asian elephants. Weekly serum samples were analyzed to determine if the cow was cycling normally and to discern the estrus period. Once normal cyclicity was established, the cow was presented to the bull for breeding during her estrus period.

This study also described some of the more typical reproductive behaviors associated with Asian elephants in captive situations. Five categories of reproductive behavior (olfactory, locomotory, pre-copulatory, aggressive and copulatory) were identified and monitored using a behavioral ethogram to quantify the results. Often, the aggressive reproductive behavior of males requires managers to identify safety parameters for the females for reproductive success without injury to her. Currently, only limited information is available to assist elephant managers in the development of these breeding programs and safety parameters for the females. This study identifies some of the typical behaviors associated with breeding Asian elephants in a captive situation and examines the relationship of confounding variables such as age, age discrepancy of the males compared to the females, and reproductive experience.

Finally, the existence of a lactational anestrus period in the Asian elephant was investigated. Researchers and elephant managers are often in dispute with animal rights organizations over early weaning of calves. Managers often assume that an earlier weaning date will allow for an earlier conception for that cow, although this assumption has not been tested. Animal rights groups believe the calves should be left with their mothers longer for the health and well-being of the calf. The information gained from this formal study of lactational anestrus could help alleviate some of the tension between these two opposing factions and potentially benefit the calves.

The duration of this proposed post-partum anestrus period was investigated. This study identifies variations in estrous cyclicity between cows with suckling young and those without suckling young, as well as differences in serum progesterone concentrations before conception and after parturition.

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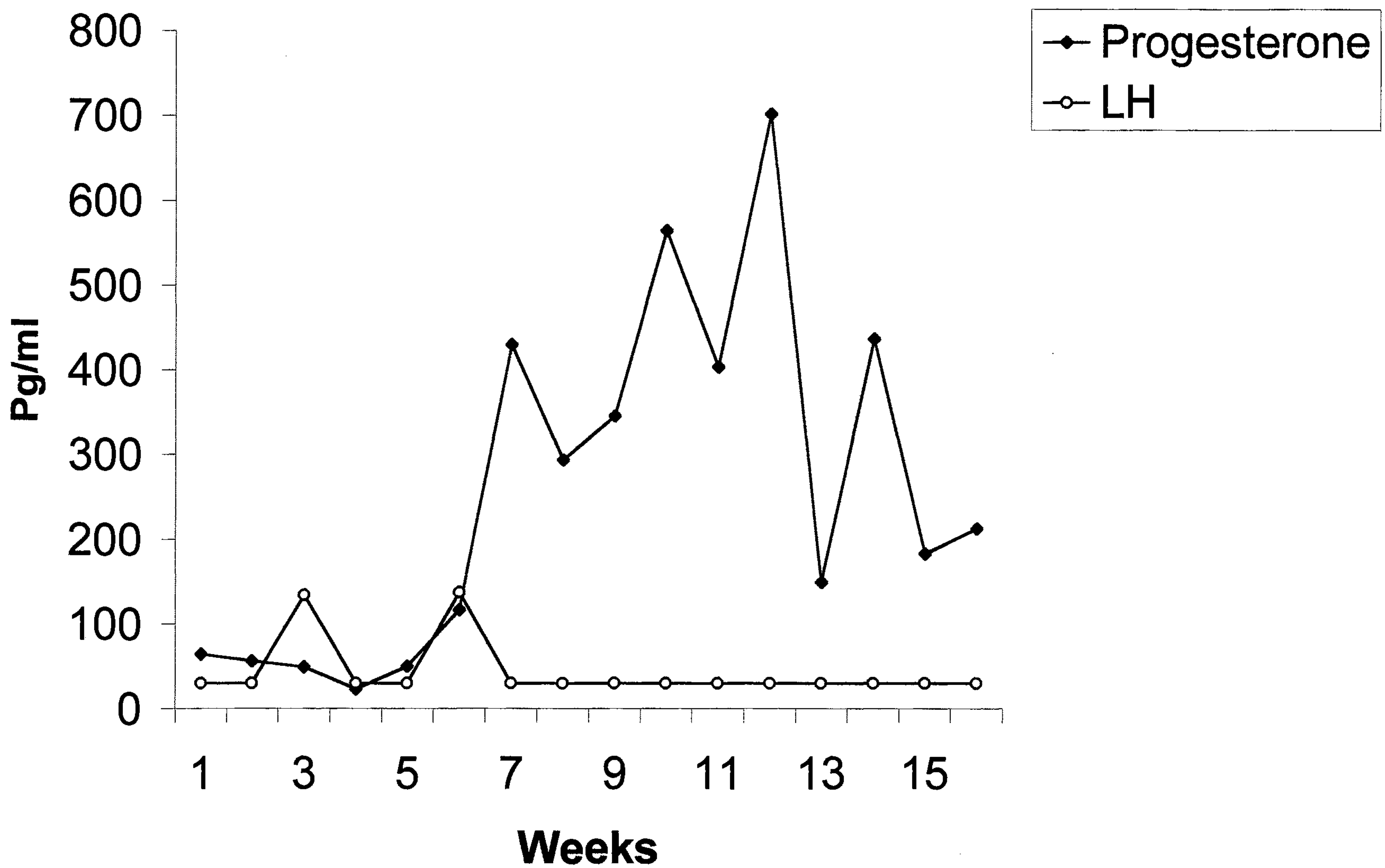

Progesterone
LH
Pg/ml
800
700
600
500
400
300
200
100
0
1
3
5
7
9
11
13
15
Weeks

CHAPTER II

ESTROUS SYNCHRONIZATION IN ASIAN ELEPHANTS

INTRODUCTION

Reproductive management

Captive and wild Asian elephant (*Elephas maximus*) populations have been steadily declining, causing this species to be listed on Appendix I of the Convention for International Trade in Endangered Species (CITES) (Keele, 2001; Wiese, 2000). Due to this decline, elephant managers and researchers have been studying all aspects of elephant reproduction in an effort to arrest this decline and increase propagation of this species (Keele and Dimeo-Ediger, 1999). Currently, the literature on estrous synchronization in elephants has had conflicting results (Sikes, 1971; Rasmussen and Schulte, 1998; Bechert et al., 1999; Leong, et al., 2003a; Slade et al., 1997). If the occurrence of estrous synchronization was reliable, it could provide elephant managers with a valuable tool for planning natural breeding and artificial insemination (Rasmussen and Schulte, 1998). Synchronized herds of females could either be transported to male holding facilities for breeding or artificially inseminated using a single male to conserve resources and increase reproduction (Beal, 1996; Holt et al., 1996; Hinrichs et al., 2000).

The average length of the estrous cycle in both African and Asian elephants is reported to be 13 to 16 weeks (Plotka et al., 1988; Mainka and Lothrop Jr., 1990; Taya et al., 1991; Hodges, 1998; Figure 1). Both species also exhibit a period of heat or behavioral estrus lasting from one to seven days that coincides with male mating interest (Moss, 1983; Poole, 1994; Carden et al., 1998). Behavioral estrus is temporally associated with the initial rise in serum progesterone concentrations from their nadir with follicular development and a concurrent elevation in luteinizing hormone (LH) concentrations (Carden et al., 1998; Brown, 2000; Hermes et al., 2000). The elevation in serum LH is the second of two increases within the follicular phase of the cycle, with the first elevation in LH release occurring approximately 20 days before the second (Hodges et al., 1998; Hermes et al., 2000; Brown, 2000). In a study of African elephants, the first LH elevation was anovulatory while the second LH elevation and initial rise in progesterone were associated with ovulation (Hermes et al., 2000).

Estrous synchronization

A natural synchronization of estrous cycles has been documented in a broad range of species including sable antelope (*Hippotragus niger*), humans (*Homo sapiens*), chimpanzees (*Pan troglodytes*) and Siberian hamsters (*Phodopus sungorus*) (McClintock, 1971; Wallis, 1985; 1992; 1994; Thompson, 1995; Cooke et al., 2000). In a study of captive chimpanzees, comparisons were made of anogenital swelling patterns within groups and between groups that were housed in the same building, but maintained separately (Wallis, 1985). A pattern of synchronization within the groups was discovered with a mean of 5.7 days discrepancy between first anogenital swellings of the individuals, but no synchronization was noted between the groups (mean discrepancy in swelling patterns between groups was 10.4 days); Wallis, 1985. Patterns of first postpartum estrus in captive and wild chimpanzees' were synchronized with those of closely associated companions, indicating that social contact may play a role in estrous synchronization (Wallis, 1985; 1992). Wallis also investigated patterns of first anogenital swelling of wild adolescent chimpanzees and found similar patterns of estrous synchronization related to social factors (Wallis, 1994).

Another common cause of reproductive synchrony is associated with seasonal variations. This type of seasonal breeding often relies on photoperiod adjustments that trigger neuroendocrine activity and is exhibited in many species including domestic sheep (Woodfill et al., 1994). Other species, such as the springbok (*Antidorcas marsupialis*), may become synchronized due to changes in ambient temperatures (Skinner et al., 2001). Several species of primates have adapted to a seasonal system of mating based on available food resources (Koford, 1965), whereas other primate species do not exhibit seasonal variation in estrous cycling (Monfort et al., 1996). In one study on African elephants in captivity, the effect of seasonality was investigated using serum progesterone and prolactin content (Bechert et al., 1999). No seasonal effect was detected, although the authors speculated that the artificial lighting in their captive environment may have influenced their findings. In another study, births for captive elephants in India were seasonal, with most births occurring in January, suggesting a seasonal estrous period (Sukumar et al., 1997).

Chemical communication between conspecifics in many species has been indicated as a source of estrous synchronization (Rekwot et al., 2001) or suppression

(Barrett et al., 1993). In ungulates, the expression of the flehmen posture is widely known to signify the detection of reproductive chemicals through the vomeronasal organ (Thompson, 1995). Recent studies have shown a similar relationship to flehmen testing in the African and Asian elephant (Rasmussen and Schulte, 1998). These authors noted that female elephants would flehmen-test each others' urine, feces and urogenital region. They hypothesized these females use the information gained from these flehmen tests to detect the reproductive state of their conspecifics.

In a landmark study involving human subjects, female subjects with close social contact developed synchronous estrous cycles (McClintock, 1971). In later studies with female rats, Mclintock and Schank found that ovulatory pheromones could suppress or synchronize estrous cycles (Schank and McClintock, 1997). In marmoset monkeys (*Callithrix*), this suppression is associated with subordinate status within the group and is maintained through sensory cues (Barrett et al., 1993). Other authors have noted this dichotomy in the social effects of conspecifics to either suppress or synchronize estrous cycles using social or chemosensory cues. One author speculated that dominant African elephant females may have suppressed the reproductive activity of subordinates with chemosensory cues (Sikes, 1971). In studies on chemosensory behavior of Asian elephant females, the authors noted some synchrony in ovulation when females were housed together, but some fluctuations also were noted (Rasmussen and Schulte, 1998; Slade, et al., 1997). Estrous synchronization was not found in studies on African elephants (Leong, et al., 2003b).

In the present study, the individual estrous patterns of pairs of Asian elephants were determined using serum progesterone analysis (Brown et al., 1991; Carden et al., 1998). To determine if estrous cycles became synchronized, the discrepancy between the onset of their individual estrous cycles within pairs were compared (Wallis, 1985).

I will test the hypothesis that:

Asian elephants exhibit synchronization of their estrous cycles when housed in close social contact with conspecifics.

MATERIALS AND METHODS

Study design

Study investigated estrous synchronization in captive Asian elephants using serum progesterone analysis. The study was designed to test whether physically separated, normally cycling elephants would, when subsequently housed together, alter their estrous cycles over time to achieve a synchronous pattern.

Study location/housing

The animals utilized in this study were housed at Dickerson Park Zoo in Springfield, Missouri. All animals were allowed variable amounts of outdoor access when ambient temperatures were suitable (> 0°C). Short bouts of outdoor access were allowed in temperatures <0°C. Each set of study animals included a resident female and a visiting female that were housed together upon arrival of the visiting female (which initiated the study period for that pair). Upon detection of the onset of estrus in either female, (as determined by a rise in serum progesterone from basal concentrations to above 100 pg/ml), the pair would be placed into the male's enclosure for breeding. Courtship lasted from 1-5 days. Each adult animal was afforded a minimum of 37.16 m^2 of space as per AZA standards.

Subjects

This study utilized 24 (12 pairs) adult female Asian elephants. Animals were not randomly paired. Pairing was determined by visiting females' arrival as recommended by the management group of the SSP for that female to be translocated to the study institution for breeding. Females used in the estrous synchronization study ranged in age from 12 to 46 years, with a mean of 26.5 years (resident mean, 27; visitor mean, 26) (Table 1). All animals in the study were exhibiting continuous estrous cycles before and during the study period (no acyclic or pregnant animals were included in the study). The determination of asynchrony prior to housing the individual animals together was confirmed using serum progesterone analysis to discern the estrous pattern of each individual for comparison within a pair. None of the paired animals were randomly in a synchronous state before the study period.

