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Reproductive control of elephants

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Introduction

CHAPTER 6 deals specifically with fertility control as a possible means of population management of free-ranging African elephants. Because methods that are described here for elephants function by preventing cows from conceiving, fertility control cannot immediately reduce the population. This will only happen once mortality rates exceed birth rates. Considering, however, that elephants given the necessary resources can double their numbers every 15 years, fertility control may have an important role to play in population management.

The first part of the chapter is devoted to the reproductive physiology of elephants in order to provide the reader with information and understanding which relate to fertility control. This is followed by examples of contraceptive methods that have been used in mammals, and a description of past and ongoing research specifically carried out in elephants. Finally guidelines for a contraception programme are provided, followed by a list of key research issues and gaps in our knowledge of elephants pertaining to reproduction and fertility control.

In this chapter we will also attempt to answer the following questions in regard to reproductive control of African elephants:

- Do antibodies to the porcine zona pellucida (pZP) proteins recognise elephant zona pellucida (eZP) proteins or is the vaccine likely to work in African elephant cows?
- Is it possible to implement a contraceptive programme using the pZP vaccine?
- Is it practical to implement such a programme?
- What contraceptive efficacy can one expect?
- Is the method safe, reversible and ethical?

- What effect does the implementation have on the behaviour of a population?
- What are the effects of contraception on behaviour?
- What are the proximate and ultimate effects of contraception?
- Given the current technology, what population sizes can be tackled?
- What are the costs involved?
- Are there alternatives to pZP for contraception of elephants?
- What developments are in the pipeline that could facilitate implementation?

Aspects of elephant reproduction that relate to reproductive control

Social organisation

Elephants live in female-dominated herds comprising an old female referred to as the matriarch together with her mature daughters and their offspring, including sexually immature male calves (Owen-Smith, 1988). Female elephants remain in their natal herds their whole lives; male elephants leave their natal groups at approximately 12–14 years or when they reach sexual maturity (Poole, 1996b). These young bulls are often driven out of their family groups by cows that bully and 'chivvy' them (Douglas-Hamilton & Douglas-Hamilton, 1975). These newly independent bulls may leave their families only to join up with another family for a few years, or go off to 'bull areas' and join up with other bulls to form bachelor herds, or they may stay in female areas moving from family to family (Poole, 1996b). These courses to sexual maturity result in mature males that live alone (13–60 per cent, Owen-Smith, 1988) or in small bachelor herds characterised by temporary associations (Owen-Smith, 1988).

The mating system of elephants can be considered as promiscuous, because males and females will mate with more than one individual during a given oestrus, but females usually have only one offspring per pregnancy (Rasmussen & Schulte, 1998). However, because only one male can be the sire, the mating system can also be described as sequential polygyny (Hollister-Smith, 2005).

Elephant communication is very complex and five main sensory receptor systems are used to communicate with other elephants and with their environment. These are tactile, visual, vibrational, auditory and chemical receptor systems (Schulte *et al.*, 2007). In recent years chemical signalling has received a lot of attention, as it appears to play an important role in elephant societies. Chemosignals (also referred to as 'honest' signals because they cannot be faked) are released in urine, temporal gland fluid, vaginal mucus, from the toe glands in the feet and from a number of other sites. Chemosignals reflect the physiological status (age, sex, reproductive and metabolic condition) of the sender, and the response of the receiver also depends on receiver status (Schulte *et al.*, 2007). Importantly, chemical signals can be used for environmental enrichment of captive elephants as well as in the resolution of human-elephant conflict. Two examples of the later are the use of musth chemosignals to deter wild Asian elephants from raiding crops (Rasmussen & Riddle, 2004), and conditioned aversion of African elephants with chilli peppers planted in between edible crops, also to prevent crop raiding (Parker & Osborn, 2006).

Reproductive physiology of African elephant cows

Puberty

In the wild, given adequate nutrition and social structure, a cow will reach puberty at about 10–12 years old. Laws (1969) found that culled elephant cows in five different parks were essentially ready to ovulate at the age of 10–11 years. However, the age at which first ovulation took place was dependent on population density (table 1). The parks studied – Mokomasi Game Reserve (MK), Tsavo National Park (TNP), Murchison Falls National Park North (MFPN), Murchison Falls National Park South (MFPS) and Budong Central Forest Reserve (BCFR) – had elephant population densities of <3, 3, 2.8, 5.9–10.3 and 6–7 per sq mile (2.6 km^2), respectively. The mean age at first ovulation was 11, 12.5, 14, 18 and 20 years respectively. He attributes these differences to density-dependent physiological, social and nutritional stresses.

The effects of density on age of ovulation, intercalving interval and incidence of anoestrus (see Gestation, intercalving interval and lactation) must be taken into account when considering methods of population control. Methods that reduce population without affecting fertility will inevitably increase reproductive and thus population growth rate. In the Luangwa Valley females reached maturity (age at first ovulation) at 14 years of age (Hanks, 1972). First ovulation typically occurs between 11 and 14 years, while the earliest recorded age at first conception is 7 years (Owen-Smith, 1988). In Saburu National, the first oestrus was recorded in an 8-year-old primiparus cow. Faecal progestin metabolite studies showed that she in fact fell pregnant during this oestrus (Wittemyer *et al.*, 2006). The age of cows at first parturition ranges from 9 to 18 years (Owen-Smith, 1988). On the other hand, in translocated populations, births have been observed in cows as young as 9, indicating the onset of puberty as early as 7–8 years, a trend commonly observed in relocated populations (Delsink *et al.*, 2006; Slotow, pers. comm. 2006).

Oestrous cycle length, length of oestrus

Oestrus persists for 2–6 days, with a minimum of 2 and maximum of 10 days, during which time a female may be mounted by several males (Western & Lindsay, 1984; Moss, 1983). The oestrous cycle lasts between 15 and 16 weeks with an 8–11 week luteal phase and a 4–6 week follicular phase (figure 1). It is considered that cows are at their most fertile during the late follicular to early luteal phase. Oestrous females may be observed in any month of the year, although oestrous frequency is highest during and following the wet season (Poole, 1987; Poole & Moss, 1989b; Brown *et al.*, 2004).

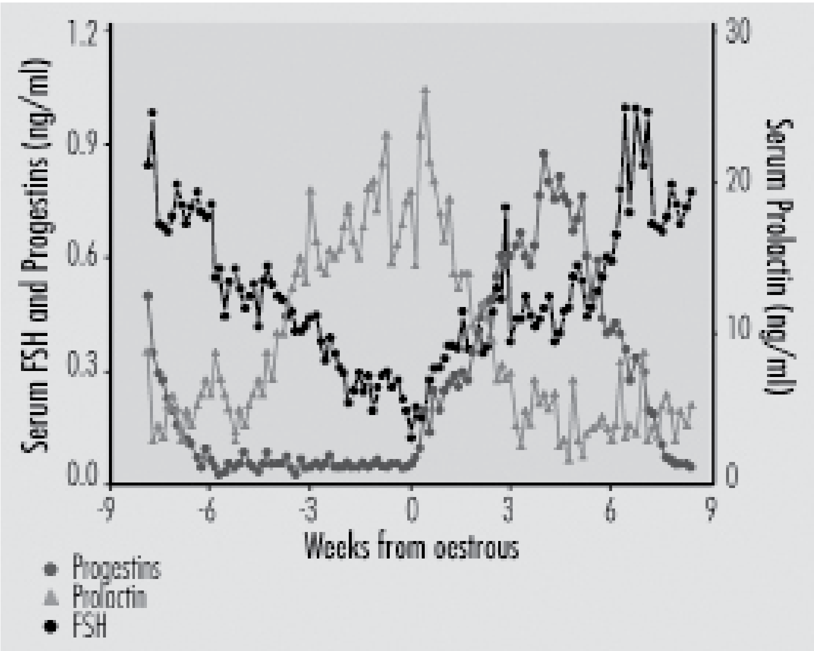

Figure 1: Mean profiles of serum progestins, prolactin, and FSH throughout the oestrous cycle in reproductively normal African elephants (n = 7 females; 15 cycles). Week 0 designates oestrus. The follicular phase is considered the period between successive luteal phases (week 6 to week 0) (Brown *et al.*, 2004)

Females remain reproductively active throughout the adult period and do not appear to display reproductive senescence (Lawley, 1994). Nevertheless, in the Luangwa Valley reproductive rate started to decrease after 40 years of age:

5 of 30 cows between 50 and 60 years old were neither pregnant nor lactating. This shows that a high percentage of animals are still reproductively active (Hanks, 1972). According to Owen-Smith (1988), fertility declines rapidly after 50 years of age. During one of the last elephant culls in the Kruger in 1995 a cow aged 55–60 had 11 placental scars, indicating that reproductive senescence was unlikely in this individual (Whyte, unpublished data).

Behaviours associated with oestrus

While this section has no direct bearing on the assessment process, it provides important background information which could be of importance in regard to future studies. One of the criticisms of contraception is that it may affect reproductive and social behaviour of breeding herds. In order to establish possible effects a sound knowledge of normal reproductive behaviour is essential.

Oestrous females exhibit conspicuous behaviour by calling loudly and frequently and by producing urine with particular olfactory components (Poole & Moss, 1989). Prior to the ovulatory phase the female uses low-frequency acoustic signals with a range of up to 8 km to attract males to her (Leong *et al.*, 2003). Five categories of oestrous behaviour were classified (Moss, 1983). The first sign of oestrous behaviour is that of 'wariness'. The female is noticeably alert and wary of males, carrying her head high and directing her gaze towards other elephants. When approached by males, the female avoids their approaches and attempts to test her reproductive status (Moss, 1983). During the ovulatory phase, bull elephants increase the frequency of genital inspections, flehmen and trunk contact towards the receptive cow between 1 and 9 days before ovulation, with most inspections occurring just a few days beforehand (Ortolani *et al.*, 2005). In Asian elephants the increase in male attention is thought to be due to the release of (z)-7-dodecene1-yl acetate in the urine during the pre-ovulatory phase. While this chemical or pheromone has not yet been detected in African elephants, it is likely that a similar compound exists, producing the same results (Rasmussen & Schulte, 1998). Male elephants detect this pheromone using the vomeronasal organ (a highly specialised and sensitive scent organ situated in the roof of the mouth) and when the concentrations are at their greatest, mating occurs after the sequence of oestrous behaviours have played out (Moss, 1983; Bagley *et al.*, 2006). This is the period just prior to ovulation. Females will often urinate on their tails and then raise them, presumably to help the spread of the olfactory cue (Freeman *et al.*, 2005). The wariness phase gives rise to the 'oestrous walk', where the cow will exhibit a distinct 'walk' back and forth in which her back is arched, tail raised and head held at an angle (Moss, 1983). It is assumed that this is a visual and chemical cue to attract males (Freeman *et al.*, 2005).

Increased vocalisations also occur when males arrive. It is thought that this is to further attract other males, allowing for female selection of the healthiest male to enable the optimum survival rate of her offspring (Vidya & Sukumar, 2005). The oestrous walk may develop into a 'chase', where both animals run. The male appears to be trying to catch the female. The female travels in a wide arc away from her group and may be separated from them for a number of hours. If the male is able to touch her with his trunk, the female will stop running, upon which he will attempt to mount her (Moss, 1983).

During oestrus, older experienced females show preferences for males in older age classes and preferably those in musth (Moss, 1983). There is a hierarchical dominance order among bulls of a particular range which is so strong that although as many as eight bulls have been seen escorting an oestrous cow, only the highest-ranking bull will mate or be accepted by the cow (Hall-Martin, 1987). In addition, females may solicit male-male competition by drawing attention to themselves in the early days of oestrus to have a wider selection pool (Moss, 1983). In the Amboseli study the female did not mate with each male in relation to his courting efforts, nor did she mate with males in proportion to the numbers in each male size/age class; furthermore, the male that she mated with was successful partly because of her own behaviour, letting herself be caught by or going into consortship with him (Moss, 1983).

Mountings occur after the 'chase' or a brief 'walk' with the oestrous female. Once the male is able to touch the female with his trunk and she has stopped, the male then places his trunk lengthways on her back, resting his head and tusks on her rump, rearing up on his back legs. The female remains standing still or may back closer to the male to facilitate intromission (Moss, 1983). The male stays mounted for about 45 seconds, while intromission lasts approximately 40 seconds. Temporary consortship occurs between the individual male and an individual female, characterised by close proximity, affinitive behaviour, and attempts to maintain exclusive copulatory behaviour (Moss, 1983). Consort behaviour may be displayed even when no oestrous walks or chases have been observed. However, the female approaches or follows the particular male if he moves away, even if other bulls are present in the area. Similarly, the bull follows the cow and remains in close proximity if she moves away (Moss, 1983).

When the oocyte is released (ovulation), the sperm will still be viable and capable of fertilisation. The ovulation fossa (follicular cavity) left behind is filled by the growing corpus luteum which secretes progesterone. Besides numerous other functions, progesterone is responsible for behavioural quiescence which lasts approximately 10 weeks.

There is also evidence to suggest that olfactory signals released during oestrus have an effect on other females, resulting in the synchronisation of ovulation in a herd. Presumably this would result in calves being born at a similar time, enabling the herd to create ‘nurseries’ which would increase individual calf survival (Archie *et al.*, 2006). The exact pheromones used are currently not known (Freeman *et al.*, 2005; Rasmussen & Schulte, 1998).

Gestation, intercalving interval and lactation

Intercalving interval (ICI) is one of the major factors that affect the rate of population growth. It responds to a number of variables: most importantly resource availability and population density. The relationship between population density and intercalving interval was clearly shown by Laws (1969) in a study of cull material from five East African parks (table 1). The relationship between resource availability and reproductive endocrinology was also shown by Wittemyer *et al.* (2006) who studied a group of wild elephants in Samburu and Buffalo Springs National Parks. They compared Normalised Differential Vegetation Index to faecal progestogens metabolite concentrations during the oestrous cycle and in pregnancy. Faecal progestogens were significantly higher in pregnant and non-pregnant cows during the wet than dry seasons (figure 2). The relationship between resource and reproduction was thus clearly shown. Manipulating the ICI by means of reproductive control therefore will influence the rate at which a population grows. In several studies of East African populations, intercalving interval ICI ranged from 2.9 to 9.1 years (Laws *et al.*, 1975). In Amboseli, ICI was 4.9 years during the period 1972–1980 (Moss, 1983), 5.6 years during dry years, and 3.5 years over a sequence of wet years (Owen-Smith, 1988). Variations in ICI occur according to region; between 3.3 and 5.5 years in Lake Manyara in Tanzania, Luangwa Valley in Zambia, Kruger National Park (Kruger) in South Africa, Gonarezhou and Hwange in Zimbabwe (Hanks, 1972; Owen-Smith, 1988).

Gestation lasts 22 months in African elephants, which accounts for approximately 50 per cent of the intercalving period. This means that for a period of up to two years after calving cows do not show an oestrous cycle (figure 3; Brown *et al.*, 2004).

	Mokomasi Game Reserve	Tsavo National Park	Murchison Falls National Park North	Murchison Falls National Park South	Budongo Central Forest Reserve
Elephants per mile^2	<3	3	2.8	5.9 and 10.3	6–7
Age at first ovulation	11	12.5	14	18	20
Intercalving interval (yrs)	4–5	6–7	6–7	8–9	no data
% anoestrous cows ≥25 years and not lactating	no data	7.9	8.6	28.6	no data

Table 1: Influence of population density on reproductive variables of five East African parks (adapted from Laws, 1969)

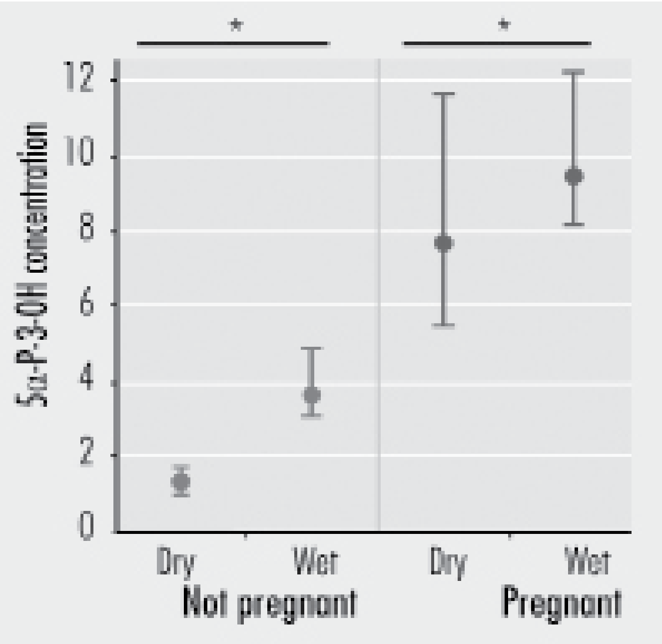

Figure 2: The median faecal 5α-pregnane-3-ol-20-one (5α-P-3-OH) concentrations (not pregnant, blue circles; pregnant, green circles) and inter-quartile ranges (error bars) of all females are presented in relation to reproductive state and season. The asterisks represent statistically significant differences between categorised individually paired median values (Wittemyer *et al.*, 2007)

Under normal conditions fertile cows would seem to lactate permanently (from one calving to the next). According to Whyte (2001), on data obtained from culling operations in the KNP from 1989 to 1992, 344 lactating cows and 350 calves less than one, one, two and three years old were found in the same populations, which had an average ICI of 54.4 months. The gestation period of 22 months added to the age of the oldest group of calves (36 months) provides a lactation period of about 58 months, indicating that African elephant cows lactate from the birth of one calf to the next. The duration of lactation recorded in the Tsavo and Murchison districts of East Africa was 4–5 and 7–8 years, respectively (Laws, 1969) but in the same paper 40.7 per cent and 73.2 per cent of pregnant cows ≥25 years of age in Tsavo and Murchison North, and Murchison South were not lactating, respectively. It seems therefore that as intercalving interval increases, the percentage of lactating cows decreases.

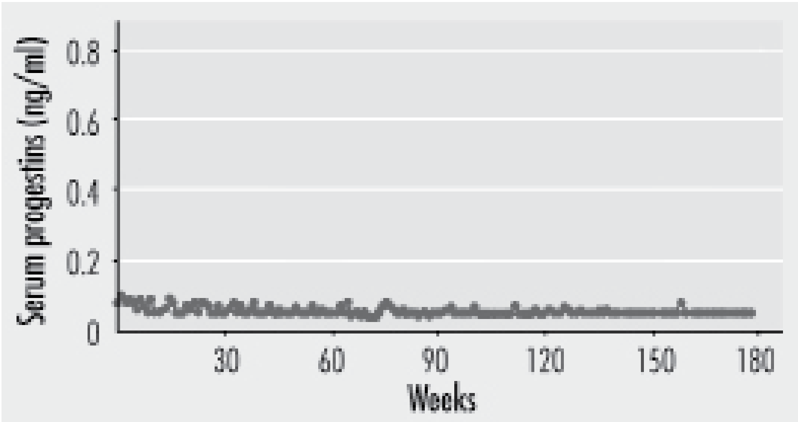

Figure 3: Serum progestin profile in a non-cycling African elephant female (Brown *et al.*, 2004)

Reproductive physiology of African elephant bulls

The social ranking of bulls and reproductive behaviour, which is driven by androgens of testicular origin, are the two most important factors that determine the likelihood of a bull being able to mate with an oestrous cow under natural conditions. A 30-year study using faecal DNA microsatellites to determine paternity of calves born in Amboseli showed that bulls 45 years and older sired 50 per cent of all calves. Furthermore, it showed that 75 per cent of progeny were sired by bulls that were in musth at the time of mating (Hollister-Smith, 2005). While physical stature and strength are known to affect ranking, there is another factor that explains why older bulls are more likely to be afforded the chance to breed. Recently Rasmussen & Riddle (2002) found distinct differences in the pheromone content of Asian elephant bulls up to and over 35 years of age. The pheromone content of the younger bulls scares off cows in oestrus, whereas in older bulls it becomes highly attractive. Granted the work was carried out in Asian bulls, but the reproductive physiology of the males of both species has been found to be very similar and is hardly likely to differ in this respect (Schulte *et al.*, 2007).

