Endocrine correlates of free-ranging African elephant (*Loxodonta africana*) treated with porcine zona pellucida vaccine

By
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DECLARATION

I, Melodie Bates, do hereby declare that the research presented in this dissertation, was conceived and executed by myself, and apart from the normal guidance from my supervisors, I have received no assistance.

Neither the substance, nor any part of this dissertation has been submitted in the past, or is to be submitted for a degree at this University or any other University.

This dissertation is presented in partial fulfilment of the requirements for the degree MSc in Production Animal Studies.

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Signed ________________________________
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5α-DHP:	5α-pregnane-3,20-dione
5α-P-3α-OH:	3α-hydroxy-5α-pregnan-20-one
BSA:	Bovine serum albumin
CITES:	Convention on Trade in Endangered Species
CL:	Corpus luteum
EIA:	Enzyme immunoassay
FP:	Follicular Phase
FSH:	Follicle stimulating hormone
GnRH:	Gonadotropin releasing hormone
KNP:	Kruger National Park
LH:	Luteinizing Hormone
LP:	Luteal Phase
MPGR:	Makalali Private Game Reserve
PBS:	Phosphate buffer saline
pZP:	Porcine zona pellucida
SD:	Standard deviation
TPC:	Threshold of Potential Concern
TPNR:	Thornybush Private Nature Reserve

ABSTRACT

Due to overpopulation of African elephants in South Africa and the consequent threat to biodiversity, the need for a method of population control has become evident. The potential use of the porcine zona pellucida (pZP) vaccine as a safe and effective means for population control is explored. While potential effects of pZP treatment on social behaviour of African elephants have been investigated, no examination of the influence of pZP vaccination on the endocrine correlates in treated females has been undertaken. The ovarian activity of free-ranging, pZP-treated African elephant females was monitored non-invasively for one year duration by measuring faecal progestagen concentrations via enzyme immunoassay. Behavioural observations were recorded for comparison with progestagen concentrations and to determine any behavioural changes surrounding the pZP vaccine darting event. Each elephant under study showed progestagen concentrations rising above baseline at some period during the study indicating luteal functionality. Average progestagen concentrations were 1.61 ± 0.46 µg/g. Within sampled females, 42.9% exhibited oestrous cycles within the range reported for African elephants, 14.3% had irregular cycles, and 42.9% did not appear to be cycling. Average oestrous cycle duration was 14.72 ± 0.85 weeks. Behavioural oestrous coincided with the onset of the luteal phase and a subsequent rise in progestagen concentrations. Focal sampling to determine activity budgets before and after the darting event revealed no significant change in behavioural activities. In the week following immunization, individual progestagen concentrations decreased significantly from overall average concentrations. Average progestagen concentrations positively correlated with rainfall and with herd dominance. No association between average individual progestagen concentrations or cyclicity status with age, lactation, or parity were detected. Earlier determination of efficacy was made indicating reproductive control was established 22 months post-treatment. Results indicate the presence of ovarian activity amongst pZP-treated female African elephants in two years following initial immunization. Further study should be aimed toward studying the long term effects of pZP vaccination on the reproductive function of female African elephants.

Chapter 1: INTRODUCTION

Rapidly expanding elephant populations in the Republic of South Africa has led to widespread concern over the resulting destruction of habitat and consequent threats to biodiversity (Fayrer-Hosken *et al*. 1999; Stout & Colenbrander 2004; Kerley & Landman 2006; Kerley *et al*. 2008). In 1990, the Convention on International Trade in Endangered Species (CITES) placed the African elephant (*Loxodonta* africana) on Appendix 1 and thereby banned international ivory trading (Fayrer-Hosken *et al*. 1997). As a result, decreases in poaching, hunting, as well as a moratorium placed on culling practices within the Kruger National Park (KNP) in 1994, allowed elephant numbers to grow unhindered (Fayrer-Hosken *et al*. 1997; Carruthers *et al*. 2008). Even after elephants were reassigned to Appendix 2 in 1997 due to the acknowledgement that elephant populations were exponentially rebounding, ethical concerns as well as the appeal for further scientific studies have prevented the use of culling as an elephant management tool (Whyte *et al*. 1999; Borchert 2006). The end result has left wildlife managers at odds over how to control elephant numbers.