Nutrition

During the study, all animals were given a fortified herbivore pelleted ration (HMS brand, Springfield, MO) and free-choice hay of varying types including brome, orchard grass, timothy and wheat hay. The animals also were given a variety of fresh fruits, vegetables, and breads. Water was always available. Because captive elephants have lower vitamin E concentrations than their wild counterparts, all animals were given an oral vitamin E supplement (three times per week).

Serum collection

Serum samples were collected weekly from all females during their respective study periods. Duration of the study period for estrous synchronization was one year per pair. Study period is normally the equivalent of three estrous cycles.

Serum was obtained by collection of whole blood via leg or ear vein in unanesthetized animals. Once the blood was collected, it remained at room temperature for 1h and then was centrifuged at 1500 x g for 20. Serum was stored in polypropylene tubes at -20°C until assayed for serum progesterone content.

Hormone analysis

Concentration of progesterone in serum from female elephants was measured via radioimmunoassay (RIA) utilizing a commercial kit (Diagnostic Products Corporation, Los Angeles, CA). An established assay protocol for serum concentrations of progesterone in Asian elephants was utilized (Carden et al., 1998).

Statistical analysis

A repeated-measures analysis of variance, (ANOVA), was used to determine if there was synchronization of estrous cycles between the females based on discrepancy in estrous onset within the pairs. The method of ranked normal deviates was used to confirm normality in the data set. To test for synchronization, weekly serum of the visiting and resident females were analyzed over one year for progesterone concentrations beginning with the new female's introduction. Three estrous cycles per annum were expected as predicted by the mean cycle length of 13-16 weeks noted in the literature. Data for each pair were aligned for analysis with the date of the initial introduction of the visiting female. Each individual of the pair was subsequently tested for the first sustained rise above 100 pg/ml of serum progesterone from follicular concentrations (below 100 pg/ml). Increase in progesterone signaled the initiation of a new estrous cycle (estrous onset and presumed ovulation). The discrepancy in weeks between the onset of estrous cycles within each pair was determined for all three cycles within the study period. Discrepancy values were then analyzed using a repeated-measures ANOVA (Wallis, 1985).

Mean cycle length, mode and range were calculated. Mean cycle length was used to calculate the average maximum discrepancy and average expected discrepancy within pairs that would be associated with the null hypothesis of asynchrony (a random dispersion of estrous onset; Wallis, 1985). Because the length of the estrous cycle of the resident or the visitor can change in either direction, the average maximum discrepancy is one-half the mean cycle length. In asynchrony, discrepancy values would be distributed evenly between zero and the maximum discrepancy value. This random dispersion should yield an average expected discrepancy of one-fourth of the mean cycle length (Wallis, 1985). Absolute changes in cycle lengths also were determined for both the resident and the visitor to determine which female (visitor or resident) may have changed to achieve synchrony.

RESULTS

The mean length of the estrous cycles during the study was 14.6 weeks (range: 7-26; mode: 16 weeks; SD, $\pm$ 3.4). With a null hypothesis of asynchrony, the average maximum discrepancy within pairs was determined to be 7.3 $\pm$ 1.7 weeks and the average expected discrepancy was determined to be 3.6 weeks. All pairs had a discrepancy value higher than the average expected value on their first cycle when initially housed together, with most pairs near the maximum discrepancy value indicating asynchronous estrous cycles. There was a significant change over time in discrepancy of estrous onset between individuals within pairs ($F = 35.5$ $(2,22)$, $p < 0.01$). Post-hoc protected dependent *t*-tests revealed that discrepancy values decreased ($p < 0.05$) from the first cycle ($m = 6.3 \pm 1.8$) to the second cycle ($m = 2.4 \pm 1.8$), and continued to decline to the third cycle ($m = 1.7 \pm 1.0$, NS). All but one pair, (6) showed a distinct trend over time toward synchrony, with a reduction in discrepancy values to below the average expected discrepancy value (Table 1).

The absolute change (increase or decrease) in cycle lengths for all resident females was a mean of 4.1 weeks, while visitor females changed a mean of 5.7 weeks (Table 2). The range of estrous cycle lengths for resident females was 8-19 weeks with a mean of 14.6 ± 2.7 and a mode of 16 weeks, while the visitors' range was 8-26 weeks, with a mean of 14.9 weeks $\pm$ 4.8 and a mode of 15 weeks (Table 2).

DISCUSSION

The results of this study documented a pattern of rapid estrous synchronization in captive female Asian elephants that have been housed together for one year, and maintenance of this synchronization to between zero and two weeks of estrous onset (Figure 2). Results demonstrate that unrelated elephants, previously unknown to each other, are capable of forming a social bond that results in estrous synchronization.

A relationship between social factors and estrous synchronization has been documented in female rats (*Rattus norvegicus*) and humans (*Homo sapiens*)(Schank and McClintock, 1997). These studies revealed that the close association of a subject with a conspecific resulted in estrous synchronization. Previous studies conducted with chimpanzees implicated the association of conspecifics as a key factor in estrous synchronization (Wallis, 1985; 1992; 1994). Wallis (1985) noted that wild female chimpanzees and their dependent offspring are dispersed throughout male territories. These females are competitors for food and may only be distantly related to one another. Even with these socioenvironmental constraints, Wallis found that wild chimpanzees exhibited estrous synchronization with their traveling companions when they emigrated to join males for mating (Wallis, 1992; 1994). Typically, related males will form bonds with each other and will cooperate to defend a territory against outside males (Wallis, 1985). Wallis (1985) noted that if there were more than one female in estrus, the dominant male could only select one female to guard from other males, thus allowing the other females to exercise mate choice among the remaining males. This system of mate choice by the estrus females and mate guarding by the males also has been noted in natural African and Asian elephant social structures (Poole, 1989; Sukumar, 1989, Payne, et al., 2003).

Asian elephants are maintained in small social herds comprising older related females and their offspring, with adult males joining the herds primarily for mating (Eisenberg et al., 1971; Sukumar, 1994). In captivity, elephants are usually not maintained in natural social structures because most animals are housed with unrelated females (Keele, 2001). Previous studies on the relationships among unrelated female Asian elephants in captivity have shown they develop close associations (Garai, 1992). Perhaps similar socioenvironmental factors (close associations and chemosensory exchange) may contribute to the development of estrous synchronization in these species (Wallis, 1985; Schulte and Rasmussen, 1999).

Other researchers have noted that most captive elephants experience more estrous cycles than their wild counterparts due to the lack of a male and subsequent breeding and pregnancy (Rasmussen and Schulte, 1998). This continuous cycling of the females may be a confounding variable in the development of estrous synchronization of captive elephants due to adjustments to the artificial environment (Rasmussen and Schulte, 1998). Conversely, because studies on the existence of estrous synchronization of wild Asian elephants have not been conducted, it is not known if this phenomenon may be a normal part of their reproductive strategies. Limited information regarding seasonal births suggests that these animals may have estrous synchronization in the wild related to environmental not social factors (Schank and McClintock, 1997)

Studies on human and chimpanzee estrous patterns have shown that a close social association is necessary for synchronization in these species (McClintock, 1971; Wallis, 1985; 1992; 1994). Chimpanzees or women that are merely housed in the same building but do not have a close association with each other, did not exhibit estrous synchronization (McClintock, 1971; Wallis, 1985). In a previous study, researchers did not find synchronization of estrous cycles in six African elephants that were housed in the same building, but there may have been some confounding variables (Leong et al., 2003a). Three of the 6 study animals were acyclic, therefore; they showed no clear pattern of progesterone cycling (Leong et al., 2003a). Although acyclic animals are common in Asian and African elephants, neither the cause nor effect of these events are clearly understood at this time (Brown and Lehnhardt, 1997; Brown et al., 1999 a,b). Additionally, the study animals were not maintained in the same groups over time and were placed individually in stalls at night, although they did have visual, olfactory and auditory contact (Leong et al., 2003 a,b). Shifting of animals between groups and separations at night may have affected development of social affiliations among the individuals or on chemosensory communication, and therefore; may have interfered with the development of estrous synchronization.

Of the twelve pairs of Asian elephants in the present study, only one pair (6; Table 1) did not synchronize upon introduction and subsequent housing. While this pair did show a distinct trend toward synchrony, they did not achieve estrous onset concentrations below the average expected value. Speculation on the cause of this apparent delay of synchronization would point toward the visiting female's inability to cope with translocation. This female was characterized as highly reactive to environmental stimuli (by caretakers at both the original and visiting institution) and required an extended time to acclimate to the new institution, handlers and conspecifics (M.A. Carden, personal observation). The female may have just needed more time to achieve synchrony. As a side note, this female copulated numerous times with the male but did not conceive during the study period.

This study supports preliminary findings of other authors that have noted some degree of reproductive synchrony in Asian elephants, although there are also instances of asynchrony (Rasmussen and Schulte, 1998; Slade et al., 1997). These previous inconsistent findings may be the result of confounding variables associated with acyclic animals, physiological problems, behavioral problems (including stress) or environmental inconsistencies such as herd structures. Further studies on estrous synchronization in wild Asian elephants would be useful to determine if this phenomenon exists in their natural social environment or is strictly a product of an artificial captive environment.

CONCLUSION

1. Asian elephants exhibited estrous synchronization over time when maintained in stable pairs.

2. Serum progesterone concentrations may be utilized to determine estrous synchronization within Asian elephants.

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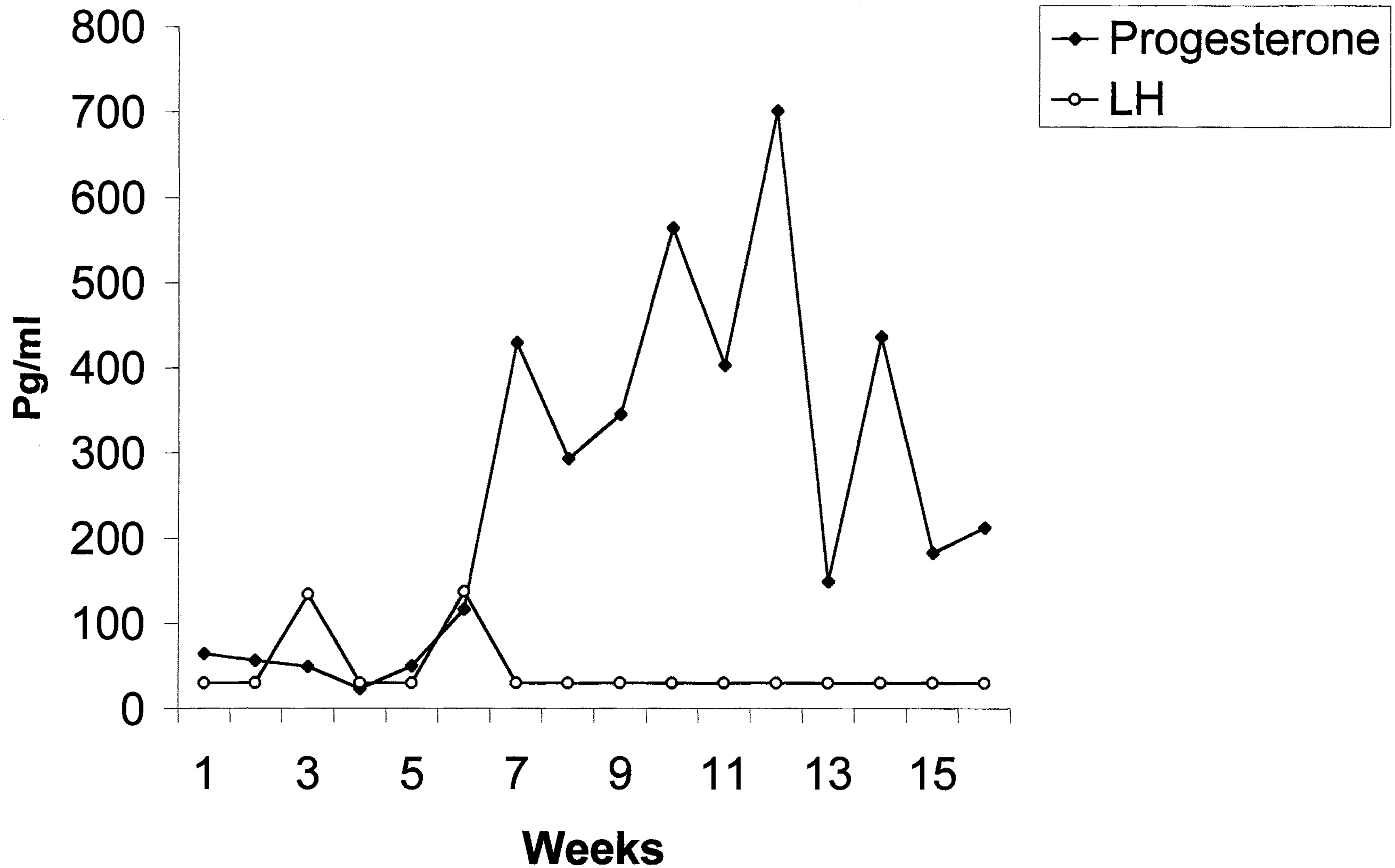
Table 1. Age of the individual pairs of Asian elephants located in Springfield, MO at the initiation of the study period and discrepancy in weeks between individuals for onset of estrous for each cycle.