The above highlights the more important factors associated with the natural selection of sires. Human interventions – particularly hunting, which targets trophy animals, but also translocation or culling of dominant bulls – potentially impact on the selection process and may affect the quality of progeny. As a result of hunting and poaching, tusk sizes have decreased in Africa. The complexity of the selection process also makes the bull a less attractive target for implementing reproductive control. If older bulls are targeted, younger, less dominant bulls will contribute more offspring than would normally be the case. In small, translocated populations with only one or two bulls, it may be an option. A watchful eye will, however, have to be kept on young bulls that have been introduced with their natal herds.

The following pages provide more insight into the reproductive behaviour of elephant bulls and link endocrinology and behaviour. This is valuable information upon which further studies related to various behaviours such as aggression, habitat degradation and reproductive control can be planned.

Puberty

In males, spermatogenesis begins between 7 and 15 years, but full sperm production is not reached until 10–17 years (Owen-Smith, 1988). In the Luangwa Valley spermatogenesis started at the mean age of 15 years, when the combined weight of the testes was 650–700 g (Hanks, 1972). According to Johnson & Buss (1967), however, the start of puberty ranged from 3 to 14 years of age. This was based on the histological appearance of the testes, whereas puberty is usually defined as the age at which sperm appear in the ejaculate. Male elephants leave their natal families at the onset of puberty, at about 14 years of age, and henceforth live in a highly dynamic world of changing sexual state, rank, associations, and behaviour, most of the time alone or in small groups of males (so-called bachelor groups) in specific bull areas (Moss, 1983; Poole, 1994; Lee, 1997). At this time, adolescent bulls produce fertile sperm and are physically able to mate successfully (Hall-Martin, 1987), giving a bull a potential reproductive lifespan of over 40 years. The age of dispersal and reproductive capability, however, may be affected by human activities (e.g. hunting, poaching and habitat loss). For example, the killing of older males may permit younger males to mate and may select for earlier dispersal by males (Sukumar, 1989; 1994; Owens & Owens, 1997). Although reproductive capability is already achieved during the teenage years, recent findings suggest that elephant bulls in the wild alternate between sexually active and inactive periods only from the age of 20 years onwards (Rasmussen, 2005). In captivity, however, the fact that elephant bulls are sometimes housed singly (http://www.elephant.se/elephant_database.php), could be the reason why reproductive activity is documented for captive bulls as young as seven years of age (Brown *et al.*, 2007).

Male reproductive behaviour and musth

Two alternative reproductive tactics are documented for sexually active bulls in the wild: (a) the sexually active non-musth tactic, a non-competitive tactic seen in less dominant, often younger males which is associated with low, prolonged investment, and (b) the musth tactic, a competitive tactic seen in dominant, often older individuals, associated with short periods of high investment (Rasmussen, 2005). Although recent findings underlined the importance of musth to male reproductive success, paternity analyses also revealed that approximately 20–25 per cent of the reproduction can be attributed to sexually active non-musth bulls (Hollister-Smith *et al.*, 2007; Rasmussen, 2005).

The phenomenon of musth was first described in adult Asian bulls in captivity (e.g. Eggeling, 1901). First studies on African elephants suggested that musth did not occur in this species (Perry, 1953; Sikes, 1971), but it has since also been shown to exist within the genus *Loxodonta* (Poole & Moss, 1981; Poole, 1987). The physical, behavioural and physiological changes associated with musth appear sporadically in adolescent Asian elephants from approximately 10–20 years of age, and periodically appear in all Asian bulls after the age of 30 (Eisenberg *et al.*, 1971; Jainudeen *et al.*, 1972a). In free-ranging African elephants, first signs of musth occurred at about 25 years of age. In the absence of older dominant bulls, however, musth may occur at an earlier age. As in the Asian elephants, older African bulls show longer and more predictable periods of musth (Poole, 1982; 1987; 1994). The long time span between the age when the first sporadic musth signs occur and the onset of relatively regular prolonged periods of musth (around 35–40 years of age) suggests that the optimal reproductive tactic (either sexually active non-musth or musth) may vary depending on ecological and/or demographic conditions during a period covering more than 10–15 years of life of a bull (Rasmussen, 2005). Although musth in general seems to be more predictable in older animals, the intensity and duration of musth is also variable and asynchronous between those bulls, and even the character of musth within such an individual can vary from year to year (Cooper *et al.*, 1990; Poole, 1987; 1994), indicating that the appearance of musth may vary depending on local conditions.

Male elephants in musth leave their normal home ranges, travel long distances and spend significantly less time feeding and resting in order to locate and associate with oestrous females (Hall-Martin, 1987; Poole, 1989b; 1994). A receptive female will preferably mate with the most dominant bull in the area, which is usually a bull in musth, because musth bulls show aggression which overrides normal social male hierarchies (Hall-Martin, 1987). During aggressive interactions, bulls in musth are invariably the winners irrespective of body size, the factor which normally determines dominance rank between males in non-musth condition (Poole, 1989a). Apparently, bulls in musth are more likely to be involved in fights, and several musth males have been killed by stronger bulls in musth (Hall-Martin, 1987; Poole, 1994). Furthermore, it is known that the presence of a dominant musth bull can suppress the physical and behavioural changes associated with musth in lower-ranking bulls (Poole, 1982).

Elephants in musth have a characteristic posture, which is particularly noticeable when they move. The head is carried well above rather than below the shoulder blades and held at such an angle that the chin looks tucked in. The ears are tense and carried high and spread (Poole, 1987; Kahl & Armstrong, 2002). Bulls in musth also repeatedly call at very low frequencies (infrasound) that travel over long distances without attenuation. African elephants in musth emit a distinct set of calls with frequencies as low as 14 Hz and sound pressure levels up to 108 decibels (Poole, 1987; 1994; Poole *et al.*, 1988). Males in musth call significantly more often when they are alone and apparently searching for female groups, than when they are in association with females (Poole, 1987). Their rumbles are often preceded or followed by listening behaviour, suggesting that they are either answering a call or calling and waiting for a response (Poole *et al.*, 1988), whereby similarly ranked musth males actively avoid one another (Poole, 1989a). Since males in musth criss-cross the range in order to locate and associate with oestrous females (Hall-Martin, 1987), it seems plausible that they may call and/or listen for other musth males' calls to avoid unexpected meetings with another equally aggressive and high-ranking bull (Poole *et al.*, 1988). Musth bulls emit further specific signals which notify other male and female elephants of their status. These musth-related signals are mostly characterised by the continuous discharge of urine in a series of discrete drops (urine dribbling) with the penis retained in sheath. This urine has a typical strong odour, especially when associated with a greenish discoloration of the penis and sheath (figure 4) (Moos & Poole, 1981; Poole, 1982; 1987; Hall-Martin, 1987). A further visual signal for a bull in musth is the copious secretion from and enlargement of the temporal glands (Jainudeen *et al.*, 1972a; Poole and Moss, 1981; Hall-Martin, 1987; Poole, 1987; Rasmussen and Schulte, 1998), unique paired modified apocrine sweat glands located in the temporal fossas. Watery secretion from the same glands is often also interpreted as a sign of musth. Elephants of all ages produce a watery secretion from the temporal glands in response to excitement (Bertschinger, unpublished data). Riddel *et al.* (2000) also described secretion of a watery liquid from the ears of captive and wild male and female African elephants of various ages. The fluid was noted when animals became excited and appeared in the form of squirts or slow dribbles. The compounds found in the secretions were also found in temporal gland secretions. Watery aural secretions have also been observed in captive elephant bulls and cows in South Africa (Bertschinger, unpublished data).

Figure 4: A – Bull showing all physical signs of musth (temporal gland swelling and secretion, urine dribbling and wet legs). B – greenish coloured sheath (photo: Bertschinger, Etosha National Park, Namibia, April 2007)

There is extensive anecdotal information from Asia that good nutrition and body condition are necessary for the successful expression of musth in elephant bulls, and that musth bulls in poor condition usually drop out of musth (Poole, 1989a, Rasmussen & Perrin, 1999). Although it seems that the musth-related weight and condition loss is largely attributed to the increased restlessness and reduced feeding activities of African elephant bulls during musth (Poole, 1982; 1989a), it has been shown that captive Asian elephants lose weight during musth even when they are chained and given normal rations of food (Poole, 1989a). Since musth is also associated with elevated androgen levels, weight loss may also be related to the increase in metabolic rate that is associated with high androgen levels (Poole, 1989a). In this respect, it could be demonstrated for an Asian elephant that musth- related changes in serum testosterone and triglyceride concentrations followed similar patterns: that lipase activity was significantly elevated immediately before and after musth and that urinary, especially albumin-like, protein concentrations increased during musth (Rasmussen & Perrin, 1999). However, the precise physiological links between body condition and musth are not clear as yet and due to the limited information available, additional data, particularly for the African elephant, are needed.

Endocrine profiles of elephant bulls

Physiologically, musth in both genera is particularly characterised by a periodic increase in androgen levels (e.g. Jainudeen *et al.*, 1972b; Poole *et al.*, 1984; Rasmussen *et al.*, 1996, Ganswindt *et al.*, 2002; 2005a). But it is still unknown which role the adrenal gland might play in this context, and if at all, whether increases in gonadal androgens precede the rise of androgens of adrenal origin, or vice versa. Recent findings also indicate that thyroid hormones might play a role in testicular steriodogenic activity (Brown *et al.*, 2007), but this possible relationship is far from clear and needs further investigations. Apart from the periodic increase in androgen levels, it has also been suggested that musth-related physical and behavioural changes are associated with elevated glucocorticoid levels, which would result from increased adrenal activity. As mentioned above, musth is known to be associated with increased restlessness and reduced feeding activities, often leading to a progressive loss of condition (Poole 1989a). This has led to the hypothesis that musth represents a form of physiological stress, and a recent invasive study showed a modest positive correlation between testosterone and cortisol concentrations in captive bulls exhibiting musth (Brown *et al.*, 2007). Contrary to this result, Ganswindt and co-workers found no clues for an elevation in glucocorticoid output during musth or any other state of sexual activity in captive and free-ranging animals (Ganswindt *et al.*, 2003; 2005a), and additionally provide evidence for a suppressing effect of the musth condition on adrenal endocrine function (figure 5; Ganswindt *et al.*, 2005b). Further research is therefore necessary to determine whether characteristic conditions associated with musth represent a form of physiological stress, and which role the hypothalamic-pituitary-adrenal axis plays. Apart from the open questions regarding hormone involvement, little is also known about the time course and time-related occurrence of musth triggers influencing the onset and duration of musth. However, it could be recently demonstrated for captive African elephants, that temporal gland secretion and urine dribbling were typically first recorded after the elevation in androgens, indicating that these physical musth-related signs are downstream effects of increased androgen concentrations. In this respect, the results of the study show that temporal gland secretion responds earlier and to lower androgen levels than urine dribbling, which manifests itself later and requires a higher level of androgen stimulation (figure 5) (Ganswindt *et al.*, 2005b).

Nevertheless, more information about what regulates musth, and what are the mechanisms underlying the associated physiological and behavioural changes, is necessary and would not only be of scientific interest, but also useful in the development of new approaches to deal with the acute management problems of elephants in the wild.

Basic methods of contraception for wildlife

If it is achievable, the ideal contraceptive must be efficacious, allow remote delivery, be reversible, produce no deleterious short– or long-term health effects, should cause no changes to social behaviours and group integrity, must not pass through the food chain, should be safe during pregnancy and finally, be affordable.

Potential contraceptive methods available for animals

The following methods, broadly speaking, can be used for contraception in animals:

- surgical (gonadectomy, vasectomy and salpingectomy)
- hormonal (oral contraceptives, depot-injections or slow-release implants)
- immunocontraception.

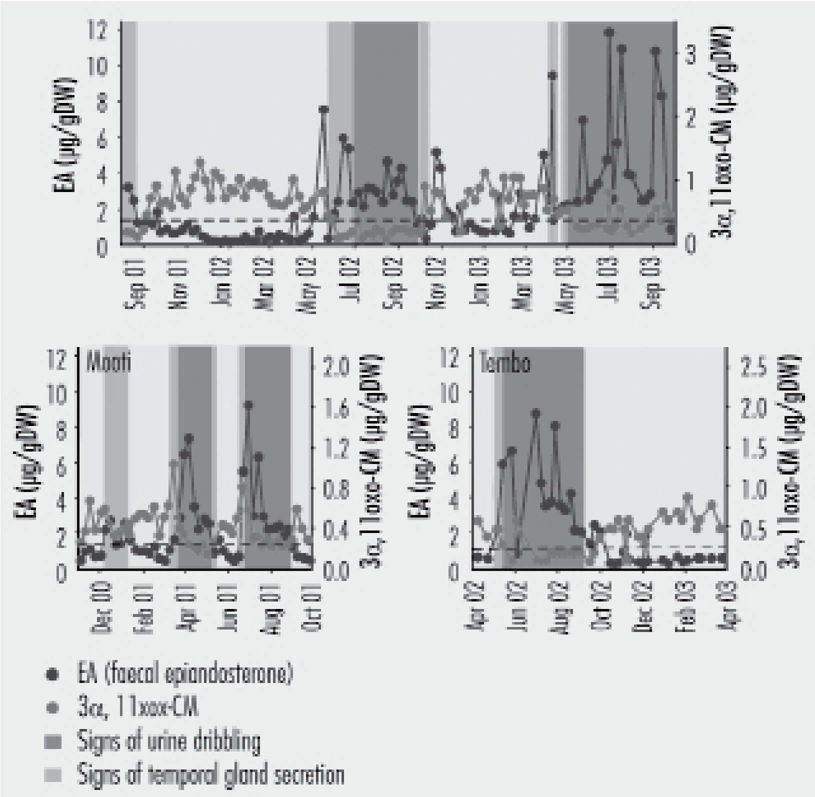

Figure 5: Profiles of faecal epiandrosterone (EA) and 3α, 11oxo-cortisol metabolites (3α, 11oxo-CM) immunoreactivity throughout a period of 11–26 months in three captive adult male African elephants (Calimero, Mooti, and Tembo). A range of months of 11–26 months has been given which addresses all three bulls. Dashed lines indicate the threshold for elevated epiandrosterone and decreased 3α, 11oxo-CM levels (Ganswindt *et al.*, 2005b)

Potential target tissues or reproductive processes that lend themselves to reproductive control were summarised by Asa (2005). Table 2 summarises methods that have been used for contraception in animals and how each method compares to the properties of an ideal contraceptive agent.

Surgical methods

Surgical methods to control reproduction in domestic species have been in use for many years. In males and females there are two options. The first is gonadectomy, which is irreversible and affects reproductive and probably territorial behaviour. The alternative is tying off the fallopian tubes in the female and vasectomy in the male, both of which leave reproductive and associated behaviours largely intact. Although vasectomies have been performed on African elephants (see *Surgical sterilisation of elephant bulls*) surgical methods are not considered practical for large numbers of wildlife. In addition they are invasive, expensive, and in elephants irreversible for practical purposes, as microsurgical techniques are needed to reverse the process.

Hormonal methods

Hormonal methods that have been used to contracept animals are oral or depot-type progestogens, oestrogens and androgens and GnRH super-agonists in the form of implants or depot-injections. Progestins in the form of long-acting implants were used extensively for contraception of large carnivores (figure 6). They are extremely effective but due to a number of serious side effects have largely gone out of use (Munson *et al.*, 2002; Munson *et al.*, 2005). The other possibility to consider, and this also applies to oestrogen implants, is that progestins may get into the food chain and affect reproductive performance of other species. A large number of bird, insect and indirectly reptile species could be exposed to such steroids through elephant faecal material should one consider such methods for elephants. Steroid implants are impractical to use since they require immobilisation of the target animal, and are too expensive.

Early attempts at contraception in wild horses relied primarily on steroids. It has long been known that exogenous androgens can exert a down-regulation of endogenous androgens and sperm production in the stallion (Blanchard, 1984). This fundamental biological strategy led to the first attempts at actual contraception aimed at free-ranging wild horses. Trials with domestic pony stallions demonstrated that six repeated monthly injections of testosterone propionate (TP) or 17-α-ethinyl oestradiol, 3-cyclopentyl ether (quinestrol), at doses of 1.7 g per 100 kg resulted in significant degrees of oligospermia and decreased motility (Turner & Kirkpatrick 1982). Practicality for application in the field, however, was limited because of the need for repeated treatments. Thus, testosterone propionate was microencapsulated in a polymer of D, L-lactide (mTP) (Southern Research Institute, Birmingham AL), permitting a sustained release after intramuscular injection for up to six months. On contact with intercellular water, the lactide coating erodes and releases the active steroid inside. The lactide coating is converted to carbon dioxide and lactic acid.

Property	Surgical methods		Steroids		GnRH agonists		Immunocontraception	
	Gonadect	Vasec/FallT	Oral	Implants	Injectable	Implants	pZP	GnRH
Contraceptive efficacy ♀	100%	100%	100%[b]	100%	≤100%	≤100%	70–100%	70–100%
Contraceptive efficacy ♂	100%	100%[a]	Poor	Poor	≤100%[c]	≤100%[c]	No	70–100%
Remote delivery	No	No	Only captive	No	Yes	No[d]	Yes	Yes
Reversible	No	No[d]	Yes	Yes	Yes	Yes	Yes	Yes
Social behaviours or organisation of groups or herds	Affects behaviour	Some in cats	Affects behaviour	Affects behaviour	No data	♀ anoestrus ♂ aggression decr.	♀ continue to cycle	♀ anoestrus ♂ aggression decr.
Deleterious short- or long-term health effects	Obesity	None	Carnivores some serious	Carnivores some serious	None	None	Local swelling	Local swelling
Contraceptive passes through the food chain	No	No	Possible	Yes	No	No	No	No
Safe to use during pregnancy	n/a	n/a	No	No	Yes/no	Yes/no	Yes	Yes
Production and/or application costs	Expensive	Expensive	Expensive	Medium	Medium	Medium	Medium	Medium

[a] Males can remain fertile for a number of weeks

[b] Only if given daily

[c] Does not work in male ungulates; remote delivery system being developed (Herbert & Vogelnest, 2007)

[d] Requires microsurgery – not feasible under field conditions and especially in megaherbivores

Gonadect = gonadectomy

Vasec = vasectomy

FallT = tying off fallopian tubes

n/a = not applicable

Table 2: Properties of an ideal contraceptive agent and evaluation of different options summarised (Bertschinger, unpublished)

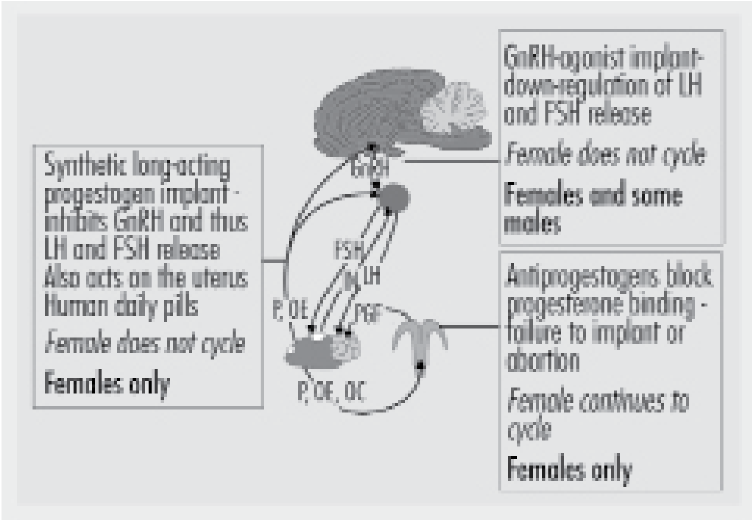

Figure 6: Sites at which hormonal contraceptives act (Bertschinger, pers. comm.)