Various solutions for population control have been proposed in addition to culling and include employing a laissez-faire approach, translocation, range expansion, sterilization, and contraceptives. Each of these options has associated positive and negative features and subsequently wildlife managers face a dilemma over selecting the best option. The search for a better alternative, where negative consequences are minimal to non-existent, is still ongoing.

The use of immunological contraceptives, namely the porcine zona pellucida (pZP) vaccine, is a fairly recent development that has shown promise as an effective and acceptable means for elephant population management (Fayrer-Hosken *et al*. 1997; Delsink *et al*. 2002). Primary concerns associated with its use involve its effects on behaviour. While preliminary behavioural studies have reported no observable behavioural abnormalities (Delsink 2006a; Delsink *et al*. 2006c), currently no physiological parameters have been examined and thus immunocontraception has not been fully embraced by scientists and the public as one possible solution to the elephant overpopulation problem. To date, there has been no attempt to confirm regular oestrous cycling associated with pZP vaccine use. In view of this fact, it is clear that evidence of factual data is needed in order to determine the legitimacy of any concerns that linger over the safety and effectiveness of utilizing the pZP vaccine as a method of controlling elephant population growth.

Measurement of progesterone metabolite concentrations detectable in the faeces (Wasser *et al.* 1996; Fieβ *et al.* 1999) allows for the attainment of quantifiable data demonstrating frequency and duration of oestrous cycles. The ability to monitor the ovarian cycle non-invasively, in addition to being more practical in the field, avoids contributing to increases in stress levels which could lead to inaccurate results (Lasley and Kirkpatrick, 1991).

In an assessment of the existing information on the elephant reproductive cycle, Hodges (1998) identified particular areas where knowledge is lacking, one of which being the need for an integrated study of behavioural and endocrine mechanisms in order to support a better understanding of the elephant's reproductive physiology. The proposed study, while examining pZP-treated individuals, offers insight on this disparity and provides an earlier estimate of efficacy of the pZP vaccine than that determined by previous studies that required two to three years to establish zero population growth (Bertschinger *et al.* 2004).

The aim of this study is to substantiate evidence of the pZP vaccine's potential as a safe and effective contraceptive through factual data.

The objectives of the study were to:

- Assay the progestagen in faecal samples collected from pZP-treated individuals;
- Use the progestagen assays results to determine the reproductive status of the pZP-treated cows on a continual basis; and
- Correlate hormonal results to behaviours observed.

Chapter 2: LITERATURE REVIEW

2.1 African Elephant Population Status and Distribution throughout Africa

Historically, it is believed that the African elephant (*Loxodonta africana*) was widely dispersed throughout the African continent, largely as a consequence of the species' adaptability to a wide range of differing climates, vegetation and ecosystems (Laws 1970; Sikes 1971). Colonization by man into various regions of Africa, land development, the Rinderpest epidemic, as well as heavy slaughter for subsistence and to make way for agricultural practices reduced much of the population by the late 1800s (Sikes 1971; Hanks 1979; Barnett 1991; Joubert 2007). Game reserves and parks were subsequently established in order to protect what few populations were left but continually increasing human population pressures have restricted areas which elephants can now inhabit (Sikes 1971; Hanks 1979; Blanc *et al*. 2007). Today, it is estimated that 22% of the African continent is inhabited by elephants (Blanc *et al*. 2007). At 39% of the continents' total, southern Africa contains the largest elephant range as well as the largest number of elephants on the continent estimated at 321,000 individuals (Blanc *et al*. 2007). While populations in Central, East and West Africa are decreasing (Harris *et al*. 2008), the approximate number of elephants in southern African has increased by over 19% (or 51,000 individuals) in the past 5 years (Blanc *et al*. 2007). In South Africa alone, populations have been shown to increase at a rate of more than 7% per year (Slotow *et al*. 2005). With Africa's high human population growth rate, it is likely that the current available range for elephants will continue to decrease leading to higher densities in smaller areas and creating greater concern over the potential impact on their habitats (Hanks 1979; Cumming *et al*. 1997; Whyte *et al*. 1998; Blanc *et al*. 2007).