Pair	Age (years) Resident	Visitor	Cycle 1 (weeks)	Cycle 2 (weeks)	Cycle 3 (weeks)
1	34	21	8	6	2
2	32	30	6	1	1
3	34	32	10	1	2
4	16	24	4	2	1
5	20	22	7	1	2
6	22	20	6	5	4
7	26	16	4	2	2
8	29	42	8	4	0
9	25	25	6	1	2
10	33	21	6	3	1
11	35	46	4	0	1
12	44	12	6	3	2
Mean	27	26	6.3 ± 1.8	2.4 ± 1.8	1.7 ± 1.0

Table 2. Absolute changes (increase and decrease) in estrous cycle lengths and ranges of cycle lengths in resident and visitor female Asian elephants located in Springfield, MO (weeks).

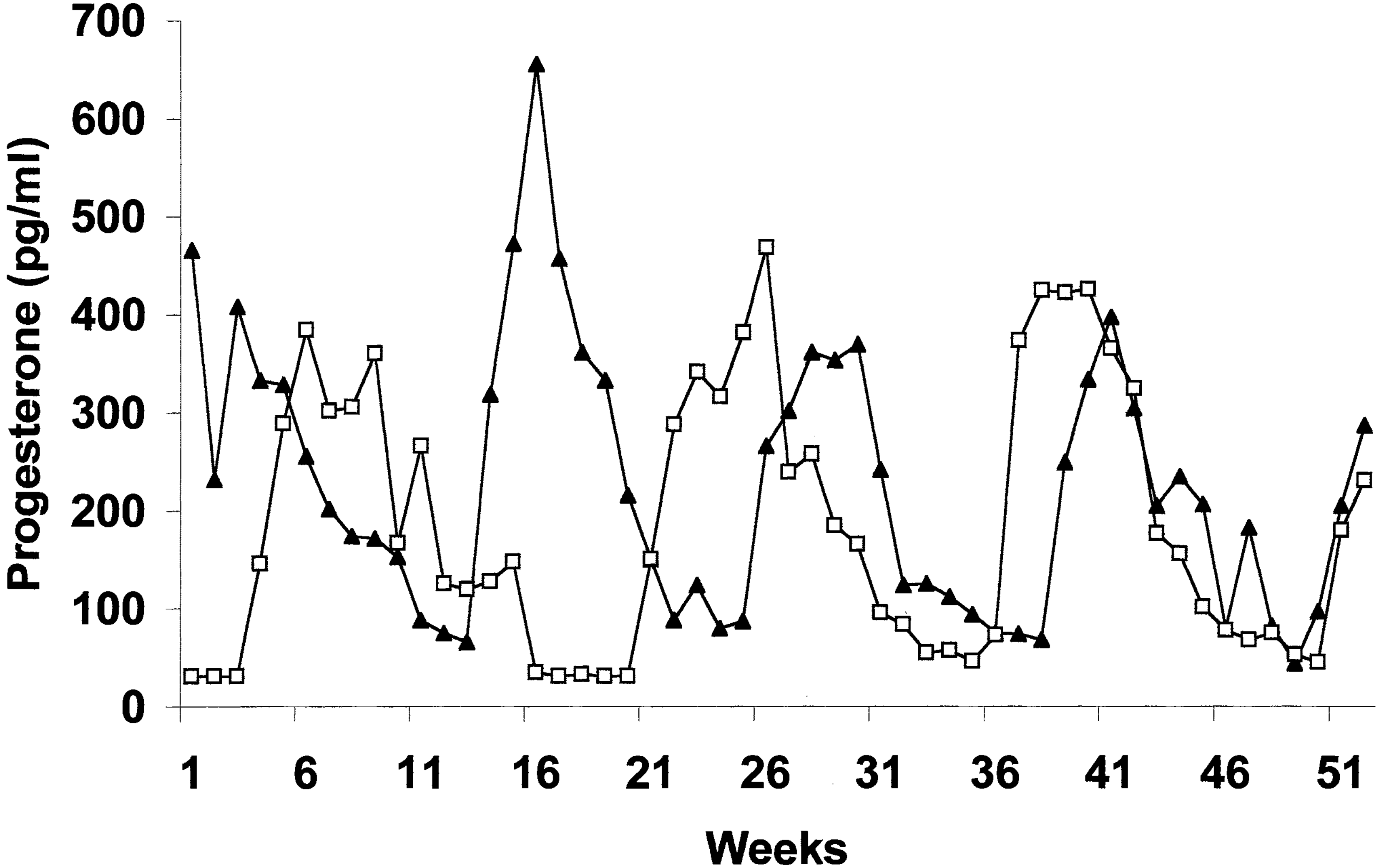
Pair	Resident cycle length changes (weeks)	Visitor cycle length changes (weeks)	Resident cycle range (weeks)	Visitor cycle range (weeks)
1	4	5	11-14	13-16
2	4	19	8-16	8-15
3	17	16	14-18	8-15
4	2	1	9-16	15-16
5	4	18	16-17	18-23
6	3	11	16-18	15-26
7	6	17	15-16	11-18
8	12	4	15-17	12-13
9	3	2	15-16	12-13
10	4	8	16-19	11-16
11	1	8	14-15	11-15
12	6	11	9-12	7-18

Figure 1. Typical weekly serum progesterone concentrations and luteinizing hormone elevations during an estrous cycle in a female Asian elephant.



Progesterone
LH
Pg/ml
800
700
600
500
400
300
200
100
0
1
3
5
7
9
11
13
15
Weeks

Figure 2. Progesterone concentrations (pg/ml) demonstrating an estrous synchronization pattern in a pair of Asian elephants housed in Springfield, MO over a one-year period.



Progesterone (pg/ml)
700
600
500
400
300
200
100
0
1
6
11
16
21
26
31
36
41
46
51
Weeks

CHAPTER III

REPRODUCTIVE BEHAVIOR IN MALE AND FEMALE ASIAN ELEPHANTS

INTRODUCTION

Behavior of captive Asian elephants

Reproductive problems in captive Asian elephants may have a fundamental explanation based in the social structure of these species (Carlstead and Shepherdson, 1994; Lindburg and Fitch-Snyder, 1994). Most of the Asian elephants in North America were captured at an early age in the wild and sold to zoos in small groups, mostly females (Schulte, 2000). These young orphan elephants did not have the opportunity to learn from older elephants within their herd (Poole et al., 1997). Although the orphan males' social development appears closely to mimic that of their wild counterparts, the females' social development seems to be altered (Poole et al., 1997). Unlike their wild counterparts, the orphan female Asian elephants do not form close social bonds (Poole, et al., 1997). This lack of social knowledge in the captive females has led to some aberrant reproductive behaviors contributing to low reproductive rates and a lack of adequate maternal behavior (Poole et al., 1979; Hutchins and Smith, 2001). Knowledge of wild Asian elephant social and reproductive behaviors could prove useful in the production of future captive herds.

Behavior of wild Asian elephants

Wild female Asian elephants are social animals that live in a hierarchical system (Gadgil and Nair, 1984; Garai, 1992; Fernando and Lande, 2000; Schulte, 2000). They live in herds consisting of a matriarch, her sisters, and their offspring (Rasmussen and Schulte, 1998; Hutchins and Smith, 2001). These herds form long-term bonds that are maintained over space and time (Garai, 1992). Asian elephant matriarchs have a knowledge of their surroundings that they use to navigate the herd to food and water (Nair, 1989; Rasmussen and Schulte, 1998; Schulte, 2000; Hutchins and Smith, 2001). Much of this survival behavior appears to be learned from the older animals in the herd (Eisenberg, et al., 1971; Gadgil and Nair, 1984; Poole et al., 1997).

Asian elephants also rely on learning for their social and reproductive behaviors (Nair, 1989). Young females learn how to react to the amorous attention of the males by watching their older kin during periods of mating (Eisenberg, et al., 1971). They quickly learn from the reactions of the older females that attempts to mate by related young males are not acceptable (Eisenberg, et al., 1971). The attention of adult males toward immature females also results in a negative response from the older, dominant females (Eisenberg, et al., 1971).

Male Asian elephants largely exist in a different social structure. Once young males reach sexual maturity, older females expel the males from the herd, theoretically to prevent inbreeding (Eisenberg et al., 1971; Rasmussen et al., 1993). These young males will often form bachelor herds that may last throughout their teens and early twenties (Eisenberg et al., 1971; Schulte, 2000; Keele, 2001). Once males are mature, they are largely solitary except when pursuing an estrus female (Rasmussen et al., 1993, Rasmussen and Schulte, 1998; Keele, 2001). When mature males have achieved sufficient size and age, they periodically exhibit a phenomenon called musth (Cooper et al., 1990; Rasmussen and Schulte, 1998). This period is characterized by a loss of appetite, drainage from the temporal glands, dribbling urine, elevated serum testosterone concentrations and increased aggression (Cooper et al., 1990; Taya, 1993; Lincoln and Ratnasooriya, 1996; Dickerman et al., 1997; Rasmussen and Schulte, 1998). Musth is a period in which the males advertise their dominance to others by scent-marking territories and engaging in male-male competition to secure mating rights to estrus females (Cooper et al., 1990; Rasmussen and Schulte, 1998). In herds of wild elephants, these musth males are dominant to all non-musth males and perform most of the mating (Rasmussen and Schulte, 1998).

Male and female Asian elephants use chemical communication to signal their reproductive or physiological state (Rasmussen et al., 1982; Rasmussen et al., 1993; Rasmussen, 1998; Rasmussen and Schulte, 1998; Lamps et al., 2001). These pheromones are usually associated with secretions from the breath, urine, feces, urogenital openings, temporal glands, inter-digital glands and ears (Rasmussen and Krishnamurthy, 2000). As females reach the pre-ovulatory state, they release pheromones that signal the males of their impending ovulation (Rasmussen, 2001). The chemicals released by the Asian females will attract males, even from a distance, and permit the males to locate the estrus females (Rasmussen and Schulte, 1998). Once males have located females, they usually approach them and inspect each one with their trunk presumably to gather relevant reproductive chemical signals to determine which females may be nearing estrus (Eisenberg, 1971; Rasmussen and Schulte, 1998). Male elephants assess the female's reproductive state by means of a modified flehmen response to urine, feces and the urogenital opening, using their vomeronasal gland (Diephuis, 1993; Rasmussen and Schulte, 1998).

Males release pheromones signaling their musth status, with a highly positive correlation in natural settings between the number of matings and incidence of musth (Perrin et al., 1996; Rasmussen and Schulte, 1998; Rasmussen, 1999; Rasmussen and Perrin, 1999; Schulte and Rasmussen, 1999). Non-musth males avoid musth males, thereby eliminating themselves from the competition (Rasmussen and Schulte, 1998). Females exhibit preferential mating with dominant musth males and evade non-musth males or related males in an effort to avoid mating with them (Rasmussen and Schulte, 1998).

Both African and Asian elephants also communicate through a complex system of infrasound that can be used by females for normal social interaction or to alert males to their reproductive state (Payne et al., 1986; Garstang et al., 1995; Larom et al., 1997; O'Connell et al., 1997; Payne, 1997; Byron et al., 1998; Ben-Ari, 1999; Langbauer, 2000; O'Connell-Rodwell, 2000; Payne et al., 2003). In African elephants, a wide range of temporal patterns have been associated with reproductive vocalizations (Leong et al., 2003 a,b). Female African elephants vocalized more in the pre-ovulatory phase of their estrous cycle than they did at ovulation (Leong et al., 2003 a,b). Calling males before ovulation would allow them to locate the females and compete for dominance before the ovulatory period (Leong et al., 2003 a,b). This pattern of pre-ovulatory calls would help insure multiple males would be in attendance for mate selection by the time of her ovulatory period (Rasmussen and Schulte, 1998; Leong et al., 2003b). Male elephants have been known to signal their musth status with a "musth rumble". In playback experiments, musth males would approach this call, while non-musth males would leave the area (Poole, 1999). Elephants are even known to produce seismic signals that can travel great distances through vocalizations, foot-stomping and locomotion (O'Connell et al., 1997; Arnason et al., 1998; O'Connell-Rodwell, 2000). This seismic communication is often associated with infrasonic calls (O'Connell et al., 1997; Arnason et al., 1998; O'Connell-Rodwell, 2000).