Experimental and control stallions in Idaho were immobilised from a helicopter and given mTP in the hip. Stallion libido and quantitative aspects of sexual behaviour, based on elimination marking behaviour (Turner *et al.*, 1981) were unaffected and breeding took place, but there was an 83 per cent reduction in foal production compared with mares bred by control stallions (Kirkpatrick *et al.*, 1982). Concerns for the safety of stallions and the dangers and high costs associated with helicopter use and immobilising drugs led to a second field trial in which the mTP was delivered remotely, to wild harem stallions on Assateague Island National Seashore, MD, (ASIS) with barbless darts, from the ground and without immobilisation. The pharmacological success of the mTP was evident, with a 28.9 per cent fertility rate for the mares accompanying the treated stallions and a 45 per cent fertility rate among the mares accompanying untreated stallions. Unfortunately, the logistics of delivering 3.0g microcapsules in four separate doses to each stallion was daunting and impractical for routine use.

Logistical difficulties in treating stallions with steroids, concerns about steroid-related pathologies, and a general concern that the treatment of wild stallions would have serious genetic consequences to the gene flow in free-ranging herds turned the focus of contraception in horses to the mare. Based on experience with persistent corpora lutea (Stabenfeldt *et al.*, 1974) and data which indicated that plasma progesterone concentrations in excess of 0.5–1.0 ng.ml^{-1} inhibited ovulation in mares (Squires *et al.*, 1974; Noden *et al.*, 1978; Palmer & Jousett 1975), attempts were made to administer contraceptive doses of progestins to wild horses. Captive wild mares in Nevada were each implanted with silastic rods containing various doses of the synthetic oestrogen ethinyl oestradiol (EE_2) or EE_2 plus progesterone (Eagle *et al.*, 1992). Animals pregnant at the time of implantation delivered healthy foals, and contraceptive efficacy ranged from 88 to 100 per cent through two breeding seasons. Endocrine studies of these mares suggested that contraception was affected by blocking ovulation and/or implantation. In a similar study, intraperitoneal implants of EE_2 alone also resulted in contraceptive efficacy of 75–100 per cent through two breeding seasons, and rates of EE_2 decline in the plasma suggested a contraceptive life of 16, 26, and 48–60 months, for 1.5 g, 3.0 g, and 8.0 g of EE_2, respectively (Plotka & Vevea, 1990).

Results achieved with oestradiol, progesterone and ethinyl oestradiol in mares brings to focus advantages and disadvantages of natural versus synthetic steroids for contraceptive purposes in the horse. Steroids native to the mare, such as oestradiol and progesterone, are required in impractically large doses due to their rapid enzymatic degradation *in vivo*. The use of some long-acting synthetic steroids such as ethinyl oestradiol may delay metabolic degradation and permit more sustained contraception.

Because of the difficulties with delivering large masses of microencapsulated steroids, dangers associated with capture and restraint of horses, surgical procedures associated with intraperitoneal implants, concern over long-term effects of steroid contraception, and passage of synthetic steroids through the food chain, attention turned to immunocontraception.

Despite the problems experienced with steroids in horse trials, in 1996 a contraceptive trial was carried out on 10 cows in KNP using slow-release oestradiol silicone implants (Compudose). Each cow received five implants providing a daily 17ß-oestradiol dose of 300 µg/animal (Goritz *et al.*, 1999). The cows were non-pregnant at the time and had small calves at foot. The results were reported by Bartlett (1997), Butler (1998) and Whyte & Grobler (1998). The implants were effective as a contraceptive and lasted at least 12 months when the uteri of all cows still showed signs of oestrogenisation. Reversibility of the method was inconclusive as some cows were still barren when the collars were removed. The major side effect of the implants is that the cows showed an almost permanent state of oestrus that lasted at least 12 months. The negative effects associated with this were constant presence of bulls, increased harassment of cows by bulls, and separation of calves from their mothers – two of the ten calves went missing and were presumed dead. On account of the side effects no further cows were made available for oestrogen-implant contraception. As in horses, the practicality and expenses related to administration of oestradiol implants in free-ranging elephants also renders the method a non-starter.

A newer and safer hormonal approach

GnRH super-agonist implants or depot formulations have replaced progestin implants in wild carnivores and to a large extent in non-human primates. Deslorelin marketed as a slow-release implant (Suprelorin®, Peptech Animal Health, Sydney) lasting 6–12 months and up to 24 months and leuprolide depot injection (Lupron Depot®, TAP Pharmaceuticals) lasting 1, 3 or 4 months have been the most commonly used products. They down-regulate the release of both LH and FSH and as such have the potential to be used in both sexes (figure 6). Suprelorin has been used successfully in cheetahs (both sexes), lionesses, leopards (both sexes) and baboon and monkey species (Bertschinger *et al.*, 2001; 2002; 2004b; 2006; 2007; 2008). Although well suited for the above species, the application in herds of female animals would be impractical since administration of implants requires immobilisation. Besides, the results with both products in ungulates have been highly variable and hardly reliable (Patton *et al.*, 2005). The possibility for remote delivery of Suprelorin® implants is, however, being researched in kangaroos and preliminary results look promising (Herbert, 2007).

Immunocontraception

GnRH vaccine

The GnRH vaccine consists of one or more molecules of GnRH conjugated to a protein molecule to render the GnRH component antigenic. This is combined with an adjuvant and, when injected, antibodies to endogenous GnRH are formed. These antibodies neutralise GnRH released from the hypothalamus, thus down-regulating the release of the gonadotrophic hormones FSH and LH (figure 7). This is effective in both male and female animals. The process is reversible and if no further boosters are administered, the antibody titres will fall until insufficient to neutralise endogenous GnRH. Vaccinated females cease to show an oestrous cycle, while in males testosterone release is inhibited (meaning the vaccine also suppresses testosterone-related aggression) and eventually also spermatogenesis.

GnRH immunocontraception was originally developed for immunocastration of cattle (Hoskinson *et al.*, 1990). One of the main reasons for further development of the GnRH vaccine, however, was to immunocastrate male piglets as an alternative to surgical castration and so control the problem of boar taint in pork (D'Occhio, 1993; Oonk *et al.*, 1998; Dunshea *et al.*, 2001; Zeng *et al.*, 2001) The vaccine has also been used to control fertility of male feral pigs (Killian *et al.*, 2006), stallions (Dowsett *et al.*, 1996; Turkstra *et al.*, 2005; Burger *et al.*, 2006), rams (Janett *et al.*, 2003), bison bulls (Miller *et al.*, 2004), white-tailed deer (Becker *et al.*, 1999; Curtis *et al.*, 2001), and others (Ferro *et al.*, 2004).

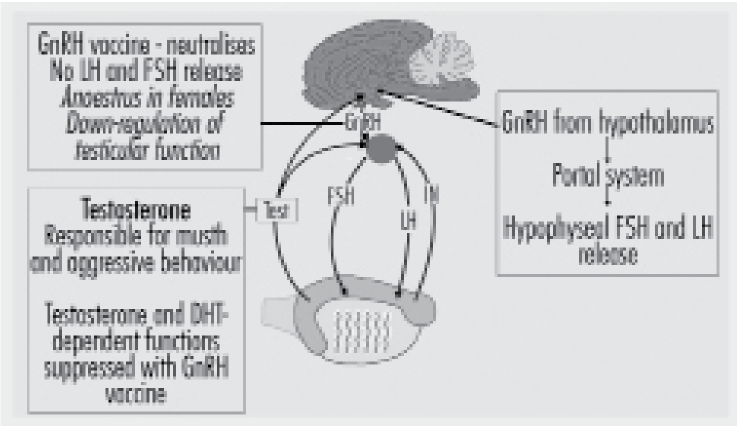

Figure 7: Endocrine control of testicular function in the male and site of action of anti-GnRH antibodies in males and females (Bertschinger *et al.*, 2004b)

Goodloe *et al.* (1997) immunised 29 wild mares on Cumberland Island National Seashore, GA, with GnRH conjugated to keyhole limpet haemocyanin (KLH). The vaccine was freeze-dried, sequestered in a solid biodegradable 0.25 calibre bullet and administered by an air-powered gun (Ballistivet, Inc., White Bear Lake, MN). After imbedding in the muscle of the target mare, the compressed compound forming the biobullet degrades over 24 hours, releasing the antigen. A total of 25 treated mares survived until the next foaling season and 17 (68 per cent) produced foals, which was not significantly different from control foaling rates.

A more recent attempt to immunise wild horses against GnRH was carried out in Nevada (Killian *et al.*, 2004). Mares received either 1 800 or 2 800 μg GnRH vaccine (National Wildlife Research Center, Fort Collins, CO) with Adjuvac adjuvant, which is a dilution of a commercial Johne's Disease vaccine (Fort Dodge, Ames, IA). Following a single breeding season, none of 18 mares, in both treatment groups, were pregnant on the basis of ultrasound evaluations.

All GnRH-treated mares had low concentrations of serum oestrogen and progesterone, which is consistent with the predicted actions of a GnRH vaccine. The largest study performed in mares so far made use of a GnRH vaccine developed for pigs (Improvac®, RnRF-protein conjugate, Pfizer Animal Health, Sandton, South Africa) (Botha *et al.*, 2008). Fifty-five mares were given a primary followed by a booster vaccination 35 days later, each containing 400 µg RnRF-protein conjugate. On Day 35 after the primary vaccination only 8 of 55 (14.5 per cent) experimental mares showed evidence of ovarian activity on clinical examination and by Day 70 all mares were quiescent. Baseline progesterone concentrations were attained by Day 42 and persisted until the end of the observation period on day 175. The application in elephants poses an exciting prospect. The vaccine is remotely deliverable, and the dose is small in terms of volume (3 ml), cheap and freely available. If the GnRH results in elephants follow the same pattern as pZP in elephants followed the pZP results in horses, it may provide an alternative method to pZP immunocontraception. One advantage that it may hold over pZP immunocontraception is that it induces anoestrus (see *Possible future developments*).

Porcine zona pellucida vaccine

The origins of porcine zona pellucida (pZP) contraceptive vaccine can be traced back to the work of Sacco & Shivers (1973) and Shivers *et al.* (1972), who demonstrated that the antibodies produced against the proteins of the porcine zona pellucida could inhibit sperm binding (figure 8). Shortly thereafter, it was discovered that spontaneous antibodies against the zona caused infertility in humans (Shivers & Dunbar, 1977) and that porcine zona proteins could block human fertilisation (Sacco, 1977). At about the same time, it was shown that these antibodies against native pZP were tissue-specific and did not cross-react with other tissues or hormones (Palm *et al.*, 1979). Collectively, these discoveries led to a surge of non-human primate research with native pZP, with an eye towards human contraception (Gulyas *et al.*, 1983; Sacco *et al.*, 1986, 1987).

Ultimately, interest in pZP for human contraception waned, for four major reasons. First, the immune systems of the target subjects were variable in their response to pZP and contraceptive efficacy was correspondingly variable. Second, the time to reversal of contraceptive effects was also quite variable and most pharmaceutical companies feared a wave of litigation by users. Third, it was discovered that in some species, such as baboons, rabbits, guinea pigs and dogs, but certainly not all species, ovarian abnormalities developed in response to the vaccine (Wood *et al.*, 1981, Mahi-Brown *et al.*, 1985, Dunbar *et al.*, 1989; Lee & Dunbar 1992). Finally, the inability to produce a synthetic or recombinant form of the native pZP, because of the difficulty in glycosylating the protein backbone, meant that large markets could never be serviced (Dunbar *et al.*, 1984).

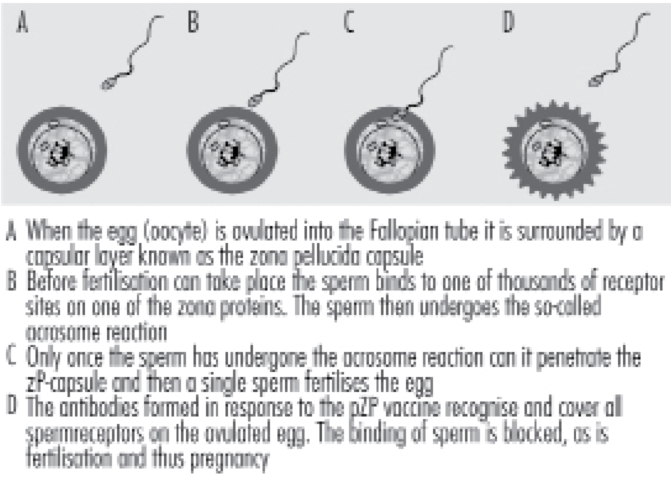

Figure 8: The proposed mechanism of pZP immunocontraception (Bertschinger *et al.*, 2004)

Liu *et al.* (1989) sparked new interest in pZP when they demonstrated that domestic mares could be rendered infertile with native pZP injections. Over the next 19 years the pZP vaccine was applied to wild horse herds in the US with a high degree of success with regard to both efficacy and safety (Kirkpatrick *et al.*, 1990, 1991, 1992, 1995a; Kirkpatrick & Turner 2002, 2003, 2007) as well as ability to achieve population effects (Turner & Kirkpatrick 2002; Kirkpatrick & Turner 2007). The work with wild horses rapidly led to the application of pZP to other species as well, including white-tailed deer (McShea *et al.*, 1997; Naugle *et al.*, 2002; Rutberg *et al.*, 2004), wapiti (Shideler *et al.*, 2002), and many species of captive exotics, in zoos (Kirkpatrick *et al.*, 1995b, 1996: Deigert *et al.*, 2003; Frank *et al.*, 2005.

Collectively, these applications of pZP to various wildlife species and their results conformed well to the hypothetical characteristics of the ideal wildlife contraceptive (Kirkpatrick & Turner, 1991). The vaccine resulted in an overall efficacy of near 90 per cent; it could be delivered remotely by means of small darts; the contraceptive effects were reversible; there were no changes in social behaviours or organisation among treated animals; no deleterious long-term health effects resulted from its use; the vaccine was protein in nature and therefore did not pass through the food chain; it was safe to administer to pregnant animals, and it could be produced and applied at relatively low costs.

Research on contraception of elephants in the Kruger National Park, 1995–2000

In 1995 the scientists associated with the pZP vaccine approached the Kruger National Park to propose a project that would test the safety and efficacy of pZP contraception on African elephants.

Since the pZP vaccine is made by purifying zona pellucida proteins derived from the ovaries of pigs, the team wanted to establish the degree of homology between porcine and elephant zona pellucida proteins (eZP) (Fayrer-Hosken *et al.*, 1999). To this end, an immunohistochemical study was conducted using elephant ovarian tissue obtained during the last Kruger culls in 1995 and rabbit-anti-pZP antibodies. After sections had been exposed to the rabbit antibodies they were rinsed and then treated with immuno-gold-labelled goat anti-rabbit antibodies rendering the antibody complex visible. The results showed distinct immuno-gold staining of the zona pellucida capsules of the elephant oocytes in the histological sections. Following this work three tractable elephant cows in zoos in North America were vaccinated with pZP in order to determine the vaccination regimen. All three cows developed antibody titres similar to those of horses that had been successfully immunocontracepted with pZP vaccine (Fayrer-Hosken *et al.*, 1997; 1999).

A field trial began in Kruger in late 1996 with the treatment of 21 female elephants and 20 control animals. The purpose of this and the second field trial with 10 cows was simply to test the contraceptive potential of the pZP vaccine. The initial primer inoculation (600 μg) was given by hand, after immobilisation, the animals were collared for later identification, and all subsequent booster inoculations were given remotely, by dart, from a helicopter. Each animal received two booster inoculations the first year, six weeks apart. A year later, 44 per cent of the treated animals and 89 per cent of controls were pregnant; this difference was statistically significant. One of the treated elephants had a 22-month foetus, indicating that the vaccine had no effects on the health of the pregnancy (refer to later trials).

The next experiment changed the timing of the booster inoculations, from six weeks apart to two and four weeks apart, and a year later only two were pregnant (20 per cent). Four of the seven cows that were non-pregnant during the first trial received a single booster inoculation. A year later ultrasound examinations revealed that none of the four pZP-boosted animals was pregnant; all were cycling and had normal looking reproductive tracts. The three that were not treated were all pregnant (Fayrer-Hosken *et al.*, 2000). The collective results of these experiments with the Kruger elephants were corroborations of previous work with wild horses, deer and a variety of captive exotic species, and suggested that pZP immunocontraception of African elephants was safe, effective and had reasonable efficacy as a population management tool.

Makalali contraception project

The Greater Makalali Private Game Reserve (GMPGR) is situated in the Limpopo Province, South Africa. Its contraception project was started in May 2000, and is the longest running same-population study (n = 7 years) on elephant immunocontraception to date.

Efficacy

Initially 18 target animals were vaccinated with 600 µg of pZP + 0.5 ml of Freund's Modified Adjuvant (Sigma Chemical Co., St Louis, MO) and two booster vaccinations of pZP (600 µg) emulsified in Freund's Incomplete Adjuvant (Sigma Chemical Co., St Louis, MO) each two to three weeks apart (Delsink *et al.*, 2002, Delsink *et al.*, 2006). By 2007, a further five target animals had been vaccinated under the same regime, totalling 23 vaccinated animals in the Makalali population. Reproductive control was demonstrated in all 23 targeted females who passed the population's average intercalving interval of 56 months (Delsink *et al.*, 2006). A 0 per cent growth rate has been maintained within this target group since August 2002.

Effects on population growth

Delsink's detailed population history (Delsink *et al.*, 2002; Delsink, 2006) allowed for the estimated rate of increase (excluding mortalities and introductions) to be determined for the population, on an individual elephant basis, using the average inter-calving interval (56 months) for the period 1994-2002. (While the programme was initiated in 2000, the first two years were not influenced by pZP, thus they were included in the intercalving rate calculation). The estimated population size for GMPGR for 2010 totals 108 animals (Delsink *et al.*, 2006) (figure 9). Contraception had a significant effect on the population's growth, as the difference between the estimated and observed population size was significantly different over time (Delsink *et al.*, 2006). The contraceptive effect over the period 2003–2010 produces an average population growth decline of 6.5 per cent, assuming all the original target animals remain on the programme and there are no further introductions or mortalities. This estimation includes the addition of eight calves from the current pre-pubertal cows that will only be contracepted after they have given birth to their first calves. The average population growth rate (excluding introductions and mortalities) from 1996 to June 2000 was 8.9 per cent, similar to the range of the model projected 6.2–8.9 per cent average annual population growth rates following introduction (Mackey *et al.*, 2006). Thus, the contraceptive will effectively reduce the population growth rate by 70 per cent for the period 2003 through 2010 (Delsink *et al.*, 2006).

Local and systemic side effects of the vaccine

Because many immunogens used for contraceptives have low antigenicity, their efficacy is dependent on concurrent delivery of potent adjuvants to stimulate an adequate immune response (Munson *et al.*, 2005). The optimal efficacy of immunocontraceptives such as pZP occurs when Freund's adjuvant is used, but Freund's adjuvant incites a marked granulomatous reaction (Munson *et al.*, 2005).

Of the 18 cows vaccinated and boosted between May and June 2000, 16 displayed local swellings – most likely abscesses (Bengis, 1993) – within 3 months after the initial vaccination (Delsink *et al.*, 2002, Delsink 2006). These swellings ranged from 20 to 100 mm in diameter and were all eventually resorbed. Only three cows developed swellings of approximately 100–120 mm in diameter after the primary vaccination in 2000. While these were still present though markedly reduced in size by the fourth annual vaccinations in 2004, they have now been completely resorbed (Delsink, 2006; Delsink, pers. obs. 2007). The reactions were not associated with lameness or with other visible ill health effects on the cows.

During subsequent annual vaccinations, Freund's Incomplete Adjuvant (less aggressive than Freund's Modified Adjuvant) was used, and no swellings greater than 50 mm were formed. The affected animals never displayed signs of irritation or discomfort as a result of the swellings, nor did they suppurate or need any treatment. In fact, the swellings that formed at the dart site were similar in appearance to those produced by thorns or other penetrating objects (Delsink, 2006).

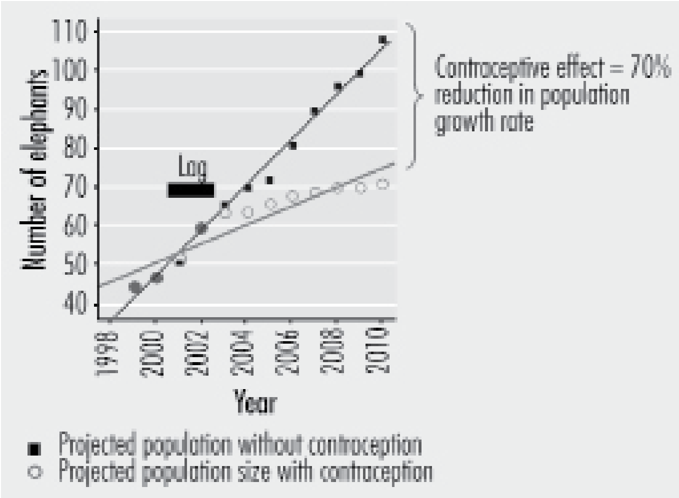

Figure 9: The effect of contraception on population size at Makalali Conservancy. The black bar above the curves indicates the lag effect before contraception as a result of elephants already pregnant prior to darting. See Delsink *et al.* (2006) for details, and text for statistical test

Behavioural effects

Effects of administration procedure on behaviour

The greatest impact of pZP implementation was on the interaction between the vaccination team and the herds during ground darting (Delsink *et al.*, 2007a). 'Avoidance' behaviour was clearly evident in the presence of and towards the darting team and was manifested by the herds either running away or remaining mobile away from the darting team (Delsink *et al.*, 2007a). Darting from the ground appeared to cause less stress than from the air, judging by the flight response and movement patterns of the elephants, but it was conducted over much longer periods (n = 25 days)(Delsink, 2006). This avoidance behaviour only continued until approximately two weeks after the last dart had been fired.

Conversely, vaccinations administered from the helicopter resulted in far greater perceivable stress in the animals. This perception was based on observations of bunching of the herd and flight patterns. The duration of disturbance, however, was much shorter than when darted from the ground, and the animals resumed normal movement patterns and appeared to be settled within a day of vaccinations (Delsink, 2006; Delsink *et al.*, 2007a).

Aerial vaccinations were far more productive, with a darting time of about 30 seconds per female (Delsink *et al.*, 2007a). Thus, the average completion of aerial vaccinations within the Makalali population is approximately 30 mins. Furthermore, the helicopter vaccinations did not compromise further monitoring initiatives, as the elephants appeared to be reacting to the helicopter, rather than to being darted (Delsink *et al.*, 2007a).

Effects on ranging patterns of clan and individual herds

Although there was avoidance behaviour, the cumulative effect of vaccine administration did not cause an overall change in the treated population's core and total ranges over a five-year period for either individual herds or the whole clan (Delsink, 2006; Delsink *et al.*, 2007b). Thus, for reserves that are largely eco-tourism driven, the implementation of a pZP programme will have little effect on game-drive and safari activities (Delsink, 2006; Delsink *et al.*, 2007b). The shorter vaccine administration demonstrated by the helicopter darting appeared to have a more consistent effect on the herds, with the least shift in core range during and after darting (Delsink, 2006; Delsink *et al.*, 2007b).

Effects on reproductive behaviour (includes effects of non-conception)

The Makalali study demonstrated that although there was an increase in the number of musth and oestrous events over the years of treatment, this change was not significant over all treatment years for all herds (Delsink, 2006; Delsink *et al.*, 2007b).

Reproductive control is achieved in the third year of treatment. This means that all females that were pregnant would have given birth and after subsequent treatments, would have been contracepted, while those that were not pregnant would be contracepted after the initial vaccination series. Therefore, there was an expectant increase in oestrus observations in the third year. However, there was no significant difference in oestrus occurrence among the years of treatment, although the greatest frequency was observed in Year 3 – the year in which reproductive control was achieved (Delsink, 2006). Therefore, there was a higher incidence of oestrus in this year, as all treated females were cycling in the same year. Calf survival was not affected by increased cycling and, in fact, no calves were lost during the entire observation period. This is similar to experience with pZP-contracepted wild horses (Kirkpatrick & Turner, 2003).

Musth was most frequent in the oldest, dominant bulls, aged 25+ years (Delsink, 2006). Musth bulls dominated consort and matings in all instances, even in presence of other bulls. Even in the absence of musth, dominant bulls still dominated all consort displays and matings, demonstrating that cow mate selection remained intact (Poole, 1996a). Thus, the treatments did not affect bull hierarchy or cow selection (Estes, 1991; Moss, 1983; Delsink, 2006; Delsink *et al.*, 2007b).

Bull association coupled to increased frequency of oestrus

Under the pZP treatment, the target animal displays normal oestrous cycles, cycling every 15–16 weeks, because although copulation still occurs, conception does not (Whyte, 2001). Therefore, under the pZP contraceptive, the frequency of mating and its accompanying disturbances is assumed to be far more frequent (Delsink, 2006). Thus, with an increased frequency of oestrus, there is the potential for change in the frequency of association of both sexually active musth and sexually active non-musth bulls with breeding herds as both sets of males compete for oestrous females (Poole, 1982; Delsink, 2006). In fact, bull association with herds decreased over the years, probably an effect of aging in this relatively young population. The decrease in herd-bull association further illustrated that the pZP implementation did not affect Makalali's bull hierarchy; that is, there were far more non-musth sexually active bulls than musth sexually active bulls and even in the absence of musth, mating and consort behaviour was highest in the three dominant musth bulls. Furthermore, the non-musth sexually active bulls did not increase their associations with the herds (Delsink, 2006; Delsink *et al.*, 2007b).

Herd fission/fusion

A change in association pattern and the proportion of time spent alone between herds was observed after the pZP implementation (Delsink, 2006; Delsink *et al.*, 2007b). The Makalali herds tended to associate less over the initial period of the study, but the integrity of the group remained strong throughout the study duration. These differences are attributed to the change in association pattern between herds – the natural formation of a bond group when the family unit becomes too large and splits along family lines (Moss, 1988) – and not to anti-fertility treatment. Furthermore, this is the general pattern of group formation in relocated herds (Slotow, pers. comm. 2006). This study demonstrates that the pZP implementation did not cause herd fragmentation, did not cause herds to become more isolated or alter matriarchal group size – management concerns raised by Whyte (2001). Furthermore, behaviour between cows and their calves at foot was recorded, and cows were never separated from their calves (Delsink, 2006; Delsink *et al.*, 2007b).

The results from the Makalali study demonstrate that there were no aberrant or unusual behaviours with the medium-term and sustained use of pZP on bull and cow societies. As demonstrated in the Kruger trials (Fayrer-Hosken *et al.*, 2000), the results of the pZP-treated cows were as expected from cows whose behavioural patterns were not affected by the treatment (Whyte, 2001); that is, there is no evidence to suggest that the pZP has any adverse effects on the behaviour of either the treated cows, their matriarchal groups or bulls (Delsink, 2006).

Continued management using contraception including reversibility

The original hypotheses of the programme have been successfully demonstrated (Delsink, 2006; Delsink *et al.*, 2006; 2007a; 2007b).While reversibility was demonstrated in the Kruger trials (after two successive years of treatment), the Makalali study aims to demonstrate reversibility in cows treated in the medium-long term. In 2005, five cows were removed from the programme to test reversibility; three cows were treated for five years, one for four years and one for three years, respectively (Delsink, pers. obs. 2007). All the cows have calved except for one who has never conceived. One of the three-year treated cows was accidentally vaccinated in 2006. However, all the other cows have been off treatment for two years (June 2007). Reversibility in 100 per cent of wild horse mares treated for one, two or three consecutive years has been demonstrated, while 68 per cent of those vaccinated for four consecutive years returned to fertility (Kirkpatrick, 2001). No mares treated for over seven years had returned to fertility at the time of publication, but they had started ovulating again (Kirkpatrick, 2001). A six-year study on white-tailed deer demonstrated that the treated does did return to fertility, though they became pregnant later in the breeding season than would normally occur (Miller *et al.*, 1999).

Other game reserves where pZP has been used

Including Makalali, immunocontraception has been applied in 10 discrete populations (table 3) (Bertschinger *et al.*, 2007). The Kapama cows belong to a captive population and were treated with a slow-release formulation of pZP. The other populations are wild and during Year 1 all cows of reproductive age (n = 103) received a primary (400 µg with 0.5 ml Freund's modified adjuvant) and two boosters (200 µg with 0.5 ml Freund's incomplete) followed by an annual booster of vaccine. Vaccines were administered remotely from the ground or helicopter with drop-out darts. The initial results of the populations vaccinated from 2000-2005 are shown in table 4.

The results of this ongoing elephant contraception project align with previous findings of the programme in Makalali (Delsink *et al.*, 2006).

During Year 1 there is no effect of immunocontraception on calving percentage (28.8 per cent, table 4). There may be an effect in Year 2 (22.5 per cent), but in Year 3 the calving percentage was reduced to 15.1 per cent. Although the data from most of the populations are incomplete at this stage, from Year 4 and onwards no calves were born to treated cows. The lag time before the effects of the vaccine are visible is due to the long gestation period of 22 months in African elephants. Taking the gestation period into account it would appear that some cows are not immediately rendered infertile after the first booster vaccination. Eight of 52 cows contracepted during Year 1 produced calves during Year 3 (>24 months).

Occasional swellings were the only side effects seen following administration of the vaccine. It should also be noted that abscesses are common following darting of elephants with immobilising agents (Bengis, 1993). The cause of the abscesses during immobilisation procedures is more than likely related to contamination of elephant skin with large numbers of bacteria originating in stagnant water, dust and sand. The dart needle merely provides a mechanical means of carrying bacteria into and below the skin. Big differences were noted in the incidence of swellings (presumed to be abscesses) at the primary vaccination site between Makalali and Thornybush, despite the same adjuvant having been used. Only 4 of 19 cows were affected at Thornybush, compared to 16 of 18 cows at Makalali. The incidence of abscesses can also be vastly different between reserves following immobilisation. According to Bengis (pers. comm.) the incidence in the South African Lowveld area is much higher than in Etosha. The latter is arid and elephants are much less likely to submerge themselves in contaminated water. This may indicate that the swellings noted after primary vaccination were bacterial abscesses rather than granulomas resulting from the adjuvant.

Excluding the largest reserve (Welgevonden; cow n = 35; total = 117), average helicopter darting time was 30 seconds per cow. Average flying time during Year 1 in Welgevonden, which is mountainous and heavily wooded in parts, was 6.9 minutes per cow.

The data available to date show that the pZP vaccine is effective in reducing the birth rate of free-ranging elephant cows. Birth rate starts to drop during the third year to reach zero during the fourth year. The safety of the vaccine has once again been demonstrated in target animals, the only side effect being occasional temporary lumps at the darting site.

Reserve & inception	Method of darting	Population detail	Year –1	1	2	3	4	5	6	7
Makalali Jun 2000	Yrs 1–3 Ground Yrs 4–7 Helicopter	Total population	45	47	52	60	64	64	67	69
		Cows contracepted	0	18	18+2	20+3	23	23	17+4	21
		Calves born	2	5	8	4	0	(3)	(2)	(1)
Mabula May 2002	Helicopter	Total population	9	11	11	11	11	11		
		Cows contracepted	0	4	4	4	4	2		
		Calves born	2	1	0	0	0	0		
Thaba Tholo Aug 2004	Helicopter	Total population	27	28	29	29				
		Cows contracepted	0	8	8	8				
		Calves born	?	1	0	1				
Shambala Jun 2004	Helicopter	Total population		10	11	12				
		Cows contracepted		4[a]	4	4				
		Calves born		1	1	0				
Phinda Jul 2004	Ground	Total population	71	77	83	90				
		Cows contracepted	0	19	19	18+3				
		Calves born	6	6	7	8				
Thornybush May 2005	Helicopter	Total population		35	38					
		Cows contracepted		19	19					
		Calves born		4[b]	4					
Welgevonden Sept 2006	Helicopter	Total population		117	129					
		Calves born		35	35					
		Cows contracepted		12	5					
Kaingo Oct 2005	Ground	Total population		9	11					
		Calves born		4[a]	4					
		Cows contracepted		2	0					
Karongwe May 2007	Helicopter	Total population	16	16						
		Calves born	0	4[c]						
		Cows contracepted	0	Too early						
Tembe E P May 2007	Helicopter	Total population		250						
		Calves born	0	75						
		Cows contracepted		Too early						

[a] One calf died after a rupture of the umbilicus

[b] These four cows only received a primary vaccination during Year 1

[c] These four cows were vaccinated once with a so-called 'one-shot' vaccine

Table 3: Summary of the elephants contracepted with pZP vaccine in 10 game reserves and the response in terms of calving data and total populations from Years 1–7 of the programme (Bertschinger *et al.*, 2007)

	Year[a]						
	1	2	3	4	5	6	7
Number of reserves	9	8	5	2	1	1	1
Cows contracepted during Year 1	111[b]	3 111	53	22	18	18	18
Calves born	32	25	8	0	0	0	0
Calving %	28.8	22.5	15.1	0	0	0	0

[a] Year contraception program was introduced

[b] Excludes the 75 cows from Tembe Elephant Park and Karangwe Private Game Reserve

Table 4: Percentage of cows vaccinated in Year 1 calving by year (Bertschinger *et al.*, 2007)

The data available so far show that 64 of 111 cows vaccinated during Year 1 produced normal, healthy calves. An additional calf died a few days after birth due to a ruptured umbilicus that is unlikely to have been caused by the pZP vaccine. Short-term reversibility after one to two years of pZP vaccination of elephant cows was demonstrated in the Kruger (Fayrer-Hosken *et al.*, 2000). However medium- to long-term reversibility still needs to be demonstrated. To prove reversibility we have now withdrawn vaccination from five cows in Makalali (three five-year treated cows, one four-year treated cow and one three-year treated cow) and two at Mabula (four-year treated). In three reserves (Makalali, Phinda and Thornybush), however, we have seen a total of 58 oestruses in a total population of 56 immunised cows. Because these data relied on behavioural observations, a number of oestruses may have been missed. The data reveal that the ovaries of these cows seen in oestrus must be functional and that there has been little or no immuno-destruction of follicular tissue. Currently there is one trial under way at Thornybush to monitor ovarian cycles more objectively than just by means of behavioural observations. Faecal progesterone metabolites will need to be used to achieve this objective (Wittemyer *et al.*, 2006). This study will be extended to include faecal monitoring of eight cows at Makalali (Bertschinger *et al.*, 2007).

Implementation/methodology

Identification of the population

In the Makalali study, it was essential that elephants could be identified individually as each elephant had to receive her primary and subsequent boosters timeously (Delsink *et al.*, 2002; Delsink, 2006). Furthermore, to ensure complete vaccine discharge, the darts were retrieved after each vaccination in the years 2000–2003. Thus, should incomplete vaccine delivery have been recorded, the target animal could be revaccinated (Delsink *et al.*, 2007).

The Makalali population was identified according to the methods described by Moss (1996), Poole (1996a, 1996b), and Whitehouse & Kerley (2002), where animals were recognised by their individual characteristics including sex, age, unique ear pattern comprising nicks, tears and holes, and the size and shape of tusks. When no distinguishing ear or tusk features were visible, ear venation patterns were completed according to the methods described by Whitehouse (2001). Other distinguishing features such as growths, lumps, scars and tail hairs were also recorded as identification criteria. The animals were sexed following the method of Moss (1996) and Hanks (1979), where head shape was observed (the profile of the head is rounded in males, steeply angled in females) when genitalia were obscured.

The elephants were aged according to accepted parameters. See box 3, Chapter 2.

The same detailed individual histories exist for the Mabula, Shambala, Thaba Tholo, Karongwe and Phinda elephants. However, no extensive individual data existed for the Thornybush, Welgevonden or Tembe elephant populations. Individual elephants in the Thornybush population were only identified after the primary vaccination was administered, using the methods described above (Delsink, pers. obs. 2005). Welgevonden elephants were individually identified during the course of Year 1 of contraception implementation. Thus, these latter populations were managed not on individual-based elephant identifications, but rather on a broader age and sex class classification – adult and sub-adult females were identified based on size, grouping compositions and head shape from the air (Delsink, pers. obs. 2006; Van Altena, pers. comm. 2007).

Darting method

At Makalali, ground darting was the original source of delivery to facilitate dart retrieval, to test vaccine efficacy and for minimal impact on the herds (Delsink, 2006). However, during the collaring procedure in 2003, 17 individuals were vaccinated from the air. This greatly improved the team's productivity, and since 2004, all vaccinations have been conducted from the air (Delsink *et al.*, 2007). In 2003, 2004 and 2005, time spent in the field decreased while the average number of darts fired per day increased (figure 10). At Makalali, the cows have been vaccinated within 30–60 minutes since 2004 (Delsink, pers. obs. 2007).

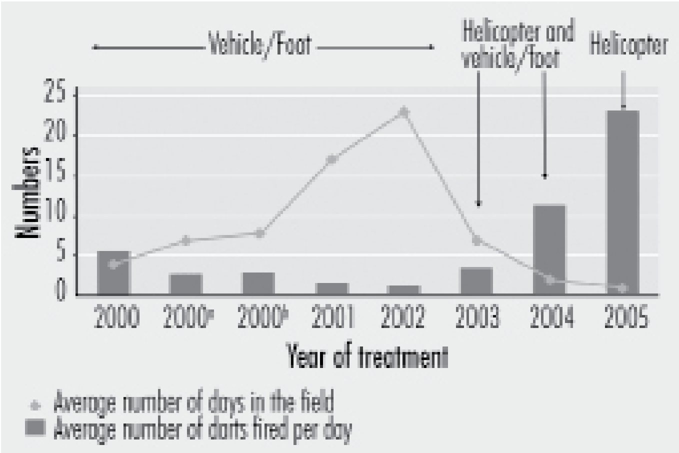

Figure 10: Efficiency in field implementation over successive treatment years 2000–2005, 2000, 2000a and 2000b reflect the primary vaccination, 1st and 2nd boosters in Year 2000, respectively. Vehicle/foot/helicopter refers to the source of vaccination delivery (Delsink *et al.*, 2007)

The experiences in the nine other reserves showed that vaccination is possible under a variety of conditions. In Phinda, which contains large areas of sand forest, ground darting was used for the first three years. The average daily rate of movement for five GPS-collared cows one week before, during the week-long contraception period and one week after contraception in Year 3 was 0.232, 0.760 and 0.250 km.h^{-1}, respectively. This showed once again that elephants settle down very soon after the darting experience but not as rapidly as following helicopter darting. In Mabula, Thaba Tholo, Shambala, Thornybush and Karongwe (all vaccinated from the helicopter), the flying time per cow was approximately the same as for 2004 in Makalali once the booster vaccinations commenced. Terrain and habitat had an influence on helicopter darting time. Average flying time during Year 1 in Welgevonden, which is mountainous and heavily wooded in parts, was 6.9 minutes per cow. In Tembe, the largest population vaccinated so far, helicopter darting was quick, provided the cows were in the open swamp area. Most, however, were darted in the sand forest, which has high tree canopies. Average darting time including total ferrying time of 90 minutes was 2.5 minutes per cow.