Reproductive behavior

Observation has been made of reproductive behavior in wild Asian elephants, but they have been largely anecdotal or of limited sample size (Slade, 1903; Norris, 1959; Eisenberg et al., 1971; McKay, 1971). In early accounts, descriptions were given of the reproductive behavior of wild Asian elephants, but not until the early seventies did a widely published analysis of Asian elephant reproductive behavior exist (Eisenberg et al., 1971). Although this study was conducted in their natural environment, the elephants were captive and the study included only one male and two females (Eisenberg et al., 1971). Even with a small sample size and a semi-captive setting, it yielded a very detailed analysis of the Asian elephants' reproductive behavior (Eisenberg et al., 1971). Eisenberg detailed several categories of reproductive behaviors classified as contact-promoting: visual, tactile, olfactory and auditory (Eisenberg et al., 1971). These researchers noted a number of reproductive behaviors that were later investigated by other researchers. They described a pattern of olfactory behavior by the male Asian elephant such as smelling the urine, feces, and anogenital regions of the females, often resulting in a flehmen response (Eisenberg et al., 1971). These authors then speculated about the existence of a pheromone that females may emit to signify their reproductive state (Eisenberg et al., 1971). The existence and composition of such an estrus pheromone ((Z)-7-dodecen-1-yl acetate) was discovered years later by other researchers (Rasmussen et al., 1996; Rasmussen et al., 1997).

Similarly, there is a paucity of information on breeding in captive animals (Eisenberg et al., 1971; Jainudeen et al., 1971; Rasmussen et al., 1982; Rasmussen et al., 1993; Poole et al., 1997; Carden et al., 1998). Although natural breeding has been accomplished in North America, it has been achieved at a limited number of institutions that have housed male elephants (Poole et al., 1997; Hutchinson and Smith, 2001). The information gained by the managers at these institutions has not been well documented or dispersed within the elephant community. For this reason, many institutions that are acquiring males are not prepared to manage a herd of breeding elephants. Long-term data on male and female behavior needs to be analyzed to assess the types of behaviors typically observed during these reproductive events. This information would assist managers who are unfamiliar with the earlier onset of sexual maturity in captive animals and the intense nature of social interactions of mature Asian elephants in the development of new breeding programs (Hutchinson and Smith, 2001; Rasmussen and Schulte, 1998). Prior knowledge of elephant reproductive behavior could be useful in determining herd size, designing facilities, and making decisions concerning the welfare of females in aggressive encounters with the much larger males.

In present study, five categories of reproductive behavior were monitored (olfactory, locomotory, aggressive, pre-copulatory and copulatory behavior) for both male and female Asian elephants. Incidences of the behavior in these categories will be related to the experience level, age and age discrepancy (absolute discrepancy between male and female age per pair) of the study animals to determine if there is a relationship between these variables.

I made the following predictions:

1. There will be an increase in the rate of olfactory, pre-copulatory and copulatory behaviors in older and more experienced males.

2. There will be an increase in the rate of aggressive and locomotory behaviors in younger and less experienced males.

3. There will be increased aggression in higher age discrepancy pairs.

4. There will be an increase in the rate of olfactory, pre-copulatory and copulatory behaviors in older and more experienced females.

5. Younger and less experienced females will experience more aggressive behavior from the males.

6. Younger and less experienced females will demonstrate higher rates of locomotory behaviors.

MATERIALS AND METHODS

Study design

Study examined the behavior of captive Asian elephants to quantify their typical mating behaviors. Male and female data were analyzed based on the age, experience level, and age discrepancy of males versus females. A multiple linear regression analysis was used to determine any relationship between the independent variables (age, experience, age discrepancy) and the reproductive behavior categories (olfactory, locomotory, pre-copulatory, aggressive and copulatory) for both males and females.

Study location/housing

The animals utilized in this study were housed at either the Tulsa Zoo in Tulsa, Oklahoma (male 3 and female 8) or Dickerson Park Zoo in Springfield, Missouri (all other subjects; Table 1 and 2). All data collection took place when ambient temperatures were between 18-27°C. When not with the bull, females were housed in social groups ranging in number from 2 to 7 individuals. Males were housed separately unless attempts at breeding were under way. Each adult animal was afforded a minimum of 37.16 m^2 of space as per American Zoological Association standards.

Subjects

Study involved 20 adult female and 4 adult male Asian elephants. All females were captured in Asia and translocated to the United States at estimated ages of 2 years old or less. Females used in the reproductive behavior study ranged in age from 18 to 39 years (Table 1). By the age of 36 years, male number 1 had courted 18 different females, of which 9 conceived (Table 2). The study period encompassed his age between 30 and 36 years and included observations of bouts with 10 females. By the age of 22 years, male number 2 had courted 10 different females, with 0 conceiving. The study period encompassed his age between 18 and 22 years and included observations of bouts with 8 females. Two males were only paired with 1 female each during the study period and were 15 and 30 years of age at the time, although both had previous courting experience (Table 2). Male numbers 1 and 2 successfully mated during the study period (4 and 1 matings, respectively). Only male number 1 successfully bred females during the study (3 out of 4 conceived).

Serum collection

Serum samples were collected weekly from all females in their respective study periods, typically lasting four months. Samples were collected to determine the timing of the individual subject's estrus period and collections for the study stopped once estrus had been identified. Estrus was defined as the period of continuous progesterone increase above 100 pg/ml following basal follicular concentrations and was correlated with the peak mating period (ovulation was presumed to occur at the initial rise in progesterone; Carden et al., 1998; Hermes et al., 2000).

Serum was obtained by collection of whole blood via leg or ear vein in unanesthetized animals. Once the blood was collected, it remained at room temperature for 1h and then was centrifuged at 1500 x g for 20. Serum was stored in polypropylene tubes at -20°C until assayed for serum progesterone content.

Hormone analysis

Concentration of progesterone in serum from female elephants was measured via radioimmunoassay (RIA) utilizing a commercial kit (Diagnostic Products Corporation, Los Angeles, CA). An established assay protocol for concentrations of serum progesterone in Asian elephants was utilized (Carden et al., 1998).

Video collection/behavior data

Behavioral data was collected with video recordings initiated at the onset of male and female cohabitation in their captive environment each day (no other animals were present in the enclosure). Pairs cohabitated for 7 to 8 hours per day. Bouts of the individual pairs were analyzed from the video recordings (by one observer) for all occurrences of selected behaviors (per second) that are listed in the reproductive ethogram (see Appendix 1). These reproductive behaviors were then converted to rates of behavior per 5 minutes for analytical purposes. This ethogram was compiled based on information obtained in the literature concerning elephant reproductive behavior and personal observation of reproductive behavior. The ethogram was divided into five major reproductive categories, (aggressive, olfactory, locomotory, pre-copulatory and copulatory behavior). The olfaction category included: smelling urine, the urogenital region, the tail, the toenails, the ears, the mouth/palatal pit region and flehmen responses. The locomotion category included: walking, the male driving the cow by pushing her while walking and following. The aggressive category included pushing from the side or behind, head on sparring, kicking and biting. The pre-copulatory behavior category included the trunk over side/back, pre-mounting kick, attempted mounting, mounting and erection.

A bout was defined by the initiation of reproductive behaviors as listed above that resulted in an erection by the male and ceased after the male no longer approached or initiated contact with the female. Although 67 hours of video recordings were taken, only the first complete bout per pair that was associated with estrus (as determined by progesterone assay) was used for analysis. Bouts ranged from 2 to 13.5 minutes and were all recorded within 20 minutes of the male and female being placed together for the day. If the male initiated any of the reproductive behaviors listed in the ethogram, but failed to achieve an erection, that recording was disqualified from the data set and the next chronological incidence of reproductive behaviors was scored for analysis. One bout from each pair was used for analysis of the reproductive behaviors (n =

20). Once the complete bouts were identified, they were viewed twice to record each second of selected behaviors listed in the ethogram for the males and females separately (all data analysis was conducted by the same person).

Statistical analysis

Serum progesterone concentrations were determined to confirm that all study females were in estrus at the time of behavioral data collection. Any recordings of bouts not associated with estrus were removed from the study to avoid confounding variables that may have been associated with estrus pheromones or behaviors.

Three independent variables were used for statistical analyses: age, age discrepancy, and experience level of the study animals. The age discrepancy variable compared the age differences within each pair and recorded these as absolute values. The experience level of each study animal was recorded as the total number of reproductive partners they had in their lifetime (current study partners were included in the figures). The reproductive behavior of the 20 females and 4 males were analyzed using multiple linear regression based on rates per five minutes of all occurrences of selected reproductive behaviors exhibited by the focal animals during bouts (olfactory, locomotory, aggressive, pre-copulatory and copulatory). Mean values for each of the 5 reproductive categories for males and females were calculated. Of the 4 males used in the study, 2 contributed 18 of the 20 data sets over a period of up to 6 years. Because the number of data collections was uneven between the males, the data were analyzed using regression analysis based on mean values.

Additionally, a regression analysis was conducted on the individual aggressive values of the males from the 20 bouts according to the number of reproductive experiences of the males at the time of the data collection (male data from all 20 bouts were used not mean values). Correlation analysis (with Pearson's correlation coefficient) was conducted to examine relationships between pre-copulatory behavior and copulation in males, male aggressive behavior and copulation; female locomotion (as possible uncooperative behavior) and aggression received by the female from the male, and the incidence of females extending their tail to one side upon approach of the male and other pre-copulatory behaviors or copulations. The tail extension category was added after preliminary viewing of the videotapes revealed that 45% of the females exhibited this behavior.

The number of times females initiated locomotory behaviors while males were exhibiting pre-copulatory and copulatory behaviors were also determined to assess the females' cooperation. These were expressed as ratios between female initiated locomotion while males were exhibiting pre-copulatory and copulatory behaviors.

RESULTS

Mean rates per five minutes of aggressive behavior for males was 15.9 ± 27.5 (sd) during bouts. Rates of male aggressive behaviors were predicted by the independent variables ($F = 6.82$, $p < 0.01$, $R^2 = 0.682$). Both experience level ($p < 0.05$) and age discrepancy ($p < 0.01$) were predictors of aggressive behavior. Less experienced males had higher rates of aggressive behavior toward the females than experienced males (Figure 1). Pairs with higher age discrepancies also had higher rates of aggressive behavior from males to females (Figure 2). Age of the male elephants was not a predictor of aggressive behavior ($p > 0.05$). The other 4 categories of reproductive behaviors: olfactory, locomotory, pre-copulatory and copulatory (mean values ± sd; 27.7 ± 32.7; 91.9 ± 34.3; 78.0 +56.8; 79.5 ± 125.1, respectively) did not show a significant relationship with age, age discrepancy or experience levels when multiple linear regressions were conducted ($p > 0.05$; Figure 3).

The incidence of male pre-copulatory behaviors and copulations were positively correlated ($r = 0.723$, $p < 0.01$) and male aggressive behavior and copulation were negatively correlated ($r = -0.676$, $p < 0.01$). When the 20 individual values for aggressive behavior were compared with the experience level of the male in each bout a moderate relationship was noted ($r^2 = 0.4$, $p < 0.01$). Clearly the small sample size and skewed proportions of data collections within the male data limit the predictive ability of these tests.

Multiple linear regression of the rate of females' reproductive behaviors showed no ($p > 0.05$) relationships between any of the three variables (female age, age discrepancy and female experience) and the reproductive categories (olfactory, locomotory, aggressive, pre-copulatory and copulatory; Table 3). All categories of female reproductive behaviors had large standard deviations from the mean. These large standard deviations indicate a lack of discernible patterns among these females in the five reproductive behavioral categories. The rate of female pre-copulatory behaviors and copulations were positively correlated ($r = 0.9$, $p < 0.01$; Figure 4). Nine of the 20 females in the study were observed extending their tail out to one side when the male approached, but of these 9 females, only 2 copulated with the male. There was a relationship ($r = 0.434$, $p < 0.05$) between extending tail and female pre-copulatory behavior, but no relationship to copulations ($p > 0.05$). There was no relationship ($p > 0.05$) between the incidence of female locomotory behaviors and aggression received.

The number of times females initiated locomotory behaviors (mean 2.6 ± 2.8 instances) while males were exhibiting pre-copulatory and copulatory behaviors (mean 5.1 ± 4.1 instances) were also determined to assess cooperation. These were expressed as ratios between instances of female initiated locomotion while males were exhibiting pre-copulatory and copulatory behaviors (copulation successful, 1.75%; copulation unsuccessful, 1.7 %).

DISCUSSION

The lack of information concerning the reproductive behavior of Asian elephants has led to misconceptions about behaviors typically observed. This study examined some typical courtship behaviors observed and addressed several hypotheses proposed among elephant managers concerning reproductive behavior in Asian elephants.