Implementation strategies for different conditions and population sizes

In small confined populations of up to 100 elephants the ideal starting point is to identify each individual animal on the property, concentrating especially on the females of breeding age. In accordance with the elephant management plan the number of cows to be immunised for contraception can be determined. Prepubertal cows can be either allowed to conceive and have their first calf or be immunised at the outset. Over time the response of the population is monitored and the contraception programme adapted according to the needs of the management plan and ongoing habitat response (figure 15). Darting is preferably carried out from a helicopter as the disturbance is brief and return to pre-darting patterns quick in comparison to ground darting. The use of GPS/radio collars placed strategically on a cow in each herd will decrease darting time but will add considerably to the cost of the programme. In time costs are likely to be reduced and collar battery-life increased. Where the population is small (4–8 cows), which allows darting to be carried out in one, maximum two days, ground darting may be considered.

Frequency of darting with regard to the primary vaccination and subsequent boosters can also be varied. The primary vaccination sensitises the animal to the antigen (in this case pZP or even GnRH), and B-lymphocytes respond by producing humoral antibodies. Some of these B-lymphocytes (also known as memory cells) remain present for years and when the animal is boosted they divide to form more active B-lymphocytes with a corresponding rise in antibodies. It stands to reason therefore that cows given a single vaccination during Year 1 followed by annual boosters will eventually develop sufficient immunity to prevent fertilisation from taking place – the process will, however, take longer (we speculate 4–6 years). This strategy could be useful to sensitise prepubertal cows but just as importantly could be employed to sensitise a translocated population where contraception is planned in the future. It would be sensible to apply this to all elephant cows being translocated to fenced smaller reserves. The cost would be minimal as the vaccination could be carried out when the cows are immobilised for translocation.

Research with pZP immunocontraception in horses has shown that time taken to reversal is approximately equal to the number of years a particular mare has been vaccinated for. If this holds true for elephant cows it may be possible to lengthen intervals between boosters after the initial three or four years. Currently we do not have data to prove such a possibility.

Returning to population size, the picture is somewhat different for larger populations where identification of individuals becomes increasingly difficult and time consuming as the population increases. Here the mass-darting approach would have to be applied. The largest population done to date was in the Tembe Elephant Park where 75 cows were immunised. Marker-darts were employed in this successful exercise which allowed the team to identify cows already darted. The same approach could be used for even larger populations, but as population size increases the percentage of cows vaccinated must decrease. Despite this it may be possible to achieve a considerable effect on growth rate of large populations. According to Page (pers. comm.), models have shown that effective contraception of 60 per cent of cows will halve population growth rate over a period of 15 years.

Costs

There is a concern that contraceptive implementation may be cost prohibitive. The Makalali study (table 5) has demonstrated that the highest costs incurred during contraception implementation are based on the helicopter costs, or more specifically, the costs of ferrying the helicopter to the site. Total annual contraceptive costs per elephant doubled from 2005 to 2006 simply because of the costs of ferrying the helicopter from Johannesburg and back during 2006 (n = 4 hours).

At Makalali, implementation costs ranged from ZAR332 to ZAR1 170 per animal including the darts, vaccine and helicopter costs. Veterinary fees vary between ZAR2 500 and ZAR3 800 per day, and helicopter rates are approximately ZAR3 800 per hour (J Bassi, BassAir, pers. comm., 2005). As such, veterinary fees can be incorporated with vaccine and dart costs on an individual elephant basis.

As can be seen from the Thornybush contraception programme, initial costs of vaccination during the first year tend to be a lot higher than during the following years. During Year 1 the average all-inclusive cost was ZAR1 639 per vaccination per cow, whereas in Years 2 and 3 it was only ZAR645 per vaccination per cow. The main reason for this was that primary vaccination procedure was also used to identify the breeding units from the helicopter. This increased helicopter flying time considerably. With the subsequent two rounds the duration decreased. Another important factor is the experience of the helicopter/spotting/darting team, which improves with each round.

	2000[3]	2001	2002	2003	2004	2005	2006
Dan-Inject® @ ZAR178 per dart[1] (Number of darts in brackets)	5 518 (31)	2 136 (12)	2 136 (12)	712 (4)	356 (2)	0	0
Pneu-Darts® @ ZAR85 per dart (Number of darts in brackets)	0	0	0	1 445 (17)	1 785 (21)	1 955 (23)	2 394 (25)
Vaccine @ US$20 per dose (2000–2002)[2] (Number of doses in brackets)	11 160 (62)	4 500 (25)	4 500 (25)	0	0	0	0
Vaccine @ ZAR100 per dose (2003–2004) (Number of doses in brackets)	0	0	0	2 300 (23)	2 300 (23)	2 300 (23)	5 273
Helicopter	0	0	0	7 524[4]	7 980[5]	10 819[6]	18 058[7]
Number of elephants vaccinated	18	20	23	23	23	23	22
Average cost of vaccinations per elephant	927	332	289	520	540	655	1 170

[1] Dan-Inject darts were used at least twice each during the 2000–2002 vaccinations, thus, the number of actual darts fired is halved in order to determine actual cost of darts for these years. Four and two Dan-Inject darts were used in 2003 and 2004 respectively.

[2] Vaccine was obtained from the USA at an exchange rate of ZAR9:US$1.

[3] The year 2000 costs were high because each animal received a primary and two booster vaccinations. A total of 62 darts were fired, including 11 revaccinations due to unsuccessful darts.

[4] Helicopter time for 2003 included 5.5 hours of flying time including ferry to Makalali. Flying time was high because it included vaccinations and the collaring and biopsy procedure of 4 and 1 elephants respectively. Therefore, costs for 2003 vaccinations are calculated at the rate per hour for 2003 (ZAR3 300) and on the number of hours flown as per 2004, i.e. 2 hours in total.

[5] Helicopter time for 2004 included 1 hour of flying time and 1 hour of ferry costs.

[6] Helicopter time for 2005 included 1 hour of flying time and 2 hours of ferry costs.

[7] Helicopter time for 2006 included 1 hour of flying time and 4 hours of ferry costs.

The above costs do not include veterinary fees or salaries for the project darts man and elephant monitor.

Table 5: Costs incurred during the Makalali vaccinations 2000-2006. Amounts are given in ZAR (amended from Delsink *et al.*, 2007a)

Surgical sterilisation in elephants

Methods

A safe, reliable and efficient technique for surgical castration of African and Indian (*Elephas maximus*) elephants has been developed (Foerner *et al.*, 1994). Although the work of Foerner *et al.* was pioneering, recent advances in elephant surgery, surgical equipment and anaesthesia, have made it feasible to consider the use of less invasive laparoscopic techniques that would avoid technical problems associated with this original procedure (Stetter *et al.*, 2005).

Surgical sterilisation of elephant cows

Laparoscopic surgery provides a direct view of internal organs and this allows for tissue manipulation via a minimally invasive procedure utilising relatively small incisions (Stetter *et al.*, 2005). In 2004, the first-ever laparoscopic sterilisations were performed on two free-ranging elephant cows at Phinda Game Reserve, South Africa (Delsink, 2006). The elephants were positioned in lateral recumbency for hand-assisted laparoscopic surgery (Stetter *et al.*, 2005). Once the surgery was complete on one side, the animals were rolled to the other side for the same procedure (Stetter *et al.*, 2005). This was the first reported abdominal surgery in free-ranging African elephants and has been considered a significant milestone. Whilst the surgery was successful, it was lengthy and the first female only returned to her herd two days after surgery (H. Genis, pers. comm. 2004). It was noted through post-operative monitoring that the surgical sites healed without complication and the treated cows showed no adverse social or behavioural issues after the sterilisation (Stetter *et al.*, 2005).

Surgical sterilisation of elephant bulls

In February 2005 Dr Mark Stetter and his team attempted the first laparoscopic vasectomies on two bulls in the Mabalingwe Game Reserve. To facilitate the procedure the bulls were suspended by straps from a crane (figure 11). In the first bull only one side could be completed, while the second bull died during the procedure. Another four bulls were operated on using the same technique in the GMPGR (Delsink, 2006). The procedure was altogether unsuccessful in one bull while in the other three the vasa deferentia could only be identified and ligated on one side. The duration of the whole procedure was approximately 5 hours per bull.

Figure 11: Suspension of elephant bull for vasectomy. Disney World Public Affairs (http://www.wdwpublicaffairs.com/PhotoAlbum)

In 2006, the team returned and successfully performed bilateral vasectomies on four of four bulls at Welgevonden Game Reserve. Procedure time was 3–4 hours per bull. In 2007, five more bulls were successfully vasectomised at Songimvelo, Mpumalanga Parks Board. The full procedure time varied from 2.5 to 3 hours. Greater efficiency was due to overcoming anatomical obstacles related to patient size, modifications in equipment, and the advancement of surgical techniques (Stetter *et al.*, 2006).

Possible applications, disadvantages and costs of bull vasectomy

Unlike castrations, vasectomies do not remove the testes and thus the treated animals should enter into musth, breed and maintain their social status (Stetter *et al.*, 2007). Recent improvements with this technique have greatly reduced the anaesthetic and surgery times and have paved the way for several animals to have surgery on a given day (Stetter, pers. comm. 2007). The potential to sterilise 10-40 breeding bulls in a small to medium-sized elephant population would provide for a significant reduction in birth rates at approximately 22 months. A further argument is that males need only be sterilised once, whereas using methods such as immunocontraception, females currently need to be treated three times in the first year and then annually, to prevent births (Bokhout *et al.*, 2005).

The limitation of vasectomy as a method for population control is that it is only suitable for smaller populations. This is dictated by cost, the invasiveness of the procedure, and the fact that it is, for all practical purposes, irreversible in elephants. The costs are summarised in table 6 (Bokhout *et al.*, 2005). In our view, the costs appear to be underestimated as they do not take into account equipment such as the crane and fieldwork to determine dominance status and locate bull areas. The average cost according Bokhout *et al.* (2005) is US$ 2 300 per bull (equivalent to ± ZAR16 100). Cows mate with sexually mature bulls during the middle of their oestrous period although they may mate with younger bulls during early and late oestrus (Moss & Poole, 1983; Poole, 1996a). This means that a high percentage of bulls would need to be treated to ensure that the females are not bred (Garrott *et al.*, 1992). Elephant populations are complex and bulls continually separate from their natal herds. These will also be potential breeders and especially with the increased frequency of heats in the population, young bulls may be afforded the chance to breed. Another disadvantage is that targeting the dominant bulls in the population will affect the natural genetic selection.

It is probable that similar levels of contraception can be achieved using a GnRH vaccine (see *The future of elephant contraception; GnRH vaccine*), which is reversible and cheaper to use.

Procedure	Cost per elephant (US$)
Capture and anaesthesia[a]	1 000
Equipment costs[b]	300
Surgery team	1 000
Total	2 300

[a] (Hofmeyr, 2003)

[b] Endoscopic instruments are calculated at US$30 000–60 000. If complete depreciation is assumed after 200 bull operations, cost per elephant = US$150–300.

Table 6: Estimated costs of laparoscopic bull vasectomy (Bokhout *et al.*, 2005)

The future of elephant contraception

Recently two papers were published that review or partly question the use of immunocontraception as a means of controlling population growth in African elephants. The first, by Perdock *et al.* (2007), is a fairly comprehensive review of the topic but with the shortcoming that the most recently quoted paper referring to elephants is from 2003. The other is by Kerley & Shrader (2007), with the rather sensational title 'Elephant contraception: silver bullet or a potentially bitter pill'. Both papers quite correctly list side effects on behaviour resulting from an increased incidence of oestrus, increased presence of bulls and a lack of calves within a breeding herd, with its possible ramifications. While short-term studies have revealed no detectable behavioural changes (Delsink *et al.*, 2004b; 2006; 2007b) in populations that have been treated with pZP vaccines, extensive studies must be carried out to investigate possible long-term effects in this species, with its highly complex social structure. Once again, the same applies to any other form of population control. Kerley & Shrader (2007) state that our understanding of contraception is now at the stage that culling was at 30 years ago. This is only partly true. First, contraceptive trials began more than 10 years ago. Second, very little work has been carried out to study short, medium- or long-term effects of culling on behaviour of remaining elephants. The indications are that they may be considerable. Some other points made by the same authors with regard to behaviour are highly speculative. These are the loss of allomothering, increased stress levels and depression caused by the presence of fewer calves, and kidnapping of calves.

Elephants are not the first gregarious species to be exposed to contraception. The best example is probably the human, where contraception has been practised for hundreds of years, particularly stringently in so-called developed nations during the last 30 or so years. Another example is the domestic dog, which is an out-and-out pack animal. Have we been concerned about the effects of contraception in these two species? The answer is not really, despite the fact that there certainly are behavioural effects. Perhaps the most controversial point made by Kerley & Shrader is the possibility of injuries to cows as a result of increased mating attempts. There are no reports in the literature of injury to African or Asian elephant cows as a result of mounting. If one observes a bull in the process of mounting the stance of the bull on his back legs is very steep, meaning that he takes most of his weight on his hind legs placing very little on the cow. Elephants are quite nimble and their ability to balance on their hind legs only is plain to see when they reach for something high up in a tree requiring this stance. Besides, in domestic species like cattle and horses, where the weight difference between male and female can be considerable, service injuries are very rare despite the fact that these species are much less nimble.

The use of a GnRH vaccine, which has distinct possibilities, will of course cause anoestrus. Other points made by Perdock *et al.* (2007) are as follows:

- **The possibility of introducing new diseases with the pZP vaccine**. The vaccine is prepared from the ovaries of healthy pigs at abattoirs that are subjected to meat inspection. The manufacture of the vaccine involves washing of the ovarian material with large volumes of buffer fluid – 1000-fold larger than volumes used to free oocytes or embryos of virus particles. The zona ghosts are finally subjected to heat (65°C for 30 minutes) and aliquots are cultured for the presence of bacteria. All aliquots tested for use have been sterile. If a few viral particles were to survive the whole process they would not constitute an infective dose, and the viruses that may be found are specific pig viruses. Repeated injections of the same virus would have to be undertaken to possibly adapt a virus to a new host. pZP vaccine has been used on more than 80 different species all over the world, in many cases on captive populations, without the appearance of a new disease. In any case, elephants could be exposed to similar diseases in the wild where they come into contact with warthogs and bush pigs, both of which may carry the same diseases as domestic pigs.
- **Selection of cows that are immunocompromised as breeders, as they do not respond to the vaccine**. As with most free-ranging African mammals, natural selection of elephants that are resistant to or develop immunities to a range is rigorous. Immunocompromised animals will not survive under African conditions.
- **Ovarian damage may result, meaning that contraceptive effects could be permanent**. As the authors mention, short-term studies have demonstrated reversibility of pZP immunocontraception (Fayrer-Hosken *et al.*, 2000). Medium- to longer-term studies are under way to test reversibility after five years of vaccination and we expect that time taken to reversal will be approximately equal to the number of years a cow has been subjected to vaccination. We do, however, already have evidence that ovarian function in terms of the occurrence of normal oestrous cycles appears to be normal, with 58 oestruses observed in 56 cows that are seen intermittently (once to twice a week) (Bertschinger *et al.*, submitted). The time taken to reversal may, however, be a considerable advantage for the implementation of immunocontraception. It could mean that the interval between boosters can be lengthened to two or even three years after the first four or so years of annual vaccination. This would improve practicality and reduce cost.

The cost of contraception (at approximately ZAR1 000 per cow) and vaccination is considerably less than indicated in the papers above. A number of the points raised in the two papers and from other quarters presume sustained use of the vaccine on 100 per cent of the population. This does not have to be the case and will certainly depend on management plan.

One-shot vaccine: formulations

One-shot vaccines are vaccine formulations that with a single administration would provide sufficient stimulus to the immune system to render the animal infertile for a year or more without any boosters. Such formulations either provide a slow continuous release of vaccine and adjuvant over time (e.g. liposomal system) or release at intervals (e.g. lactide-glycolide copolymer pellets, Turner *et al.*, 2002). The major advantages of one-shot vaccines are that they would be more practical and cheaper to administer, and provide less disturbance to the population being contracepted. The improved practicality would mean that much larger populations could be tackled with greater average efficacy. The lactide-glycolide copolymer pellets have been tested extensively in horses. The one-year formulation reduced fertility rates to 10.7 per cent in 266 hand-injected mares and to 25 per cent in 114 dart-injected mares. The fertility rate after the two-year formulation varied from 5.2 to 31.6 per cent in a total of 96 mares (Turner *et al.*, 2007). It should be remembered that annual boosters following the one-shot vaccine will further increase contraceptive efficacy.

One-shot vaccine: trial in captive elephants

A trial was performed on three captive elephant cows using the traditional vaccine (fluid) for primary vaccination and three different formulations of lactide-glycolide pellets that release after 1, 3 and 12 months respectively. The cows were hand-injected at two different sites – one for the fluid vaccine and one for the pellets. They were bled two months later (one month post-release of the one-month pellets) for antibody titre determination and the results compared to six cows one month after their first traditional booster vaccination (table 7). The titres of all three one-shot-vaccinated cows were higher than the cows treated with the traditional method (Van Rossum, 2006; Turner *et al.*, 2007). The trial is ongoing.

One-shot vaccine: trial in free-ranging elephants

This trial began in May 2007, when four cows were vaccinated from the helicopter. They will be captured at 3–6 month intervals during 2007–2008 to assess antibody titres and contraceptive effect (Bertschinger, pers. comm.).

Vaccine and cow	Anti-pZP antibody titre at 1:270 dilution (absorbency)
Traditional vaccine with boosters	
Cow 1	0.246
Cow 2	0.918
Cow 3	0.915
Cow 4	0.969
Cow 5	0.970
Cow 6	0.354
One-shot pellet vaccine	
Setombi	1.138
Bubi	1.431
Nandi	1.058

Table 7: Antibody titres of elephant cows vaccinated with either the traditional method or the one-shot pellet vaccine

Possible future developments

The one-shot pellet vaccine needs to be tested more extensively on free-ranging elephants to determine efficacy, duration of contraceptive effect and reversibility. An additional change to the lactide-glycolide copolymer pellets will extend the effect of the one-shot vaccine from two to three years after administration of a single dart (Turner *et al.*, 2008). Melodie Bates (MSc student 2006–2008, Faculty of Veterinary Science, University of Pretoria) is conducting a project in Thornybush Private Reserve to more objectively assess oestrous cycles and stress response of contracepted cows. She will monitor faecal progestins (cycle) and glucocorticoids (stress) to achieve this. Indirectly, if one can show that contracepted cows have ovarian cyclic activity, it will show that they have normal ovarian function. Evidence of this in at least four reserves is available through less reliable behavioural observations.

GnRH vaccine

Experience in elephant bulls

In 2003 a trial was initiated to test the efficacy of a GnRH vaccine (GnRH-tandem-dimer-ovalbumin conjugate, Pepscan Systems, Lelystad, The Netherlands; Oonk *et al.*, 1998) to control aggressive behaviour and musth in captive and free-ranging bulls (DeNys, 2005). Initially five captive bulls were vaccinated. Behaviour and faecal epiandrosterone were monitored in all bulls before the primary vaccination (Stage 1), after the primary, first and second booster vaccinations (Stages 2, 3 and 4) and two and four months after the second booster (Stages 6 and 7). Prior to vaccination two bulls were aggressive while the three others were not. During Stage 1 the behaviour of aggressive and non-aggressive bulls corresponded with faecal epiandrosterone concentrations of the two groups (figure 12). The vaccine produced encouraging results, with the two aggressive bulls showing a behavioural improvement and all bulls remaining non-aggressive during the remainder of the six-month observation period. The effect of the vaccine on one of the aggressive bulls (Thembo) is shown in figure 13. This bull is now 24 years old, vaccinated at six-monthly intervals, is non-aggressive and has yet to come into musth (Bertschinger *et al.*, 2004c).

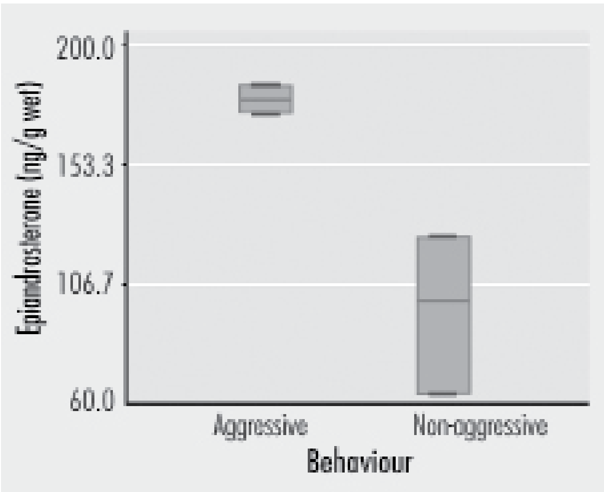

Figure 12: Epiandrosterone levels of non-musth aggressive and non-aggressive bulls during Stage 1 (DeNys, 2005). Two-sample t-test showed a significant difference between the two groups (t = 3.483, dF = 3, p = 0.04)

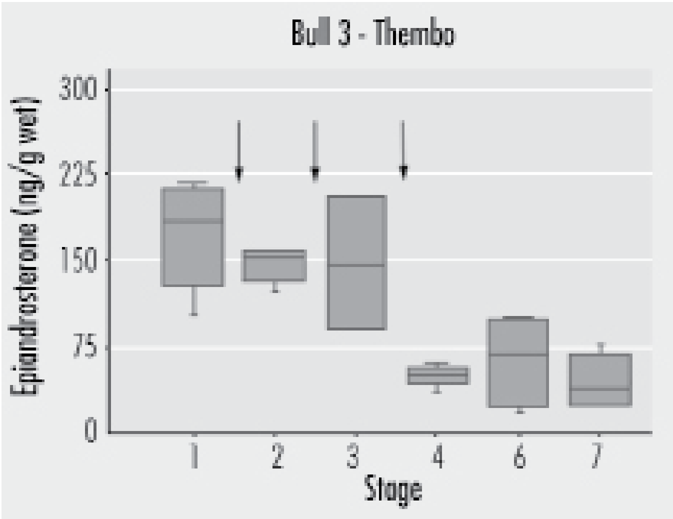

Figure 13: Faecal epiandrosterone concentrations of bull Thembo before (Stage 1) and after the primary, first and second booster (Stages 2, 3 and 4) GnRH vaccinations (arrows), and two and four months after the second booster (Stages 6 and 7, respectively) (DeNys, 2005). Stages 4-7 differed significantly from Stage 1 (AVOVA; dF = 5, F = 11.029, $p < 0.001$)

There are now 15 captive bulls varying from 10 to 23 years of age on the GnRH vaccination regime. The suppressive effect of the vaccine on behaviour and faecal epiandrosterone concentrations lasts 6-9 months. Furthermore, three free-ranging bulls in musth were vaccinated and all three went out of musth a week to 10 days after the first booster vaccination (Bertschinger, unpublished data).

Furthermore, the vaccine has been able to postpone musth and subdue aggressive behaviour in one adult Asian and one adult African elephant in Bowman Zoo (Canada) (Bertschinger & Korver, unpublished data). The African elephant died later unrelated to the vaccination, and on post mortem had small testes. This may well indicate that the vaccine not only suppressed testosterone production but also spermatogenesis. GnRH vaccines are known to be effective in down-regulating spermatogenesis and thus decreasing testicular size in a number of species such as cattle (Hoskinson *et al.*, 1990), pigs (Dunshea *et al.*, 2001; Zeng *et al.*, 2001; Killian *et al.*, 2006), stallions (Dowsett *et al.*, 1996; Turkstra *et al.*, 2005; Burger *et al.*, 2006), rams (Janett *et al.*, 2003) and bison bulls (Miller *et al.*, 2004). Testis size is largely determined by the diameter of individual seminiferous tubules and a decrease in spermatogenesis reduces the diameter of each tubule and thus the size of the testis. In the Asian bull musth has been postponed by at least 15 months as a result of GnRH vaccination (figure 14).

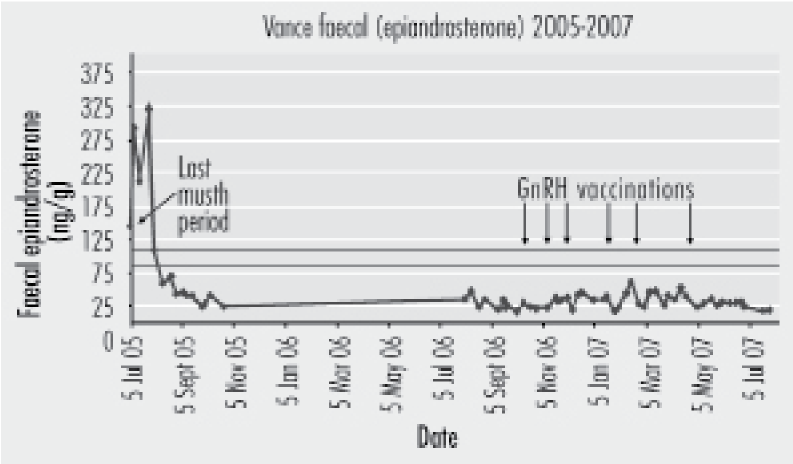

Figure 14: Faecal epiandrosterone concentrations (ng.g^{-1}) of a 35-year-old Asian elephant bull before and after repeated GnRH vaccinations (arrows) (Bertschinger & Korver, unpublished data)

Possible application in elephant cows

One of the criticisms of GnRH vaccines is that they cause anoestrus (lack of cyclic activity), and in herd animals this could be regarded as an undesirable effect. From a behavioural point of view, anoestrus in horses may interfere with interactions between mares and the herd stallion and affect herd integrity. On the other hand, mares are seasonal breeders, cycling from spring to autumn with a gestation period of 11 months. Ten to 14 days post-foaling (foal heat) they often reconceive, body condition allowing. This means that normally they will cycle only once a year. African elephant cows commence cycling about two years after calving and probably fall pregnant during the first post-calving oestrus (Brown *et al.*, 2004). So which is better; anoestrus with GnRH vaccine or continuous cycling following pZP immunocontraception?

Only four elephant cows have been treated with GnRH vaccine, and as yet it is too early for conclusive results. In domestic mares the vaccine has been applied by various people, mostly with success (Garza *et al.*, 1986; Dalin *et al.*, 2002; Imboden *et al.*, 2004; Elhay *et al.*, 2007). A trial with probably the largest single group of mares to be treated with a GnRH vaccine was recently carried out in South Africa (Botha *et al.*, 2007). Fifty-five mares were vaccinated twice at an interval of five weeks. Following the primary and booster vaccinations 85 per cent and 100 per cent of mares entered anoestrus respectively. Given the results in mares, the vaccine should be tested more extensively in free-ranging elephants. Non-invasive cycle monitoring using faecal progestogens, which will allow early establishment of vaccine efficacy, is possible (Wittemyer *et al.*, 2007).

Proximate and ultimate effects of contraception

Modelling effects on population

Contraception rate

The contraception rate affects the growth of elephant populations and consequently the density of elephants on the landscape. The key elements of the modelling, using the Addo Elephant National Park (Addo) as an example, reveal that as the contraception rate is increased the population growth rate declines such that under a 100 per cent contraception regime the resultant declining growth rate will result in population extinction in the medium term (50–100 years) (Castley *et al.*, 2007). This assumes that the contraceptive treatment is 100 per cent effective in preventing pregnancy. A decline in the number of individuals in populations is expected only at contraception rates above 77 per cent of all breeding females in the population. Despite implementation of such contraceptive regimes the elephant density will remain above recommended densities in the short to medium term (table 8). This is park dependent, but is certainly the case in Addo, and similar responses may be found for other confined populations that have high growth rates (Slotow *et al.*, 2005). Increasing levels of contraceptive treatment in the population will also result in an overall aging effect on the population, with a higher number of individuals being represented in older age classes over time (Mackey *et al.*, 2006). This is likely to have social, behavioural and possibly ecological implications (Kerley & Shrader 2007).

Effects on sex ratio, age structure

The effects of contraceptive treatments on population demographics need to be carefully considered before implementing any contraception strategy as a means to control population growth. It may be necessary to highlight that the objectives to (a) control elephant population growth, and (b) maintain healthy viable elephant populations may be mutually exclusive but certain measures can be implemented to adopt an adaptive management approach that considers both of these objectives.

Scenario	Growth rate (%)	Population size after 15 years (2020)	Elephant density (recommended max. is 0.5 km^{-2})
Control	5.07	766	3.11
25%	3.78	643	2.58
50%	2.45	529	2.12
60%	1.84	484	1.94
75%	0.82	416	1.67
80%	0.44	393	1.67
100%	–1.29	303	1.22

Table 8: Elephant population growth rates for the Addo over a 15-year period to illustrate the possible effects of variable rates of continuous contraceptive treatments (assumes 100 per cent contraceptive efficacy). Starting population size was 354 elephants in 2004 in all cases. The size of the elephant camp in 2020 is expected to be 249 km^2 after further consolidation (Castley *et al.*, 2007)

As stated previously, the elevation in contraception rates results in an aging population. Furthermore, this also results in the population slowly becoming dominated by females, given that the mortality rates for males (using the Addo population as our model) are higher than for females. Although the trends observed in the Addo population may not be transferable to other elephant populations in general, the model can be applied to those cases where demographic parameters are available to determine specific population responses in these situations. Notwithstanding any lack of generalisation of the detected trends in Addo, the model is still able to highlight potential areas of concern for protected area managers in relation to elephant management requirements. The model does not consider the possible impacts of changing herd dynamics on future mortality, as the design is too simple to cater for dynamic variability in population parameters.

Nonetheless under all contraceptive scenarios the populations become skewed towards females as well as older individuals. At higher rates of contraception the proportional representation of individuals within certain age classes is significantly different (Chi-square tests) to that expected from growth within a population that is not subjected to contraception (i.e. a control model).

Scenario	Growth rate	Density @50 yr	Mean density	Age class (mean % over 50 years) – ♂/♀											
				0 ♀	0 ♂	1–9	1–9	10–19	10–19	20–29	20–29	30–44	30–44	45–59	45–50
Control	5.14	17.01	6.36	3.27	3.13	21.73	20.55	13.99	12.89	7.87	5.98	5.79	2.90	2.05	0.03
Contiguous															
25	3.84	9.13	4.27	2.73	2.73	18.73	18.73	14.25	12.31	9.12	6.31	7.47	3.44	3.52	0
50	2.49	4.75	2.94	2.17	2.17	15.81	15.81	13.87	11.93	10.22	7.04	9.49	4.37	6.59	0
75	0.65	1.91	1.9	1.35	1.35	11.29	11.29	12.25	10.53	10.98	7.53	11.75	5.39	15.92	0
77	0.46	1.74	1.83	1.25	1.25	10.63	10.63	11.93	10.25	10.99	7.54	11.98	5.50	17.72	0
100	–3.04	0.3	1.13	0	0	0	0	0	0	0	0	0	0	100	0
First calf															
25	3.97	9.71	4.44	2.84	2.84	18.98	19.98	14.25	12.32	9.02	6.24	7.29	3.36	3.32	0
50	2.78	5.56	3.17	2.30	2.30	16.43	16.43	13.98	12.05	10	6.89	9.15	4.20	5.77	0
75	1.12	2.41	2.13	1.53	1.53	12.28	12.28	12.62	10.86	10.77	7.40	12.10	5.50	12.72	0
77	0.95	2.22	2.05	0.68	0.68	7.82	7.82	11.54	9.88	12.50	8.56	15.98	7.25	17.01	0
100	–2.15	0.47	1.3	0.03	0.03	0.56	0.56	1.28	1.08	3.94	2.69	18.10	7.80	63.90	0
90% Efficacy															
25	3.96	9.66	4.43	2.83	2.83	18.97	18.97	14.26	12.32	9.02	6.24	7.30	3.36	3.34	0
50	2.79	5.49	3.18	2.31	2.31	16.49	16.49	14.00	12.08	10	6.89	9.04	4.16	5.73	0
75	1.27	2.6	2.13	1.61	1.61	12.83	12.83	12.97	11.16	10.91	7.49	11.21	5.15	11.81	0
77	1.12	2.42	2.11	1.55	1.55	12.45	12.45	12.81	11.03	10.95	7.52	11.38	5.23	12.68	0
90	0.02	1.4	1.69	1.07	1.07	9.50	9.50	11.26	9.67	10.84	7.42	12.13	5.57	21.65	0
100	–1.11	0.79	1.41	0.62	0.62	6.29	6.29	8.70	7.46	9.39	6.42	11.19	5.13	37.67	0

Scenario	Growth rate	Density @50 yr	Mean density	Age class (mean % over 50 years) – ♂/♀											
				0 ♀	0 ♂	1–9	1–9	10–19	10–19	20–29	20–29	30–44	30–44	45–59	45–50
Stepwise															
100/80/77	–0.54	1.06	1.52	0	0	7.70	7.70	10.60	9.62	10.92	7.61	12.27	5.75	28.03	0
100/80/60	0.09	1.45	1.68	0	0	9.46	9.46	12.31	10.79	11.86	8.24	11.79	5.52	20.41	0
100/77/60	0.19	1.53	1.72	0	0	9.80	9.80	12.43	10.89	11.82	8.22	11.91	5.58	19.38	0
100/75/50	0.58	1.85	1.84	0	0	10.90	10.90	13.17	11.55	11.98	8.34	11.56	5.41	16.01	0
100/72/60	0.36	1.66	1.78	0	0	10.33	10.33	12.61	11.04	11.74	8.18	12.07	5.66	17.83	0
100/75	–0.17	1.27	1.67	1.46	1.46	4.85	4.85	11.38	9.83	10.26	6.87	10.54	4.86	33.47	0
100/50	1.02	2.3	2.08	2.58	2.58	7.28	7.28	14.53	12.59	11.26	7.55	11.18	5.15	17.76	0
Staggered															
100/75–25 (repeat)	0.4	1.7	1.83	2.53	2.53	3.74	3.74	19.44	17.03	10.14	6.52	11.31	5.47	17.45	0

Table 9: Modelled population response (growth rate, density, age/sex structure) by an African elephant population in the Addo Elephant National Park to a range of contraceptive scenarios

Furthermore, under a 100 per cent contraceptive scenario there is sequential extinction of all age classes for both male and female cohorts, such that after 50 years the entire population comprises females aged 45–59 (table 9). The sex- and age-specific responses under a 100% contraception scenario are indicative of the possible implications of adopting such a management approach. Although managers may not have the local extinction of a population as an objective, the use of a 100 per cent contraceptive regime may be argued to maximise the reduction in population growth. It is clear from the model developed at Addo that this should not be considered without a complete appreciation for the possible consequences of such action.

At this stage Castley's model has not looked specifically at the breeding unit structure and impacts on the herd dynamics. Again, it would be feasible in Addo (and potentially the Makalali population, and other areas where the entire population history is known) to determine the possible effects of contracepting certain aged females and only certain percentages within family groups

Adaptive management of elephants through modelling

The scenarios that have been discussed above present data based on standard contraceptive regimes that are simple in that they are continuous regimens of a single treatment option. Given that the need to reduce population growth and density would result in significant changes to population structure, Castley *et al.* (2007) investigated the possibility of achieving the dual objectives as stated previously by manipulating the frequency and amount of contraceptive treatments administered. Their model is able to incorporate any number of variable contraceptive scenarios, but the ones tested were based around potential to administer a single-dose, long-lasting vaccine (three years), and hence adopted a three-year cycle in the various models. The various stepwise and staggered models produce a diversity of management options to choose from in order to achieve the desired objectives, and the best model would need to be considered for the circumstances of a specific park.

Despite some models producing similar growth rates, the resultant changes to the population structure were quite variable. The model is also rather deterministic in the sense that it follows a standard series of cycles and does not therefore build any stochasticity into the modelling scenarios. However, it still highlights that it may be possible to manipulate elephant populations through an adaptive experimental approach to see how populations respond while maximising reductions in population growth at the same time. As with any management alternatives, though, there are some trade-offs to be made. Building variability into the model, such that the population maintains a healthy structure, reveals that it may not be possible to simultaneously reduce the growth rate sufficiently over the long term. As a result it may be necessary to combine long-term contraception strategies with more intensive population reduction strategies such as culling. The benefit of the modelling reveals, however, that contraception may be effective in extending the period between consecutive culls.

Genetic diversity

Monitoring and research on the effect of contraception on the genetic diversity of contracepted elephant populations is required.

Habitat biodiversity effects and integrated management options

The Addo model, as well as common sense, tells us that contraception cannot have an immediate effect on a population. Assuming a 100 per cent efficacy, which is probably achievable in populations of up to 1 000 elephants, no more births will occur three years after implementation of a contraceptive programme. Population decline is then dependent on mortality rate, which in turn is dependent on age structure of the population and environmental factors like rainfall and disease. Contraception should be seen as a tool that can be used to prevent rather than cure overpopulation problems with elephants. On the other hand, where an overabundance of elephants is already present, whether perceived or real, contraception can significantly curb continued population growth rate. This is clearly visible in table 9 above, where using the Addo model even a 60 per cent efficacy restricts the total population to 484 instead of 776 over 15 years, having started with 354 elephants. By the same token, contraception will not prevent an already existing problem of habitat modification. Used as a preventive measure, it will restrict population growth and so also habitat impact. Used where an overabundance is already present, it will reduce further modification by an ever-growing population. Contraception can be combined with any of the three other management options – culling, translocation and creation of additional habitat, for example, the creation of transfrontier parks. One of the effects of each of these three management options is an increased reproductive rate as a result of density decrease (data or modelling needed). An effective way to combat the response is the use of contraception.

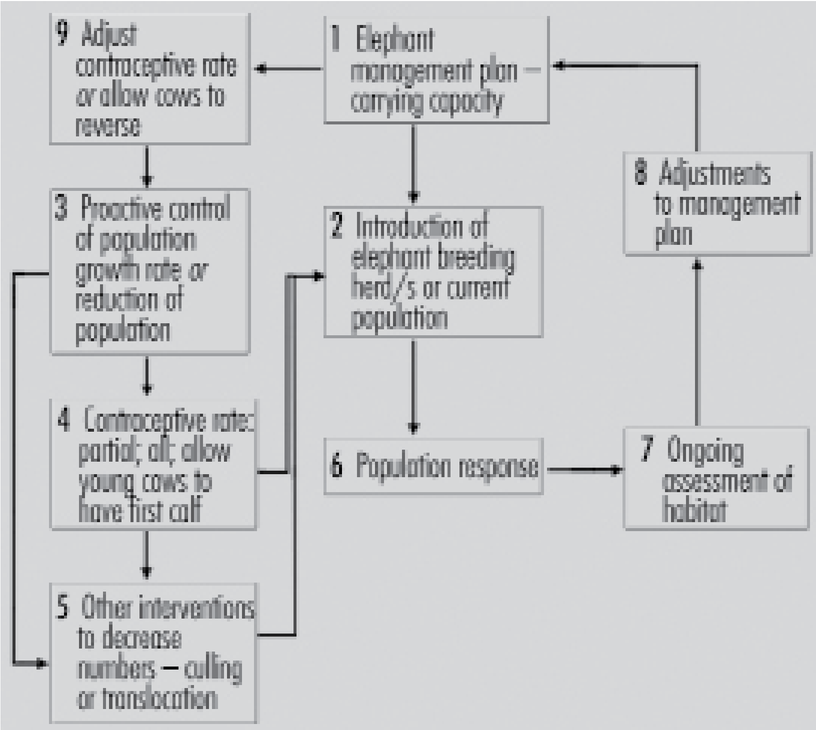

Figure 15: Adaptive management plan for the implementation and ongoing use of contraception using pZP vaccine for population control of elephants

Guidelines for implementation of a contraception programme

- Figure 15 suggests an adaptive management plan required for the implementation and ongoing use of pZP vaccine for elephant population control.
- Step 1 is central to the programme and is the elephant management plan for the reserve. It determines the approximate carrying capacity of the reserve. Step 1 also dictates the level of intervention necessary in the opinion of the managers.
- In Step 2 elephants are either introduced, or the reserve already has elephants that need managing, or more elephants are added
- In Step 3 the contraceptive rate is determined, including decisions with regard to individual age groups such as young cows. Details of implementation will be determined by population size and habitat conditions.