The females in this study showed no significant relationship between the age, age discrepancy and experience level variables with the 5 reproductive behavior categories. In the wild, the females' mating behavior appears to be largely limited to signaling the male of her reproductive state and the selection of a mate (Rasmussen and Schulte, 1998). In a previous study on Asian elephants, researchers noted that females selected musth males over non-musth males for mating (Rasmussen and Schulte, 1998). The females in this study were all housed in a captive environment and were not given the opportunity to select their mate. In 19 of the pairs, there were 2 males housed at the study institution and managers "selected" the male for the female, although the female had visual, auditory and olfactory contact with the non-selected male. In addition, all males were in musth before the observation period, therefore another aspect of mate selection was already determined for the females. The effect of the removal of the mate selection process from the female is unknown, and may have been a confounding variable because the female may have selected the other male. This confounding may account for some of the higher incidences of aggressive behavior received by a female if she was forced by managers to remain in the presence of a male that she would not have selected. The female may have been rebuffing the male in some way not discerned by this study, thus soliciting aggressive behavior from the male. Additionally, orphaned females have altered social patterns when compared to their wild counterparts (Poole et al., 1997), perhaps having a confounding effect on their reproductive behavior as well.

An interesting observation was the relationship of tail extensions to pre-copulatory behaviors. A similar behavior has been previously noted in Asian elephants (Eisenberg et al, 1971). Although 45% of the study females demonstrated this behavior (9 out of 20 females), these females represented 40% of the pairs that copulated (2 out of 5). Although the sample size for copulating females is low, it would initially appear that the tail extension behavior may be a signal to the males that the female is receptive to mating as in the horse and dog (Senger, 1997). The rate of pre-copulatory behaviors observed in the males and females were highly correlated to the incidence of copulation. This relationship implies that a certain amount of pre-copulatory behavior is required to accomplish copulation.

In this study, males were the primary initiators of reproductive behaviors. Previous studies on Asian elephants have shown a high percentage of courtship behaviors being initiated by the males when the females were in estrus (Poole et al., 1997). The only significant relationship observed between independent variables and a behavior category was with male aggressive behavior. Less experienced males showed a higher rate of aggressive behavior toward the females (both in analysis of means and in individual bout values). The males with higher age discrepancy values between themselves and their mate also showed higher rates of aggressive behavior. This type of antisocial behavior has been noted in previous studies in which "orphan" young male elephants in the wild exhibit highly aggressive behavior toward other species. This highly aggressive behavior was attributed to the lack of dominance by older males, allowing younger males to experience musth at younger ages without competition (Slotow and van Dyk, 2001).

Interestingly, the relationship between age and aggressive behavior was not significant, supporting the logic that the higher level of aggressive behavior was due to a lack of social development and experience in the study males or lack of suppression by dominant or older musth males. Other studies of Asian elephant courtship behavior have noted that in competition between older and younger males for mating rights, older males are more successful and younger males concede without a fight (Eisenberg et al., 1971). The discrepancy between the results of this study and the results noted in the literature are based upon the variation between a captive situation, with no true competition between the males for mating rights, and a wild situation in which the males must fight for mating rights.

CONCLUSION

1. Aggressive behavior in male Asian elephants toward females increases with increasing age discrepancies between males and females.
2. Aggressive behavior in male Asian elephants toward females decreases with increasing numbers of reproductive experiences.
3. Male aggressive behavior is negatively correlated with successful mating in Asian elephants.

4. A strong positive relationship between the rate of pre-copulatory behaviors and copulation were noted in Asian elephants.

5. Tail extension behaviors were correlated with the rate of female Asian elephant pre-copulatory behaviors.

6. No relationship was noted between experience level or age discrepancy and olfactory, locomotory, pre-copulatory or copulatory behaviors of male Asian elephants.

7. No relationship between age and rates of all categories of reproductive behavior were determined in male Asian elephants.

8. No relationship was found between age, age discrepancy or experience and all categories of reproductive behavior in female Asian elephants.

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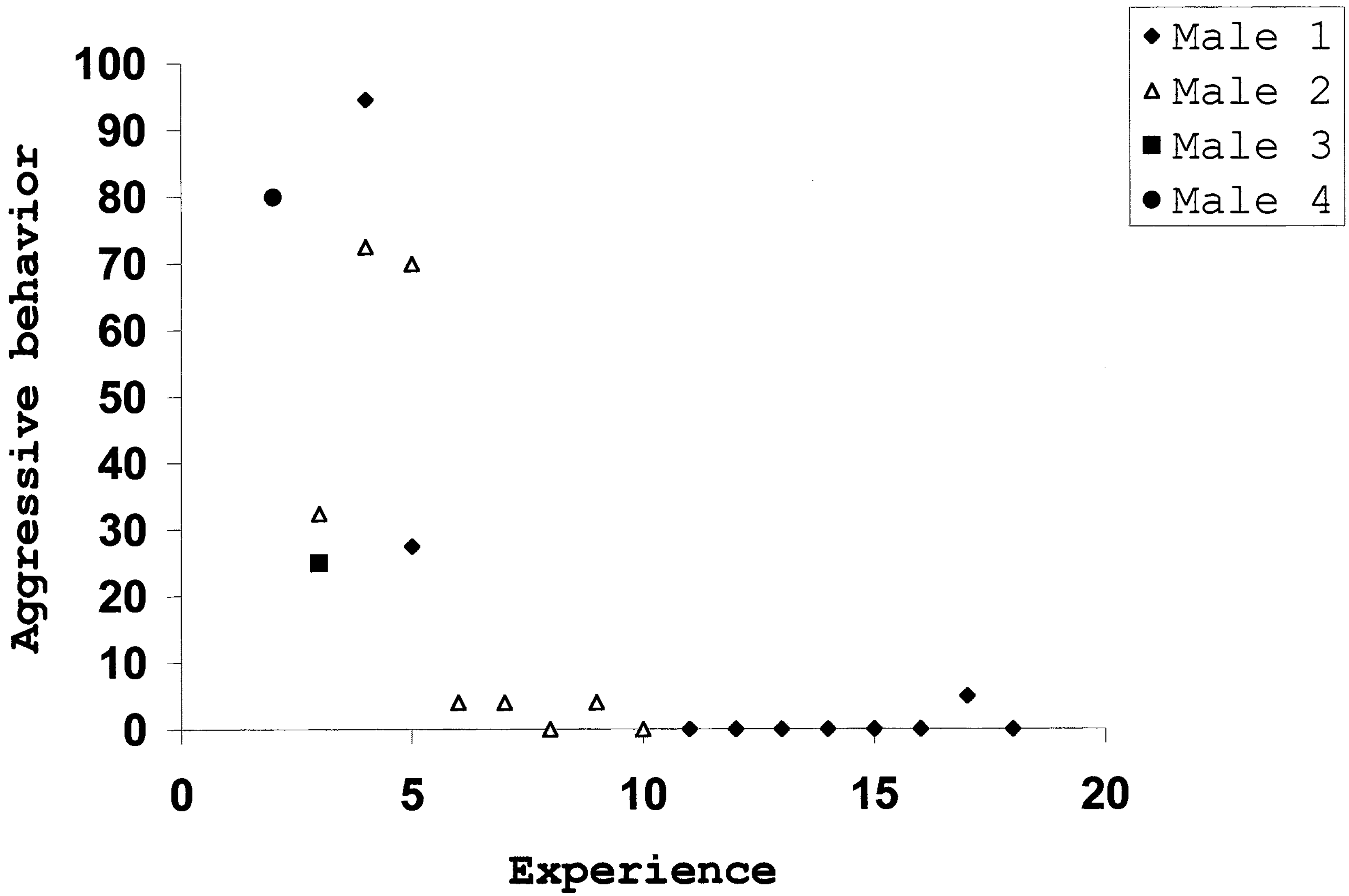
Table 1. Asian elephant's age, number of males courted, copulations and conceptions and age discrepancy (a-the absolute difference in age between the male and female within a pair). Female 9 was housed in Tulsa, OK, all other females were housed in Springfield, MO.

Female	Age (years)	Lifetime number of males courted, copulations and conceptions	Age discrepancy in years (a)
1	18	1/1/0	16
2	18	2/1/0	2
3	20	1/1/1	12
4	22	1/1/1	8
5	23	1/0/0	11
6	23	2/0/0	5
7	26	1/0/0	7
8	28	1/0/0	8
9	29	1/0/0	1
10	29	2/1/1	7
11	30	1/1/0	0
12	30	1/0/0	0
13	30	1/0/0	6
14	31	2/1/0	13
15	31	2/1/1	12
16	32	1/1/1	5
17	32	1/0/0	5
18	32	2/1/1	10
19	33	1/0/0	2
20	39	3/2/1	24

Table 2. Asian elephants' age range during the study period, lifetime number of females courted, copulations and conceptions and birth origin. Male 3 was housed in Tulsa, OK, all other males were housed in Springfield, MO.

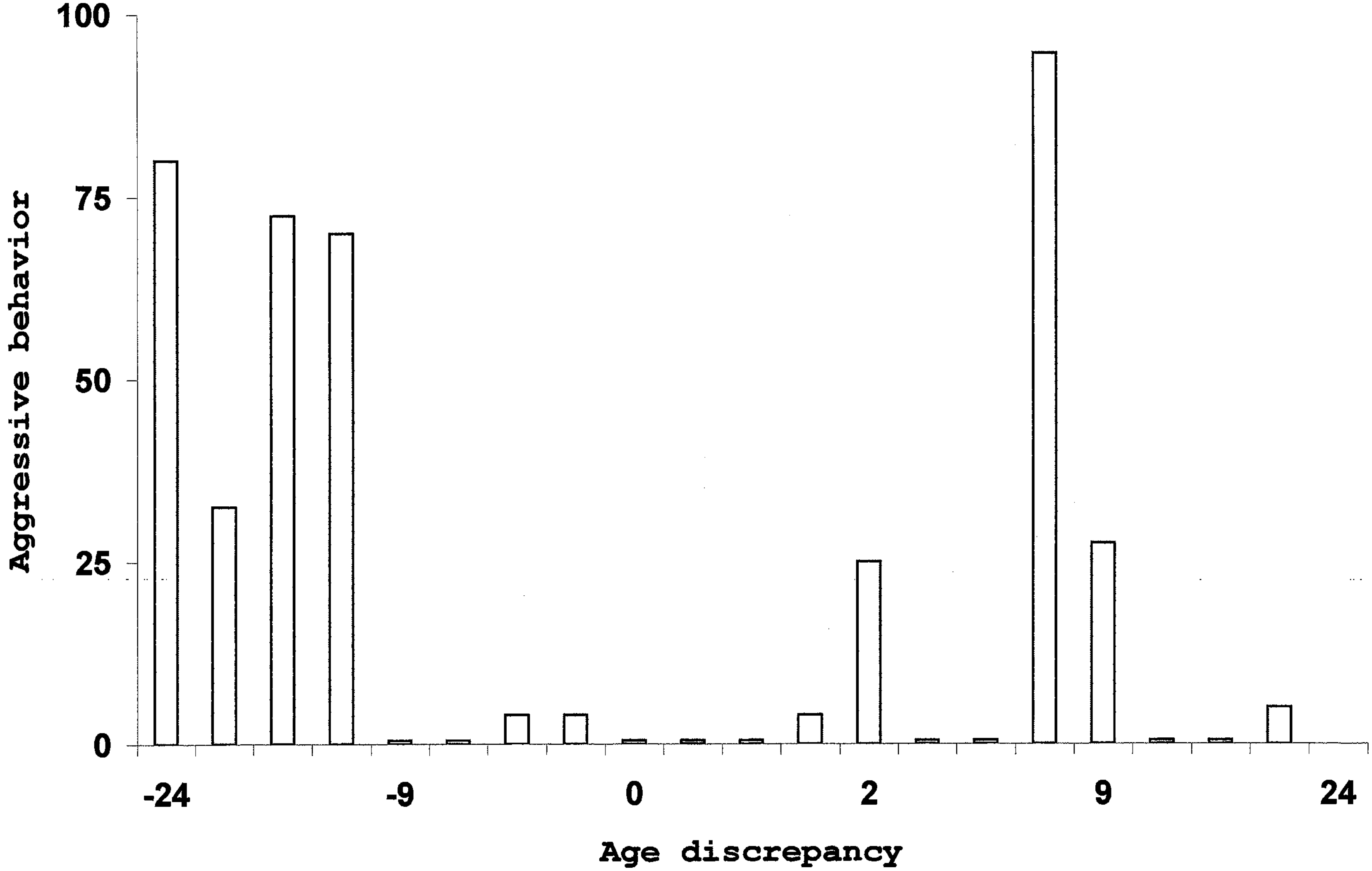
Male number	Ages during study period	Lifetime number of females courted/copulations/conceptions	Wildborn or captive born
1	30-36 years	18/9/9	Wild
2	18-22 years	10/5/0	Captive
3	30 years	3/3/2	Wild
4	15 years	2/1/1	Wild

Figure 1. Aggressive behavior of male Asian elephants during courtship bouts with 20 females based on the experience level of the males (rate per five minutes). Males were housed in Tulsa OK and Springfield, MO.