- Step 4 is an additional step that may be required to reduce elephant population according to the requirements of the elephant management plan, bearing in mind the conditions laid down in the National Norms and Standards for Elephants in South Africa
- The population response is monitored (Step 5) bearing in mind that contracepted cows may continue to calve for up to three years following the primary vaccination.
- Parallel to Step 5 there will be an ongoing monitoring of the habitat (Step 6).
- In Step 7, according to the population response and habitat condition, adjustments to the management plan are made.
- In Step 8 the contraceptive rate is adjusted to suit reserve needs. Examples are: increasing the contraceptive rate as a result of habitat deterioration; allowing individual cows to reverse; adding young cows that have calved for the first time to the contraception programme.

Key research issues and gaps in the knowledge

pZP vaccine

- Vaccine production
  - improve production and safety of native vaccine
  - develop glycosylated synthetic vaccine.
- In vitro tests
  - develop in vitro tests which will assist in getting quicker answers than the live elephant model.
- Reversibility
  - medium- to long-term reversibility with return to fertility
  - ovarian function assessed by faecal steroid assays
  - ovarian histology
  - T-cell response in immunised cows.
- Development and testing of one-shot/delayed release formulations in elephants. Test responses with antibody titres and contraceptive efficacy.

GnRH vaccine

- Test vaccine in population of cows – consider comparative trial with pZP.
- Long-acting formulations.

- Reversibility.
- Vaccinate bulls to test affects on spermatogenesis.
- Vaccinate adult bulls to investigate changes in chemical signalling.

Behavioural effects

- Measure stress by means of faecal steroids and behavioural changes in response to
  - administration
  - repeated oestrous cycles
  - presence of bulls
  - lack of calves.
- Behavioural effects of
  - cycling/anoestrus and presence of bulls
  - lack of calves on various aspects of family unit behaviour, integrity and movement.
- Need to apply fertility control to some large test populations to test the effects on population dynamics/behaviour.

Gaps in of knowledge of elephant reproduction and related behaviour

- Female reproductive physiology – what happens to infertile cows? How many cows cycle in a herd? How soon do they cycle after calving? How long does lactation anoestrus last and how can chemical signals be used to control movements and mate selection and many more?
- Male reproductive physiology – what are the triggers for musth? Why does a bull go out of musth? Can chemical signals be used to control their reproduction and movements? Are all adult bulls in fact potentially fertile?

References

Archie, E.A., C.J. Moss & S.C. Alberts 2006. The ties that bind: Genetic relatedness predicts the fission and fusion of social groups in wild African elephants. *Proceedings of the Royal Society B: Biological Sciences* 273, 513–522.

Asa, C.S. 2005. A primer of reproductive processes: potential target tissues or processes for contraceptive intervention. In: C.S. Asa & I.J. Porton (eds) *Wildlife contraception.* The John Hopkins University Press, Baltimore, 30–52.

Bagley, K.R., T.E. Goodwin, L.E.L. Rasmussen & B.A. Schulte 2006. Male African elephants, *Loxodonta africana*, can distinguish oestrous status via urinary signals. *Animal Behaviour* 71, 1439–1445.

Bartlett, E. 1997. Jumbo birth control drives bull elephants wild. *New Scientist* 154, 5.

Becker, S.E., W.J. Enright & L.S. Katz 1999. Active immunization against gonadotropin-releasing hormone in female white-tailed deer. *Zoo Biology* 18, 385–396.

Bengis, R. 1993. Care of the African elephant *Loxodonta africana* in captivity. In: A.A. McKenzie (ed.) *The capture and care manual*. Wildlife Decision Support Services cc and The South African Veterinary Foundation, Lynnwood Ridge and Menlo Park, 506–511.

Bertschinger, H.J., C.S. Asa, P.P. Calle, J.A. Long, K. Bauman, K. Dematte, W. Jöchle & T.E. Trigg 2001. Control of reproduction and sex related behaviour in exotic carnivores with the GnRH analogue deslorelin: preliminary observations. *Journal of Reproduction and Fertility, Supplement* 57, 275–283.

Bertschinger, H.J., T.E. Trigg, W. Jöchle & A. Human 2002. Induction of contraception in some African wild carnivores by down-regulation of LH and FSH secretion using the GnRH analogue deslorelin. *Reproduction, Supplement* 60, 41–52.

Bertschinger, H.J., J.F. Kirkpatrick, R.A. Fayrer-Hosken, D. Grobler & J.J. van Altena 2004a. Immunocontraception of African elephants using porcine zona pellucida vaccine. In: B. Colenbrander, J. de Gooijer, R. Paling, S. Stout, T. Stout & T. Allen (eds) *Proceedings of an Expert Consultation on the Control of Wild Elephant Populations*, Utrecht University, 45–47.

Bertschinger, H.J., L.J. Venter & A. Human 2004b. Treatment of aggressive behaviour and contraception of some primate species and red pandas using deslorelin implants. *Advances in Eyhology* 38, 24.

Bertschinger, H.J., A.K. Delsink, J.F. Kirkpatrick, D. Grobler, J.J. van Altena, A. Human, B. Colenbrander & J. Turkstra 2004c. The use of pZP and GnRH vaccines for contraception and control of behaviour in African elephants. In: B. Colenbrander, J. de Gooijer, R. Paling, S. Stout, T. Stout & T. Allen (eds) *Proceedings of an expert consultation on the control of wild elephant populations*, Utrecht University, 69–72.

Bertschinger H.J., M. Jago, J.O. Nöthling & A. Human 2006. Repeated use of the GnRH analogue deslorelin to down-regulate reproduction in male cheetahs (*Acinonyx jubatus*). *Theriogenology* 66, 1762–1767.

Bertschinger, H.J., A. Delsink, J.J. van Altena, D. Grobler, J.F. Kirkpatrick, M. Bates & T. Burke 2007. Effects of porcine zona pellucida immunocontraception on annual calving percentages of six discrete African Elephant populations. 6th International Conference on Fertility Control for Wildlife. York, UK, 3–5 September, 48.

Bertschinger, H.J., M.A. de Barros Vaz Guimarães, T.E. Trigg & A. Human 2008. The use of deslorelin implants for the long-term contraception of lionesses and tigers *Wildlife Research,* in press.

Blanchard, T.L. 1984. Some effects of anabolic steroids – especially on stallions. *The Compendium* 7, 1–8.

Bockhout, B., M. Nabuurs & M. de Jong 2005. Vasectomy of older bulls to manage elephant overpopulation in Africa. *Pachyderm* 39, 97–103.

Botha, A.E., M.L. Schulman, H.J. Bertschinger, A.J. Guthrie, C.H. Annandale & S.B. Hughes 2008. The use of a GnRH vaccine to suppress mare ovarian activity in a large group of mares under field conditions. *Wildlife Research*, in press.

Brown, J.L., S.L. Walker & T. Moeller 2004. Comparative endocrinology of cycling and non-cycling Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants. *General and Comparative Endocrinology* 136, 360–370.

Brown, J.L., M. Somerville, H.S. Riddle, M. Keele, C.K. Duer & W. Freeman 2007. Comparative endocrinology of testicular, andrenal and thyroid function in captive Asian and African elephant bulls. *General and Comparative Endocrinology* 151, 153–162.

Burger, D., F. Janett, M. Vidament, R. Stump, G. Fortier, I. Imboden & R. Thun 2006. Immunocastration against GnRH in adult stallions: effects on semen characteristics, behaviour and shedding of equine arteritis virus. *Animal Reproduction Science* 94, 107–111.

Butler, B. 1998. Elephants: trimming the herd. *BioScience* 48(2), 76–81.

Castley, J.G., G.I.H. Kerley & M.H. Knight 2007 (in prep.). Elephant population dynamics at different contraception levels using the Addo Elephant National Park as a model.

Cooper, K.A., J.D. Harder, D.H. Clawson, D.L. Fredrick, G.A. Lodge, H.C. Peachey, T.J. Spellmire & D.P. Winstel 1990. Serum testosterone and musth in captive male African and Asian elephants. *Zoo Biology* 9, 297–306.

Curtis, P.D., R.L. Pooler, M.E. Richmond, L.A. Miller, G.F. Marrfeld & F.W. Quimby 2001. Comparative effects of GnRH and porcine zona pellucida (pZP) immunocontraceptive vaccines for controlling reproduction in white-tailed deer (*Odocoileus virginianus*). *Reproduction* 60, 131–141.

Dalin, A.M., Ø. Andresen & L. Malmgren 2002. Immunization against GnRH in mature mares: antibody titres, ovarian function, hormonal levels and oestrus behaviour. *Journal of Veterinary Medicine* 49, 125–131.

Deigert, F.A., A. Duncan, R. Lyda, K. Frank & J. F. Kirkpatrick 2003. Immunocontraception of captive exotic species. III. Fallow deer (*Cervus dama). Zoo Biology* 22, 261–268.

Delsink, A.K., J.J. van Altena, J.F. Kirkpatrick, D. Grobler & R. Fayrer-Hosken 2002. Field applications of immunocontraception in African Elephants (*Loxodonta africana*). *Reproduction* 60, 117–124.

Delsink, A., H.J. Bertschinger, J.F. Kirkpatrick, H. De Nys, D. Grobler & J.J. van Altena 2004a. Contraception of African elephant cows in two private conservancies using porcine zona pellucida vaccine, and the control of aggressive behaviour in elephant bulls with GnRH vaccine. In: B. Colenbrander, J. de Gooijer, R. Paling, S. Stout, T. Stout & T. Allen (eds) *Proceedings of an expert consultation on the control of wild elephant populations*, Utrecht University, 69–72.

Delsink, A.K., H.J. Bertschinger, J.F. Kirkpatrick, D. Grobler, J.J. van Altena & R. Slotow 2004b. The preliminary behavioural and population dynamic response of African elephants to immunocontraception. In: J.H.A. de Gooijer & R.W. Paling (eds) *Proceedings of the 15th Symposium on Tropical Animal Health and Reproduction: Management of Elephant Reproduction*, Faculty of Veterinary Medicine, University of Utrecht, The Netherlands, 19–22.

Delsink, A.K. 2006. The costs and consequences of immunocontraception implementation in African elephants at Makalali Conservancy, South Africa. M.Sc. thesis, University of Kwa-Zulu Natal, Durban.

Delsink, A.K., J.J. van Altena, D. Grobler, H. Bertschinger, J.F. Kirkpatrick & R. Slotow 2006. Regulation of a small, discrete African elephant population through immunocontraception in the Makalali Conservancy, Limpopo, South Africa. *South African Journal of Science* 102, 403–405.

Delsink, A.K., J.J. van Altena, D. Grobler, H. Bertschinger, J.F. Kirkpatrick & R. Slotow 2007a. Implementing immunocontraception in free-ranging African elephants at Makalali Conservancy. *Journal of the South African Veterinary Association* 78(1), 25–30.

Delsink, A.K., J.F. Kirkpatrick, J.J. van Altena, D. Grobler, H. Bertschinger & R. Slotow 2007b. Lack of social and behavioural consequences of immunocontraception in African elephants. 6th International Conference on Fertility Control for Wildlife. York, UK, 3–5 September, 31.

De Nys, H.M. 2005. Control of testosterone secretion, musth and aggressive behaviour in African elephant (*Loxodonta africana*) bulls using a GnRH vaccine. MSc thesis, University of Pretoria.

D'Occhio, M.J. 1993. Immunological suppression of reproductive functions in male and female mammals. *Animal Reproduction Science* 33, 345–372.

Douglas-Hamilton, I. & O. Douglas-Hamilton 1975. *Among the elephants*. Collins, London.

Dowsett, K.F., L.M. Knott, U. Tshewang, A.E. Jackson, D.A. Bodero & T.E. Trigg 1996. Suppression of testicular function using two dose rates of reversible water soluble gonadotropin-releasing hormone (GnRH) vaccine in colts. *Australian Veterinary Journal* 74, 228–235.

Dunbar B. S., V. Lee, S. Prasad, D. Schwanhn, E. Schwoebel, S. Skinner & B. Wilkins 1984. The mammalian zona pellucida: its biochemistry, immunochemistry, molecular biology, and developmental expression. *Reproduction, Fertility and Development* 6, 331–347.

Dunbar B.S., C. Lo & V. Stevens 1989. Effect of immunization with purified porcine zona pellucida proteins on ovarian function in baboons. *Fertility and Sterility* 52, 311–318.

Dunshea, F.R., C. Colantoni, K. Howard, I. Mc Cauley, P. Jackson, K.A. Long, S. Lopaticki, E.A. Nugent, J.A. Simons, J. Walker & D.P. Hennessy 2001. Vaccination of boars with GnRH vaccine (Improvac) eliminates boar taint and increases growth performance. *Journal of Animal Science* 79, 2525–2535.

Eagle, T.C., E.D. Plotka, R.A. Garrott, D.B. Siniff & J.R. Tester 1992. Efficacy of chemical contraception in feral mares. *Wildlife Society Bulletin* 20, 211–216.

Eggeling, H. 1901. Über die Schläfendrüse des Elephanten. Biol. *Centralblatt* 21, 443–453.

Eisenberg, J.F., G.M. McKay & M.R. Jainudeen 1971. Reproductive behaviour of the Asiatic elephant (*Elephas maximus*). *Behavior* 38, 193–225.

Elhay, M., A. Newbold, A. Britton, P. Turley, K. Dowsett & J. Walker 2007. Suppression of behavioural and physiological oestrus in the mare by vaccination against GnRH. *Australian Veterinary Journal* 85(1/2), 39–45.

Estes, R.D. 1991. *The behaviour guide to African mammals*. Russel Friedman Books CC, South Africa.

Fayrer-Hosken, R.A., P. Brooks, H.J. Bertschinger, J.F. Kirkpatrick, J.W. Turner & I.K.M. Liu 1997. Management of African elephant populations by immunocontraception. *Wildlife Society Bulletin* 25, 18–21.

Fayrer-Hosken, R.A., P. Brooks, H.J. Bertschinger, J.F. Kirkpatrick, D. Grobler, N. Lamberski, G. Honneyman & T. Ulrich 1999. Contraceptive potential of the porcine zona pellucida vaccine in the African elephant (*Loxodonta africana*). *Theriogenology* 52, 835–846.

Fayrer-Hosken, R. A., D. Grobler, J.J. Van Altena, J.F. Kirkpatrick & H. Bertschinger 2000. Immunocontraception of African elephants. *Nature* 407, 149.

Ferro, V.A., M.A.H. Khan, D. McAdam, A. Colston, E. Aughey, A.B. Mullen, M.M.Waterston & M.J.A. Harvey 2004. Efficacy of an anti-fertility vaccine based on mammalian gonadotrophic releasing hormone (GnRH-I) – a histological comparison in male animals. *Veterinary Immunology and Immunopathology* 101, 73–86.

Foerner, J.J., R.I. Houck & J.H. Olsen 1994. Surgical castration of the elephant (*Elephas maximus and Loxodonta africana*). *Journal of Zoo Wildlife Medicine* 25, 355.

Frank, K.M., R.O. Lyda & J.F. Kirkpatrick. 2005. Immunocontraception of captive exotic species. IV. Species differences in response to the porcine zona pellucida vaccine and the timing of booster inoculations. *Zoo Biology* 24, 349–358.

Freeman, E. 2005. Investigation of behavioural and socio-environmental factors associated with reproductive acyclicity in African elephants (*Loxodonta africana*). PhD thesis, Department of Environmental Science and Policy, George Mason University, Fairfax, VA.

Ganswindt, A., M. Heistermann, S. Borragan & J.K. Hodges 2002: Assessment of testicular endocrine function in captive African elephants by measurement of urinary and fecal androgens. *Zoo Biology* 21, 27–36.

Ganswindt, A., R. Palme, M. Heistermann, S. Borragan & J.K. Hodges 2003. Non-invasive assessment of adrenocortical function in male African elephant (*Loxodonta africana*) and its relation to musth. *General and Comparative Endocrinology* 134, 156–166.

Ganswindt, A., H.B. Rasmussen, M. Heistermann & J.K. Hodges 2005a. The sexually active states of free-ranging male African elephants (*Loxodonta africana*): defining musth and non-musth using endocrinology, physical signals, and behaviour. *Hormones and Behaviour* 47(1), 83–91.

Ganswindt, A., M. Heistermann & J.K. Hodges. 2005b. Physical, physiological and behavioural correlates of musth in captive African elephants (*Loxodonta africana*). *Physiological and Biochemical Zoology* 78(4), 505–514.

Garrott, R.A., D.B. Siniff, J.R. Tester, T.C. Eagle & E.D. Plotka 1992. A comparison of contraceptive technologies for feral horse management. *Wildlife Society Bulletin* 20, 318–326.

Garza, F., D.L. Thompson, D.D. French, J.J. Wiest, R.L. St George, K.B. Ashley, *et al.* 1986. Active immunization of intact mares against gonadotropin-releasing hormone: differential effects on secretion of luteinizing hormone and follicle-stimulating hormone. *Biology of Reproduction* 35, 347–352.

Goodloe, R.B., R.J. Warren & D.C. Sharp 1997. Sterilization of feral and captive horses: a preliminary report. In: P.N. Cohn, E.D. Plotka & U.S. Seal (eds) *Contraception in Wildlife.* Edwin Mellon Press, Lewiston, NY, 229–246.

Goritz, F., T.B. Hildebrandt, R. Hermes, S. Quandt, D. Grobler, K. Jewgenow, M. Rohleder, H.H.D. Meyer & H. Hof 1999. Results of hormonal contraception program in free-ranging African elephants. In: *Verhandlungsbericht des 39 Internationalen Symposiums uber die Erkrankungen der Zoo- und Wildtiere,* Institut fur Zoo- und Wildtierforschung, Berlin, 39–40.

Gulyas, B.J., R.B.L. Gwatkin & L.C. Yuan 1983. Active immunization of cynomogolus monkeys (*Macacca fascicularis*) with porcine zona pellucida. *Gamete Research* 4, 299–307.

Hall-Martin, A.J. 1987. Role of musth in the reproductive strategy of the African elephant (*Loxodonta africana*). *South African Journal of Science* 83, 616–620.

Hanks, J. 1972. Reproduction of elephant, *Loxodonta africana*, in the Luangwai Valley, Zambia. *Journal of Reproduction and Fertility* 30, 13–26.

Herbert, C.A., L. Vogelnest 2007. Catching kangaroos on the hop: development of a remote delivery contraceptive for marsupials. *Proceedings of 6th International Conference on Fertility Control for Wildlife*, 3–5 September 2007, York, UK, 15.

Hollister-Smith, J.A., J.H. Poole & E.A. Archie 2007. Age, musth and paternity success in wild male African elephants, *Loxodonta africana. Animal Behaviour* 74, 287–296.

Hoskinson, R.M., R.D. Rigby, P.E. Mattner, V.L. Huynh, M.J. D'Occhio, A. Neish, T.E. Trigg, B.A. Moss, M.J. Lindsey, G.D. Coleman & C.L. Schwartzkoff 1990. Vaxstrate: an anti-reproductive vaccine for cattle. *Australian Journal of Biotechnology* 4**,** 166–170.

Imboden, I., F. Janett, D. Burger, M. Hässig & R. Thun 2004. Influence of immunization against GnRH on cycling activity and estrous behaviour in the mare. *Theriogenology* 66(8), 1866–1875.