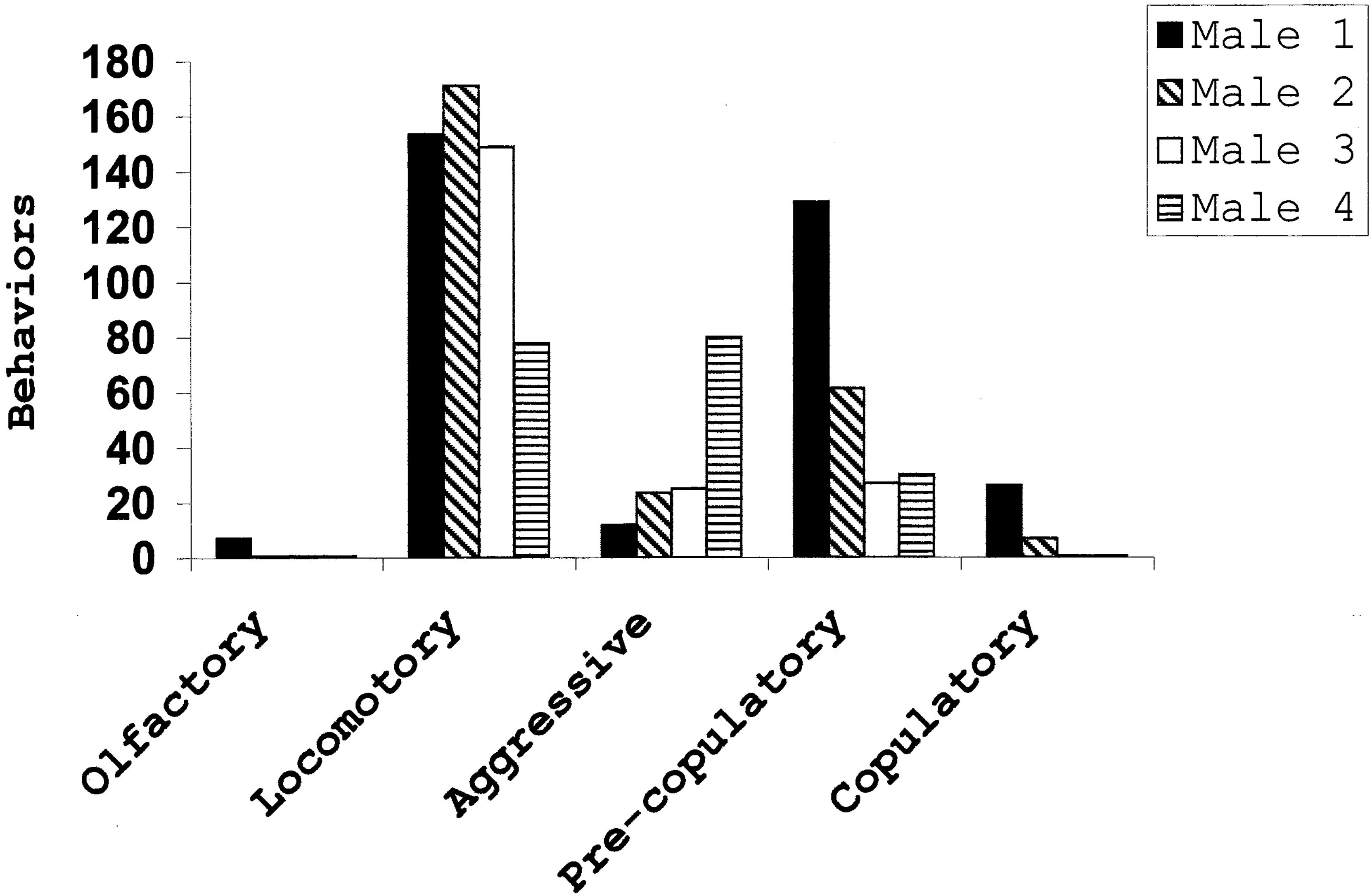
Aggressive behavior
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
Experience
Male 1
Male 2
Male 3
Male 4

Figure 2. Aggressive behavior of male Asian elephants during courtship bouts based on the age discrepancy of the males to the females (rate per five minutes). Negative values reflect a younger male than female. Positive values reflect an older male than female. Males were housed in Tulsa OK and Springfield, MO.



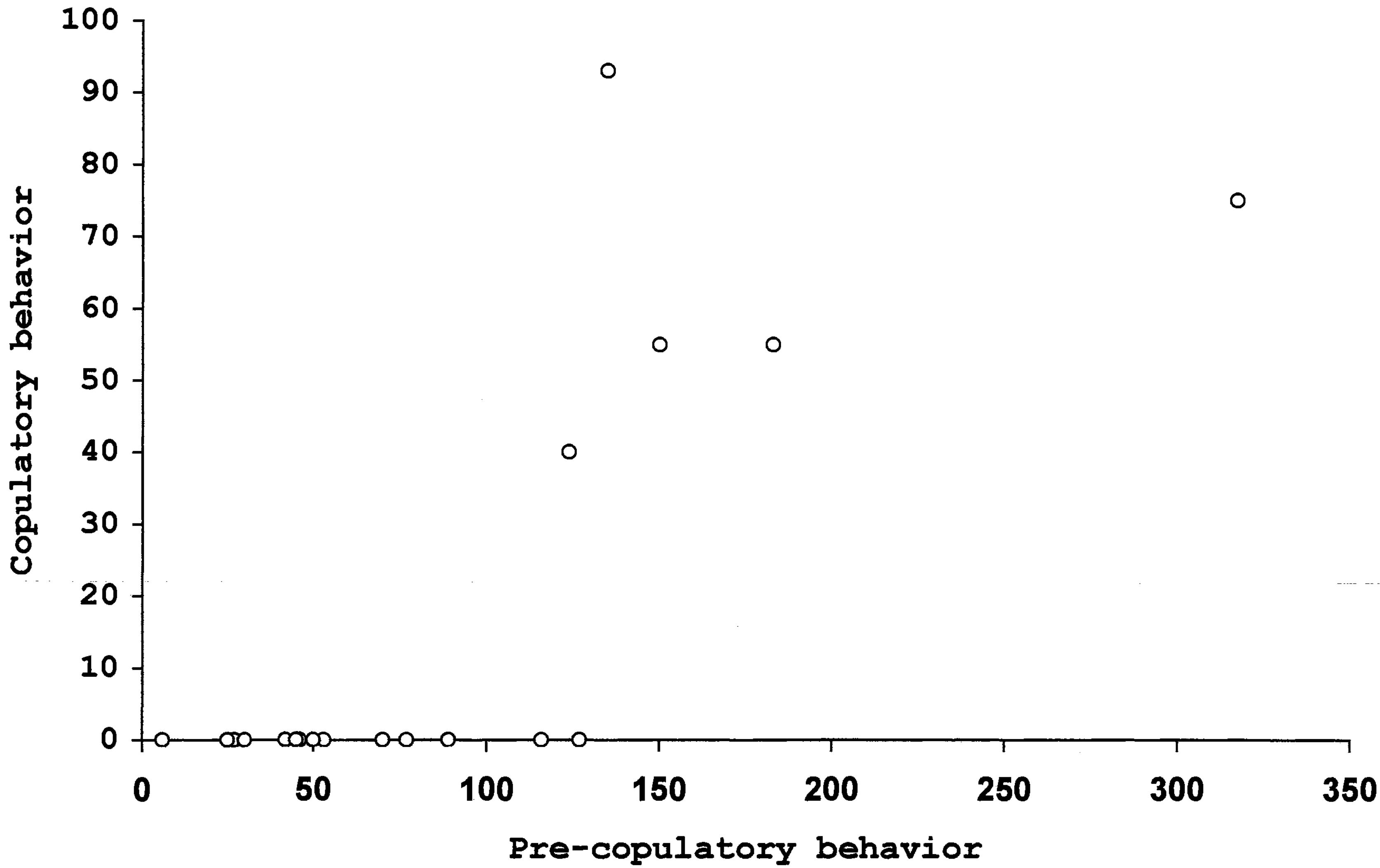
Aggressive behavior
100
75
50
25
0
-24
-9
0
2
9
24
Age discrepancy

Figure 3. Incidence of five reproductive behaviors in four male Asian elephants during courtship bouts (rate per five minutes). Males were housed in Tulsa OK and Springfield, MO.



Male 1
Male 2
Male 3
Male 4
180
160
140
120
100
80
60
40
20
0
Behaviors
Olfactory
Locomotory
Aggressive
Pre-copulatory
Copulatory

Figure 4. Incidence of pre-copulatory behaviors and copulation of twenty female Asian elephants during courtship bouts (rate per five minutes). Females were housed in Tulsa OK and Springfield, MO.



Copulatory behavior
100
90
80
70
60
50
40
30
20
10
0
0
50
100
150
200
250
300
350
Pre-copulatory behavior

CHAPTER IV

LACTATIONAL ANESTRUS IN ASIAN ELEPHANTS

INTRODUCTION

Fecundity and fatality

The current fecundity rate of captive Asian elephants in North America is estimated at 1 to 2% and the projected needed to maintain a viable captive population is 7 to 8% (Hutchins and Smith, 2001). Factors associated with this low birth rate include a high incidence of dystocia, resorption of the fetus, stillbirth and calves retained in utero (Kurt and Mar, 1996; Niemuller et al., 1998; Taylor and Poole, 1998; Keele and Dimeo-Ediger, 1999). Many delivery attempts of captive primiparous elephants end with stillbirth. For older cows, dystocia is more prevalent and this problem often ends with unsuccessful caesarean sections in which neither the mother or calf survive (Keele and Dimeo-Ediger, 1999). To date, there has not been any successful caesareans performed on elephants. In all cases both the calf and cow has died.

Since 1962, > 100 (exact figures are not given) Asian elephant calves have been produced in the North American captive population (Keele and Dimeo-Ediger, 1999). Of these calves, there has been > 30 % mortality rate due to stillbirth or death of the infant before 30 days of age (Kurt and Mar, 1996; Taylor and Poole, 1998; Keele and Dimeo-Ediger, 1999). If calves are successfully delivered, they face a high rate of infant mortality due to maternal rejection and/or aggression (Schulte et al., 2000). Most captive females never learned natural social behaviors due to their early removal from the wild and also from a lack of experience with calves in their social group in captivity (McComb et al., 2001). Without learning these behaviors from older cows within their herd, orphaned females often do not develop maternal behaviors and respond to calves with aggression or indifference. Few calves have survived that were fostered by humans (Ensley et.al., 1994).

Other lethal dangers are diseases such as tuberculosis or the elephant herpes virus (Richman et al., 1999; Richman et al., 2000a,b; Hutchins and Smith, 2001). The herpes virus has a high rate of mortality among young elephants, with only 3 successful treatments being reported of 9 confirmed cases in North America (Metzler et al., 1990; Ossent et al., 1990; Keele and Dimeo-Ediger, 1999; Richman et al., 2000 a,b; Fickel et al., 2001; Ryan and Thompson, 2001; Schaftenaar et al., 2001; personal communication).

Tuberculosis in captive elephant populations has increased elephant mortalities and is a public health risk (Larsen et al., 2000; Mikota et al., 2000; Har et al., 2001; Mikota et al., 2001). Outbreaks are being addressed by researchers and disease control agencies internationally, with a high mortality rate for elephants of all ages (8 of 9 confirmed cases) (Mikota et al., 1994; Richman et al., 2000; Mikota et al. 2001). The lack of a reliable indicator for tuberculosis in elephants makes the current infection rate of the captive population unknown (Mikota et al., 1994).

The high mortality rate among infant elephants is a substantial factor in the population decline of captive Asian elephants and needs to be addressed if the population is to become sustainable (Wiese, 2000; Keele, 2001). Mikota reported that of the 88 elephant deaths recorded from 1908 to 1991, 32 were sub-adults or younger, 28 were adults and 22 were of unknown age (Mikota et al., 1994). Coupled with this problem is the need to increase the reproductive output of the existing population of Asian elephants in North America. In an effort to increase reproductive output, some institutions have enacted a policy of early calf weaning designed to decrease the duration of the lactational anestrus period. This early calf weaning policy has met with strong opposition from animal rights groups that feel it is unnecessary and both physically and socially detrimental to the calf. Study used an endocrine monitoring protocol to investigate the nature of lactational anestrus in the Asian elephant.

Reproductive diagnostic methods

Reproductive status of females has been determined through the analysis of serum progesterone, luteinizing hormone (LH), follicle stimulating hormone and estradiol concentrations (Brown et al., 1991; Czekala et al., 1992; Brown et al., 1995; Carden et al., 1998; Brown, 2000). Serum progesterone content has proven to be a highly reliable indicator of the female Asian elephant's reproductive status (Hess et al., 1983; Plotka et al., 1988; de Villiers et al., 1989; Taya et al., 1991; Wasser et al., 1996; Schwarzenberger et al., 1997; Carden et al., 1998, Brown et al., 1999). Weekly analysis for serum progesterone concentrations can be utilized to determine if a female is cycling properly or is acyclic (Heistermann et al., 1997; Schulte et al., 2000).