Jainudeen, M.R., G.M. McKay & J.F. Eisenberg 1972a. Observations on musth in the domesticated Asiatic elephant (*Elephas maximus*). *Mammalia* 36, 247–261.

Jainudeen, M.R., C.B. Katongole & R.V Short 1972b. Plasma testosterone levels in relation to musth and sexual activity in the male Asiatic elephant, *Elephas maximus. Journal of Reproduction and Fertility* 29, 99–103.

Janett, F., U. Lanker, M. Jörg, M. Hässig & R. Thun 2003. Castration of male lambs by immunisation against GnRH. *Schweizer Archiv fürTierheilkunde* 145, 291–299.

Johnson, O.W. & I.O. Buss 1967. The testis of the African elephant (*Loxodonta africana*). II. Development, puberty and weight. *Journal of Reproduction and Fertility* 13, 23–30.

Kahl, M.P. and D.B. Armstrong 2002. Visual displays of wild African elephants during musth. *Mammalia* 66, 159–171.

Kerley, G.I.H. & A.M. Shrader 2007. Elephant contraception: silver bullet or a potentially bitter pill? *South African Journal of Science* 103, 181–182.

Killian, G., L.A. Miller, N.K. Diehl, J. Rhyan & D. Thain 2004. Evaluation of three contraceptive approaches for population control of wild horses. In: R.M Tirron & W.P. Gorenzel (eds) *Proceedings of the 21st Vertebrate Pest Conference,* University of California, Davis, 263–268.

Killian, G., L. Miller, J. Rhyan & H. Doten 2006. Immunocontraception of Florida feral swine with a single-dose GnRH vaccine. *American Journal of Reproductive Immunology* 55, 378–384.

Kirkpatrick, J.F, A. Perkins & J.W. Turner 1982. Reversible fertility control in feral horses. *Journal of Equine Veterinary Science* 2, 114–118.

Kirkpatrick, J.F. & J.W. Turner 1991. Reversible fertility control in non-domestic animals. *Journal of Zoo and Wildlife Medicine* 22, 392–408.

Kirkpatrick, J.F. & A. Turner 2002. Reversibility of action and safety during pregnancy of immunizing against porcine zona pellucida in wild mares (*Equus caballus*). *Reproduction* (Suppl. 60), 197–202.

Kirkpatrick J.F. & A. Turner 2003. Absence of effects from immunocontraception on seasonal birth patterns and foal survival among barrier island horses. *Journal of Applied Animal Welfare Science* 6, 301–308.

Kirkpatrick, J.F. & A. Turner 2007. Immunocontraception and increased longevity in equids. *Zoo Biology* 25, 1–8.

Kirkpatrick, J.F., I.K.M. Liu & J. Turner 1990. Remotely delivered immunocontraception in feral horses. *Wildlife Society Bulletin* 18, 326–330.

Kirkpatrick, J.F., I.K.M. Liu, J. Turner & M. Bernoco 1991. Antigen recognition in mares previously immunized with porcine zonae pellucidae. *Journal of Reproduction and Fertility* (Suppl. 44), 321–325.

Kirkpatrick, J.F., I.K.M. Liu, J.W. Turner, R. Naugle & R. Keiper 1992. Long-term effects of porcine zonae pellucidae immunocontraception on ovarian function of feral horses (*Equus caballus*). *Journal of Reproduction and Fertility* 94, 437–444.

Kirkpatrick, J.F., R. Naugle, I.K.M. Liu, M. Bernoco & J.W. Turner 1995a. Effects of seven consecutive years of porcine zona pellucida contraception on ovarian function in feral mares. *Biology of Reproduction Monograph Series 1, Equine Reproduction VI*, 411–418.

Kirkpatrick, J.F., W. Zimmermann, L. Kolter, I.K.M. Liu & J.W. Turner 1995b. Immunocontraception of captive exotic species. I. Przewalski's horse (*Equus caballus*) and banteng (*Bos javanacus*). *Zoo Biology* 14, 403–413.

Kirkpatrick, J. F., P.P. Calle, P. Kalk, I.K.M. Liu, M. Bernoco & J.W. Turner 1996. Immunocontraception of captive exotic species. II. Formosan sika deer (*Cervus nippon taiouanus*), Axis deer (*Cervus axis*), Himalayan tahr (*Hemitragus jemlahicus*), Roosevelt elk (*Cervus elaphus roosevelti*), Munjac deer (*Muntiacus reevesi*), and Sambar deer (*Cervus unicolor*). *Journal of Zoo and Wildlife Medicine* 27, 482–495.

Lawley, L. 1994. *The world of elephants*. Michael Friedman-Fairfax Publishing, New York.

Laws, R.M. 1969. Aspects of reproduction in the African elephant, *Loxodonta africana. Journal of Reproduction and Fertility* Supplement 6, 193–217.

Laws, R.M., I.S.C. Parker & R.C.B. Johnstone 1975. *Elephants and their habitats.* Clarendon Press, Oxford.

Lee, V. & B.S. Dunbar 1992. Immunization of guinea pigs results in polycystic ovaries. *Biology of Reproduction* (Suppl. 46), 131 (abstract).

Lee, P. 1997. Reproduction. In: G. Rogers & S. Watkinson (eds) *The illustrated encyclopedia of elephants.* Salamander Books Ltd., London, 64–77.

Liu, I.K.M., M. Bernoco, M. Feldman 1989. Contraception in mares heteroimmunized with pig zonae pellucidae. *Journal of Reproduction and Fertility* 85, 19–29.

Leong, K.M., A. Ortolani, L.H. Graham & A. Savage 2003. The use of low-frequency vocalizations in African elephant (*Loxodonta africana*) reproductive strategies. *Hormones and Behavior* 43, 433–43.

Liu, I.K.M., M. Bernoco, M. Feldman 1989. Contraception in mares heteroimmunized with pig zonae pellucidae. *Journal of Reproduction and Fertility* 85, 19–29.

Mackey, R.L.; B.R. Page, D. Duffy & R. Slotow 2006. Modelling elephant population growth in small, fenced, South African reserves. *South African Journal of Wildlife Research* 36, 33–43.

Mahi-Brown, C.A., R. Yanagimachi, J. Hoffman & T.T. Huang 1985. Fertility control in the bitch by active immunization with porcine zonae pellucidae: use of different adjuvants and patterns of estradiol and progesterone levels in the estrous bitch. *Biology of Reproduction* 32, 671–722.

McShea W.J., S.L. Monfort, S. Hakim, J.F. Kirkpatrick, I.K.M. Liu, J.W. Turner, L. Chassy & L. Munson 1997. Immunocontraceptive efficacy and the impact of contraception on the reproductive behaviors of white-tailed deer. *Journal of Wildlife Management* 61, 560–569.

Miller, L.A., B.E Johns & J. Killian 1999. Long-term effects of PZP immunization on reproduction in white-tailed deer. *Vaccine* 18, 568–574.

Miller, L.A., J.C. Rhyan & M. Drew 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. *Journal of Wildlife Diseases* 40(4), 725–730.

Moss, C.J. 1983. Oestrous behaviour and female choice in the African elephant. *Behavior* 86, 167–196.

Moss, C. 1996. Getting to know a population. In: K. Kangwana (ed.) *Studying elephants*. African Wildlife Foundation, Kenya, 58–74.

Munson, L., I.A. Gardener, R.J. Mason, L.M. Chassy & U.S. Seal 2002. Endometrial hyperplasia and mineralization in zoo felids treated with melengestrol acetate contraceptives. *Veterinary Pathology* 39, 419–427.

Munson, L., A. Moresco & P.P. Calle 2005. Adverse effects of contraceptives. In: C.S. Asa & I.J. Porton (eds) *Wildlife contraception*, The John Hopkins University Press, Baltimore, 66–82.

Naugle, R., A.T. Rutberg, H.B. Underwood, J.W. Turner & I.K.M. Liu 2002. Field testing of immunocontraception on white-tailed deer (*Odocoileus virginianus*) on Fire Island National Seashore, U.S.A. *Reproduction* (Suppl. 60), 143–153.

Noden, P. A., W.D. Oxender & H.D. Hafs 1978. Early changes in serum progesterone, estradiol, and LH during prostaglandin F2α-induced luteolysis in mares. *Journal of Animal Science* 47, 666–761.

Oonk, H.B., J.A. Turkstra, W.M. Schaaper, J.H. Erkens, M.H. Schuitemaker-de Weerd, A. van Nes, J.H. Verheijden & R.H. Meloen 1998. New GnRH-like peptide construct to optimize efficient immunocastration of male pigs by immunoneutralization of GnRH. *Vaccine* 16, 1074–1082.

Ortolani, A., K. Leong, L. Graham & A. Savage 2005. Behavioral indices of estrus in a group of captive African elephants (*Loxodonta africana*). *Zoo Biology* 24, 311–329.

Owens, M. & D. Owens. 1997. Can time heal Zambia's elephants? *International Wildlife* 27, 28–35.

Palm, V.S., A.G. Sacco, F.N. Snyder & M.G. Subramanian 1979. Tissue specificity of porcine zona pellucida antigen(s) tested by radioimmunoassay. *Biology of Reproduction* 21, 709–713.

Parker, G.E. & F.V. Osborn 2006.Growing chilli as a means of reducing human-wildlife conflict in Zimbabwe. Oryx, in press.

Patton, M.L., W. Jöchle & L.M. Penfold 2005. Contraception of ungulates. In: C.S. Asa & I.J. Porton (eds) *Wildlife Contraception*. The John Hopkins University Press, Baltimore, 149–167.

Perdock, A.A., W.F. de Boer, T.A.E. Stout 2007. Prospects for managing African elephant population growth by immunocontraception: a review. *Pachyderm* 42, 97–107.

Perry, J. S. 1953. The reproduction of the African elephants, *Loxodonta africana*. *Philosophical Transactions of the Royal Society B* 237, 93–149.

Poole, J.H. & C.J. Moss 1981. Musth in the African elephant. *Nature* 292, 830–831.

Poole, J.H. 1982. Musth and male-male competition in the African elephant. Ph.D. thesis, Cambridge University, UK.

Poole, J.H., L.H. Kasman, E.C. Ramsay & B.L. Lasley. 1984. Musth and urinary testosterone concentrations in the African elephant (*Loxodonta africana*). *Journal of Reproduction and Fertility* 70, 255–260.

Poole, J.H. 1987. Rutting behavior in African elephants: The phenomenon of musth. *Behavior* 102, 283–316.

Poole, J.H., K. Payne, W.R. Langbauer Jr. & C.J. Moss 1988. The social contexts of some very low frequency calls of African elephants. *Behavioural Ecology and Sociobiology* 22, 385–392.

Poole, J.H. 1989a: Announcing intent: the aggressive state of musth in African elephants. *Animal Behaviour* 37, 140–152.

Poole, J.H. 1989b. Mate guarding, reproductive success and female choice in African elephants. *Animal Behaviour* 37, 842–849.

Poole, J.H. 1994. Sex differences in the behavior of African elephants. In: R.V. Short & E. Balaban (eds) *The differences between the sexes*. Cambridge University Press, Cambridge, 331–346.

Poole, J. 1996a. The African Elephant. In: K. Kangwana (ed.) *Studying elephants*. African Wildlife Foundation, Kenya, 1–8.

Poole, J. 1996b. *Coming of age with elephants*. Hodder & Stoughton, London.

Palmer, E. & B. Jousett 1975. Urinary oestrogen and plasma progesterone levels in non-pregnant mares. *Journal of Reproduction and Fertility* (Suppl. 23), 213–221.

Plotka, E.D. & D.N. Vevea. 1990. Serum ethinylestradiol (EE_2) concentrations in feral mares following hormonal contraception with homogenous implants. *Biology of Reproduction* 42 (Suppl. 1), 43.

Rasmussen, H.B. 2005. Reproductive tactics of male African savannah elephants (*Loxodonta africana*). Ph.D. thesis, Oxford University, UK.

Rasmussen, L.E.L., A.J. Hall-Martin & D.L. Hess 1996. Chemical profiles of male African elephants, *Loxodonta africana*: Physiological and ecological implications. *Journal of Mammalogy* 77, 422–439.

Rasmussen, L.E.L. & B.A. Schulte 1998. Chemical signals in the reproduction of Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants. *Animal Reproduction Science* 53, 19–34.

Rasmussen, L.E.L. & T.E. Perrin 1999. Physiological correlates of musth: lipid metabolites and chemical composition of exudates. Physiology and Behavior 67, 539–549.

Rasmussen, L.E.L. & S.W. Riddle 2002. Meliferous matures to malodorous in musth. *Nature* 415, 975–976.

Rasmussen, L.E.L. & S.W. Riddle 2004. Development and initial testing of pheromone-enhanced mechanical devices for deterring crop raiding elephants: a positive conservation step. *Journal of the Elephant Management Association* 15, 30–37.

Riddle, H.S., S.W. Riddle, L.E.L. Rasmussen, T.E. Goodwin 2000. First disclosure and preliminary investigation of a liquid released from the ears of African elephants. *Zoo Biology* 19, 475–480.

Rutberg, A.T., R.E. Naugle, L.A. Thiele & I.K.M. Liu 2004. Effects of immunocontraception on a suburban population of white-tailed deer *Odocoileus virginianus. Biological Conservation* 116, 243–250.

Sacco, A.G. 1977. Antigenic cross-reactivity between human and pig zona pellucida. *Biology of Reproduction* 16, 164–173.

Sacco, A.G. & C.A. Shivers 1973. Effects of reproductive tissue-specific antisera on rabbit eggs. *Biology of Reproduction* 8, 481–490.

Sacco, A.G., D.L. Pierce, M.G. Subramanian, E.C. Yurewicz & W.R. Dukelow 1987. Ovaries remain functional in squirrel monkeys (*Saimiri sciureus*) immunized with porcine zona pellucida 55,000 macromolecule. *Biology of Reproduction* 36, 481–490.

Sacco, A.G., M.G. Subramanian, E.C. Yurewicz, F.J. DeMayo & W.R. Dukelow 1986. Heteroimmunization of squirrel monkeys (*Saimiri sciureus*) with a purified porcine zonae antigen (PPZA): immune response and biological activity of antiserum. *Fertility and Sterility* 39, 350–358.

Schulte, B.A., E.W. Freeman, T.E. Goodwin, J. Hollister-Smith & L.E.L. Rasmussen 2007. Honest signalling through chemicals by elephants with applications for care and conservation. *Applied Animal Behavior Science* 102, 344–363.

Shideler, S.E., M.A. Stoops, N.A. Gee, J.A. Howell & B.L. Lasley 2002. Use of porcine zona pellucida (pZP) vaccine as a contraceptive agent in free-ranging Tule elk (*Cervus elaphus nannodes*). *Reproduction* (Suppl. 60), 169–176.

Shivers, C.A., S.B. Dudkiewiez, L.E. Franklin & E.F. Russell 1972. Inhibition of sperm-egg interaction by specific antibody. *Science* 178, 1211–1213.

Shivers, C.A. & B.S. Dunbar 1977. Autoantibodies to zona pellucida: A possible cause for infertility in women. *Science* 197, 1182–1184.

Sikes, S.K. 1971. *The natural history of the African elephant*, Weidenfeld & Nicolson, London.

Slotow, R., M.E. Garai, B. Reilly, B. Page & D. Carr 2005. Population dynamics of elephants re-introduced to small fenced reserves in South Africa. *South African Journal of Wildlife Research* 35, 1–10.

Squires, E.L., B.C. Wentworth & O.J. Ginther 1974. Progesterone concentration in blood of mares during the estrous cycle, pregnancy and after hysterectomy. *Journal of Animal Science* 39, 759–767.

Stabenfeldt, G.H., J.P. Hughes, J.W. Evans & D.P. Neely 1974. Spontaneous prolongation of luteal activity in the mare. *Equine Veterinary Journal* 6, 158–163.

Stetter, M., D. Grobler, J.R. Zuba *et al.*, 2005. Laparascopic reproductive sterilization as a method of population control in free-ranging African elephants (*Loxodonta africana*). Proceedings AAZV, AAWV, AZA Nutrition Advisory Group, 199–200.

Stetter, M., D. Hendrickson, J. Zuba et al. 2006. Laparoscopic vasectomy as a potential population control method in free ranging African elephants (*Loxodonta africana*). *Proceedings International Elephant Conservation and Research Symposium*, 177

Sukumar, R. 1989. *The Asian Elephant: ecology and management*. Cambridge University Press, Cambridge.

Sukumar, R. 1994. *Elephant days and nights: Ten years with the Indian Elephant*. Oxford University Press, Delhi.

Turkstra, J.A., F.J.U.M. Meer, J. van der Knaap, P.J.M. Rottier, K.J. Teerds, B. Colenbrander & G.H. Meloen 2005. Effects of GnRH immunization in sexually mature pony stallions. *Animal Reproduction Science* 86, 247–259.

Turner, J.W., A. Perkins & J.F. Kirkpatrick 1981. Elimination marking behavior in feral horses. *Canadian Journal of Zoology* 59, 1561–1566.

Turner, J.W. & J.F. Kirkpatrick. 1982. Androgens, behaviour and fertility control in feral stallions. *Journal of Reproduction and Fertility* (Suppl. 32), 79–87.

Turner, A. & J.F. Kirkpatrick. 2002. Effects of immunocontraception on population, longevity and body condition in wild mares (*Equus caballus*). *Reproduction* (Suppl. 60), 187–195.

Turner, J.W., I.K.M., Liu, D.R. Flanagan, K.S Bynum & A.T. Rutberg 2002. Porcine zona pellucida (pZP) immunocontraception of wild horses (*Equus caballus*) in Nevada: a 10 year study. *Reproduction* Supplement 60, 177–186.

Turner, J.W., A.T. Rutberg, R.E. Naugle, M.A. Kaur, D.R. Flanagan, H.J. Bertschinger & I.K.M. Liu 2008. Controlled-release components of pZP contraceptive vaccine extend duration of infertility. *Wildlife Research*, in press.

Van Rossum, R.J.W. 2006. pZP-immunocontraception in the African elephant (*Loxodonta africana*). Excellence track masters thesis, Utrecht University.

Vidya, T.N.C. & R. Sukumar 2005. Social organization of the Asian elephant (*Elephas maximus*) in southern India inferred from microsatellite DNA. *Journal of Ethology* 23, 205–210.

Western, D. & W.K. Lindsay 1984. Seasonal herd dynamics of a savanna elephant population. *African Journal of Ecology* 22, 229–244.

Whitehouse, A.M. 2001. *The Addo elephants: conservation biology of a small, closed population*. PhD thesis, University of Port Elizabeth, Port Elizabeth.

Whitehouse, A.M. & G.I.H. Kerley 2002. Retrospective assessment of long-tern conservation management of elephants in Addo Elephant National Park, South Africa. *Oryx* 36, 243–248.

Whyte, I. 2001. *Conservation management of the Kruger National Park elephant population.* PhD thesis, University of Pretoria, Pretoria.

Whyte, I.J. & D.G. Grobler 1998. Elephant contraception in the Kruger National Park. *Pachyderm* 25, 45–52.

Wittemyer, G., A. Ganswindt & K. Hodges 2007. The impact of ecological variability on the reproductive endocrinology of wild female African elephants. *Hormones and Behavior* 51, 346–354.

Wood, D.M., C. Liu & B.S. Dunbar 1981. Effect of alloimmunization and heteroimmunization with porcine zonae pellucidae on fertility in rabbits. *Biology of Reproduction* 25, 439–450.

Zeng, X.Y., J.A. Turkstra, D.F.M. Wiel, D.Z. van de Guo, X.Y. Liu, R.H. Meloen, W.M.M. Schaaper, F.Q. Chen, H.B. Oonk & X. Zhang 2001. Active immunization against gonadotropin-releasing hormone in Chinese male pigs. *Reproduction in Domestic Animals* 36, 101–105.