Asian elephants have an estrous cycle lasting 13 to 16 weeks (Plotka et al., 1988; Mainka and Lothrop Jr., 1990; Hodges, 1998). The estrous cycle comprises an extended luteal phase typically lasting 8 to 10 weeks and a shorter follicular phase lasting 4 to 6 weeks (Brown et al., 1991; Magunna, 1995; Hodges, 1998; Bechert et al., 1999). Estrus or the heat period varies from 1 to 7 days between individual animals with an average of 3 days when the male elephants are interested in mating with the females (Carden et al., 1998). Estrus is correlated with the first sustained increase in serum progesterone from basal concentrations (Carden et al., 1998). In African elephants, studies have shown a correlation between a similar period of estrus with the initial rise in progesterone, a LH elevation and ovulation confirmed by ultrasonography (Hermes et al., 2000). Although male elephants are capable of detecting when the cow is in estrus, researchers must rely on serum progesterone or LH analysis for estrus detection (Carden et al., 1998, Brown and Lehnhardt, 1997). Elephant managers have successfully used daily serum progesterone concentrations and elevations in LH to monitor for timed breeding and artificial insemination (Carden et al., 1998, Brown et al., 1999).

Progesterone concentrations that remain elevated beyond the length of a normal luteal phase, based on weekly serum sampling, indicate conception has taken place (Olsen et al., 1994, Neimuller et al., 1997; Carden et al., 1998). Gestation typically lasts from 20 to 22 months in elephants

(McNeilly et al., 1983; Brown and Lehnhardt, 1995). During gestation, serum progesterone usually remains elevated at or above luteal-phase concentrations; with incidental declines into the follicular range (McNeilly et al., 1983; Greyling et al., 1998). Measurement of serum progesterone content also can be used to approximate the time of parturition. Due to the variation in gestation lengths, daily monitoring of progesterone concentrations usually begins 1 month before the estimated parturition date to watch for a precipitous decline. This decline is usually noted 3 days before birth, which gives the staff much needed time to prepare (Brown et al., 1997; Carden et al., 1998).

Lactational anestrus

Lactational anestrus is known to be regulated by suckling postpartum (McNeilly, 1980; Williams et al., 1996). In cattle, the suckling calf attenuates gonadotropin secretion and increases the duration of lactational anestrus (Williams et al., 1996).

Because elephant calves typically begin eating solid foods at 3 to 6 months of age, cessation or reduction of suckling may influence the duration of the lactational anestrus period (Nair, 1989). Previous studies have indicated mean calving intervals ranging from 3 to 9 years, which would indicate the earliest conception was approximately 14 to 16 months postpartum (Laws, 1967; Rasmussen and Schulte, 1998). Other authors noted a cow with a calf experienced only a twelve-week lactational anestrus period (Brown and Lehnhardt, 1995). Another study described two cows that did not nurse their calves (one died, the other was bottle-fed at her mother's side) and resumed cycling at 8.5 and 10.5 weeks, respectively (Olsen et al., 1994). Yet another study revealed a cessation of cyclicity that had still not resumed at 12 weeks postpartum (Mainka and Lothrop Jr., 1990), whereas others report an average range of 45 to 46 weeks of anestrus (Olsen et al., 1994). Clarification of the range of lactational anestrus may assist managers in determining natural breeding schedules for cows. More concise information also may demonstrate whether the practice of early calf weaning is necessary to bring about cessation of anestrus in the lactating cow. The present study assessed serum progesterone concentrations as an indicator of estrous activity to investigate the proposed disruption of these cycles associated with lactation. Females were monitored both preconception and postpartum to detect any change in serum progesterone concentrations within the individual animals. Additionally, postpartum serum progesterone concentrations were compared between females having live births (lactating) with females that had stillbirths (non-lactating).

I made the following predictions:

1. There is no difference in serum progesterone concentrations in nursing and non-nursing Asian elephants during their first year postpartum.
2. There is no difference in the duration of postpartum anestrus in nursing and non-nursing Asian elephants.

MATERIALS AND METHODS

Study design

An investigation into two statuses of lactation were used in this study. In the first trial, pre-conception and postpartum serum progesterone concentrations were compared within the individual subjects to determine if there was a difference in luteal concentrations. In the second trial, concentrations of serum progesterone were used to compare the luteal phase of lactating females with non-lactating females for one-year postpartum. This trial compared females that had given birth to live calves versus females with stillbirths.

Study location/housing

The animals were housed at the Dickerson Park Zoo in Springfield, Missouri. All were allowed variable amounts of outdoor access when ambient temperatures were suitable (> 0°C). Short bouts of outdoor access were allowed in temperatures < 0°C. Females were housed in social groups ranging in number from 2 to 7 individuals. Males were housed separately unless attempts at breeding were under way. Each adult animal was afforded a minimum of 37.16 m^2 of space as per American Zoological Association standards.

Subjects

Females used in the lactational anestrus study ranged in age from 16 to 32 years (n = 8) at the initiation of the study period. These females were divided into two subsets based on lactational status. Five females delivered live offspring at parturition and were subsequently suckled by their calves (Table 1). Three females delivered stillborn calves and were, therefore, not being suckled (Table 1). Non-lactating female number 7 was the only one bred back during the study (41 weeks postpartum). Female number 5 was the only female that had her calf weaned prior to the end of the study (weaning at 46 weeks).

Nutrition

During the study, all animals were given a fortified herbivore pelleted ration (HMS, Springfield, MO) and free-choice hay of varying types including brome, orchard grass, timothy and wheat hay. The animals also were given a variety of fresh fruits, vegetables, and breads. Water was always available. Because captive elephants have lower vitamin E concentrations than their wild counterparts, all animals were given an oral vitamin E supplement (three times per week).

Serum collection

Serum was collected from all females weekly during their respective study periods. Sample collections for the lactational anestrus study were for one year before conception and one-year postpartum.

Serum was obtained by collection of whole blood via leg or ear vein in unanesthetized animals. Once the blood was collected, it remained at room temperature for 1h and then was centrifuged at 1500 x g for 20. Serum was stored

postpartum cycle than nonlactating cows. No main effect of time ($F = 2.31$, $p > 0.05$) or lactational status was noted ($F = 0.043$, $p > 0.05$). In both groups progesterone concentrations before the first postpartum cycles were depressed with non-lactating females' at half the concentration of lactating females'.

Pre-conception versus postpartum estrous cycles in lactating females

Using a repeated-measures analysis of variance, no significant variations in serum progesterone occurred in luteal concentrations of lactating females over time ($F = 1.22$, $p > 0.05$). When comparing the cycles, the period prior to the first postpartum cycle has depressed concentrations from all other cycles both preconception and postpartum (Figure 2).

Pre-conception versus postpartum estrous cycles in non-lactating females

Using a repeated-measures analysis of variance, no variation in serum progesterone occurred in luteal concentrations of non-lactating females over time ($F = 5.67$, $p > 0.05$). When comparing the cycles, the period prior to the first postpartum cycle has depressed progesterone concentrations from all other cycles both preconception and postpartum (Figure 3).

DISSCUSSION

Several species of mammals have been noted to have a period of lactational anestrus (Brown and Lehnhardt, 1995; Williams et al., 1996). This period of lactational anestrus in women is characterized by high concentrations of prolactin and follicle stimulating hormone, with low concentrations and pulses of luteinizing hormone, LH (McNeilly, 1980). McNeilly noted that if the requisite frequency and duration of suckling is maintained, LH secretions were suppressed. As suckling decreases in the latter stages of development, secretion of LH increases, allowing for follicular development and the resumption of estrous cycling with a subsequent rise in progesterone concentrations (McNeilly, 1980). These studies support the findings of other researchers in a study on an African elephant (Brown and Lehnhardt, 1997). In that study, elevated prolactin concentrations and depressed progesterone concentrations in an African elephant were related to chronic anovulation (Brown and Lehnhardt, 1997). The results in my study supports these findings. The effects of a calf suckling seem to be primarily in the first few months of the calf's life and seem rapidly to lose their effect on the cow's cycle.

Because Asian elephant females have individual variability in their concentrations of serum progesterone, comparison of pre-conception with postpartum concentrations removes the variation among females from the analysis (Carden et al., 1998). Analysis of within-sample variance showed an initial irregularity in progesterone patterns in postpartum females for both study groups (live birth and stillbirth) prior to the resumption of normal cyclicity. This early suppression of serum progesterone is partially due to postpartum effects, although a two-fold difference in concentrations does show some lactational effect. This indicates that the duration of suckling may be more consistent during this time and, therefore; may cause a more pronounced suppression of the estrous cycle.

The incidence and length of lactational anestrus in elephants recorded in the literature is varied and can be affected by the occurrence of stillbirths (Moss, 1983; Brown and Lehnhardt, 1995, Niemuller et al., 1997). The incidence of stillbirths in elephants has been reported in many studies, with rates between 5.5 and 30% reported (Brown and Lehnhardt, 1995; Niemuller, et al., 1997; Keele, 2001). In another study, authors noted that female mammals that lose their offspring early in lactation tend quickly to resume cycling and conceive (Wasser et al., 1996). My study indicates that it is unnecessary to wean a calf early to cause a more rapid resumption of estrous cycling in the female, because both nursing and non-nursing females resume normal cyclicity at 20.3 to 23.6 weeks, respectively. This practice would undoubtedly be detrimental to the calf and counterproductive to the survival of the captive population. Previous studies have been largely anecdotal or with limited sample sizes, therefore, this information adds to the database and gives a broader picture of the reproductive events surrounding lactation.

CONCLUSIONS

1. Asian elephants exhibit a postpartum anestrus period.
2. Lactating Asian elephants resume typical estrous cyclicity in a mean of 23.6 weeks postpartum (based on serum progesterone analysis).
3. Non-lactating Asian elephants resume typical estrous cyclicity in a mean of 20.3 weeks postpartum (based on serum progesterone analysis).

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Table 1. Life history data on eight Asian elephants housed in Springfield, MO.

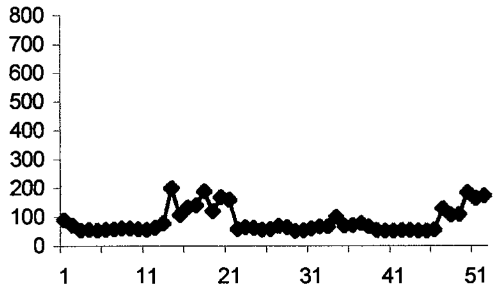
Female	Age at parturition (years)	Reproductive history	Calf status at parturition
1	27	Multiparous	Live birth
2	31	Multiparous	Live birth
3	16	Primiparous	Live birth
4	19	Multiparous	Live birth
5	22	Primiparous	Live birth
6	21	Primiparous	Stillbirth
7	32	Primiparous	Stillbirth
8	30	Primiparous	Stillbirth

Table 2. Length of time until 1st and 2nd postpartum cycles and duration of 1st cycle (weeks), in eight Asian elephants housed in Springfield, MO.

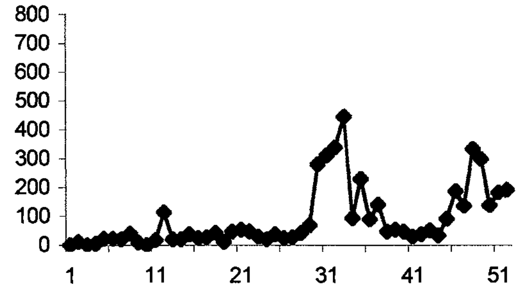
Female	Weeks until 1st cycle	Duration of 1st cycle (weeks)	Weeks until 2nd cycle
1	14	33	47
2	30	16	46
3	20	16	36
4	29	18	47
5	20	16	36
6	15	14	29
7	19	13	32
8	27	11	38

Figure 1. Postpartum progesterone concentrations (pg/ml) in lactating (live birth; n = 5) versus non-lactating (stillbirth; n = 3) female Asian elephants over one year (housed in Springfield, MO).

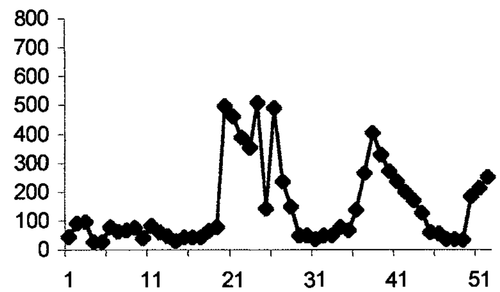
Female 1



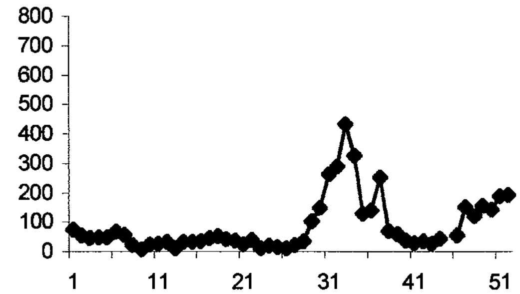
Female 2



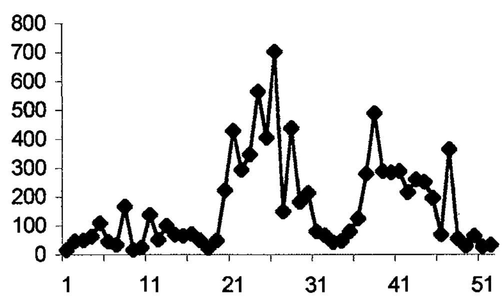
Female 3



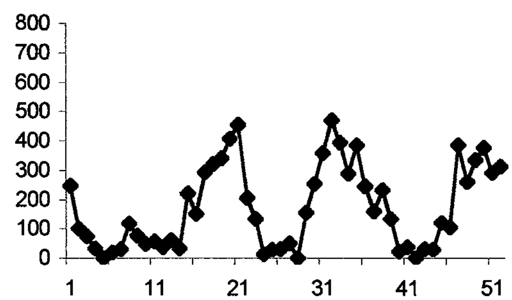
Female 4



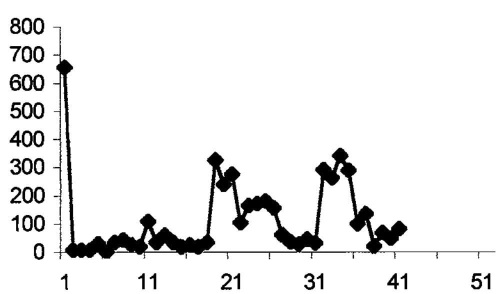
Female 5



Female 6



Female 7



Female 8

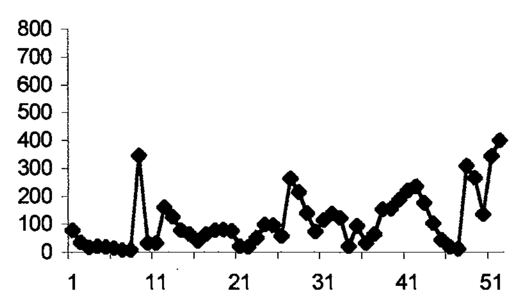
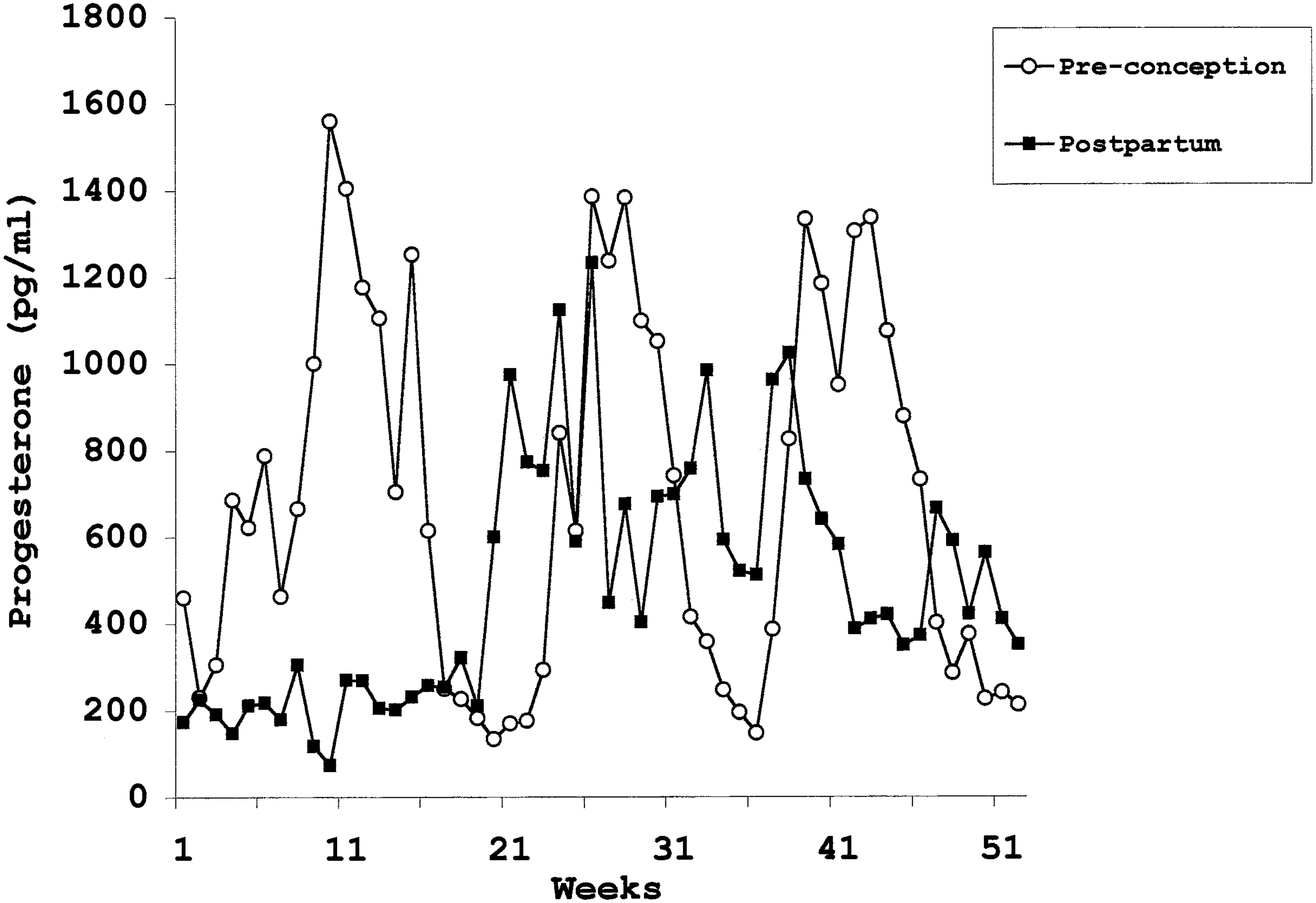
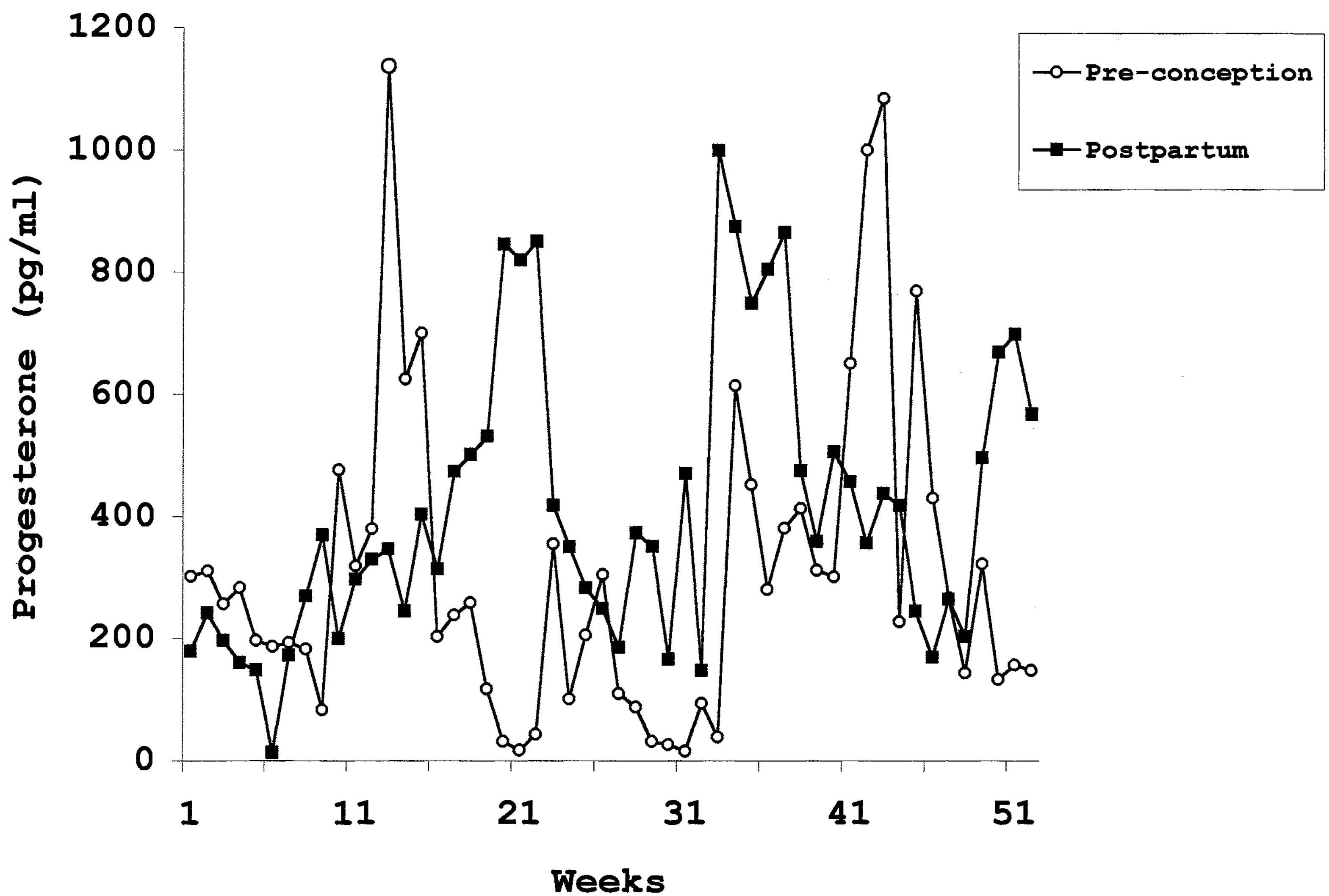


Figure 2. Pre-conception and postpartum progesterone (pg/ml) concentrations in lactating female Asian elephants for one year (housed in Springfield, MO).



Pre-conception
Postpartum
Progesterone (pg/ml)
0
200
400
600
800
1000
1200
1400
1600
1800
1
11
21
31
41
51
Weeks

Figure 3. Pre-conception and postpartum progesterone (pg/ml) concentrations in non-lactating female Asian elephants for one-year (housed in Springfield, MO).



Progesterone (pg/ml)
1200
1000
800
600
400
200
0
1
11
21
31
41
51
Weeks
Pre-conception
Postpartum

APPENDIX 1

ELEPHANT REPRODUCTIVE ETHOGRAM:

Olfaction-

Smell urine-placing tip of trunk in or near urine of another subject

Smell urogenital region-placing tip of trunk on or near urogenital region of another subject

Smell tail-placing tip of trunk near tail (without grasping) of another subject

Smell toenails-placing tip of trunk on or near toenails of another subject

Smell ear-placing tip of trunk on, in or near the ear canal of another subject

Smell mouth/palatal pit-placing the tip of trunk on, in or near the mouth or sulcus region of another subject

Flehmen test-placing the tip of the trunk on the roof of the subject's own mouth and blowing as a result of contact with another subject or her secretions/eliminations

Locomotion-

Driving-the male forcing the female to move forward by pushing her with his head on her rump or side

Walking-either the male or female moving forward on their own, (without contact from another subject)

Following-either the male or the female moving toward another moving subject

Aggressive-males give, females receive aggressive behavior

Pushing- the male uses his head to push the female from the side/behind while the subjects are stationary (they are not walking at this time)

Head to head sparring-the male pushes with his head on the head of the female, (their trunks are commonly intertwined)

Kicking-the male strikes the female with his foot

Biting-the male places the female's ear or tail in his mouth and crushes it

Pre-copulatory behavior-

Trunk over side or back-the male places his trunk on top of the female's back (either from the side or rump) without pushing

Pre-mounting kick-the male uses his leg lightly to strike the inside of the female's legs while he is mounted

Attempted mount-the male rears up on his hind feet in an effort to place his front feet on the rump of the female, but misses her rump or the female moves away prior to making contact

Mount-the male rears up on his hind feet and places his front feet on the rump of the female

Erection-the male has a fully enlarged penis

Copulation-the act of intromission of the penis into the urogenital tract of the female

\#2

VITA

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Candidate for the Degree of

Doctor of Philosophy

Thesis: REPRODUCTIVE STRATEGIES AND BEHAVIOR OF THE ASIAN ELEPHANT (*ELEPHAS MAXIMUS*)

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Biographical:

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Professional Experience: Raptor Attendant, Dickerson Park Zoo, January 1985-June 1986; Animal Nursery Caretaker, Dickerson Park Zoo, June 1986-July 1988; Elephant, Small Mammal and Bird Zookeeper, Dickerson Park Zoo, July 1988-July 1992; Elephant and Large Mammal Zookeeper, Dickerson Park Zoo, July 1992-May 1995; Elephant and Reptile Zookeeper, Dickerson Park Zoo, May 1995 to present.