2.2 Reproductive Physiology of the African Elephant

Elephant societies are comprised of matrilineal family units of between 2 to 30 adult females and their offspring (Lee 1991; McComb *et al*. 2001; Archie *et al*. 2008). The oldest female in the breeding herd, known as the matriarch, is primarily responsible for the defence of the herd as well as directing the herd to valuable resources thus enhancing reproductive success by ensuring survival and increasing fitness (Dublin 1983; Lee 1991; McComb *et al*. 2001). Sub-adult or adolescent females within the herd also cooperate in rearing young calves acting as allomothers by assisting in the protection of calves as well as teaching foraging and social behaviour, consequently allowing the biological mother to devote more time to self-maintenance (Dublin 1983; Lee 1991). With an average lifespan of up to 60 years (Stuart and Stuart 1997), age of 1st conception recorded as low as 9 years (Bertschinger *et al*. 2008), and inter-calving intervals ranging between 3 to 9 years (Whyte *et al*. 1998; Bertschinger *et al*. 2008), a single elephant cow can theoretically produce up to 17 offspring in her lifetime. Thus, the capacity for rapid population growth in the African elephant is great and the cooperative behaviour demonstrated amongst related females in a herd promotes longevity.

2.3 Limiting Factors to Population Growth

Limiting factors to population growth include emigration, fire, drought, disease, natural mortality, and man – specifically poaching, culling, hunting, and habitat loss due to human encroachment on elephant range (Laws 1970; Blanc *et al*. 2007; van Aarde and Jackson 2007). Increased interference from humans has made many of these limiting factors non-existent. The fencing of protected areas, fire manipulation and the artificial provision of water halted much of the elephants’ traditional migration patterns thus allowing higher densities of elephants to have more concentrated impact on their habitats (Eckhardt *et al*. 2000; van Aarde *et al*. 2006; van Aarde and Jackson 2007). Furthermore, an international ban on ivory trade was imposed by the Convention on Trade in Endangered Species (CITES) in 1990 following a decline in elephant numbers due to poaching, causing a reversal of the downward population trend (Fayrer-Hoskins *et al*. 1997). All of these factors have encouraged the increase in elephant numbers within the majority of southern Africa.

2.4 Consequences of Large Elephant Populations

As elephant populations continue to expand expeditiously, increasing concern over the impact on habitat and biodiversity mounts. With an optimal daily food intake calculated at approximately 6% of body weight equating to 300 kg daily for an average-sized bull and 170 kg for an average-sized cow (Laws 1970; Stuart and Stuart 1997), the potential damage to their habitat at high population densities is considerable. While elephants can have positive influences on the land they inhabit, such as promoting seed dispersal (Kerley and Landman 2006), reducing bush encroachment (Scholes *et al*. 2007), and making both browse and water in dry riverbeds accessible to other inhabitant species (Makhabu *et al*. 2006; Scholes *et al*. 2007), the negative effects generated by high densities are now being commonly reported throughout South Africa (Trollope *et al*. 1998; Whyte *et al*. 1999; Eckhardt *et al*. 2000; Duffy *et al*. 2002; Jacobs and Biggs 2002; Whyte 2003). Conversion of woodlands to grasslands (Hanks 1979; Whyte *et al.* 1998; Western and Maitumo 2004), erosion leading to siltation of waterholes due to loss of tree cover (Cumming *et al*. 1997; Foggin 2003) and homogenization of tree structure leading to a risk of species specific mortality (Jacobs and Biggs 2002) are a few of the concerns brought forth. Furthermore, as elephant populations have the capacity to markedly transform habitats (Caughley 1976), other inhabitant species are put at risk thereby reducing biodiversity. In a study comparing a densely elephant populated area to an adjacent elephant-excluded area, Cumming *et al*. (1997) showed that diversity of plant, bird and insect species was notably reduced. In order to avoid destruction of habitat and consequent threats to species diversity, managers are now investigating options for population control.

2.5 Population Control Mechanisms for the African Elephant

2.5.1 Laissez-Faire Approach

The option to allow nature to take its course has its appeal in that it is morally acceptable to those opposed to lethal intervention and that it does not necessitate the use of park funds (Delsink

2006a). Additionally, resilience and resistance, essential to population persistence, are maintained via natural disturbances (van Aarde and Jackson 2007). However, the reality remains that humans have already interfered by erecting fences that hinder natural ecological processes, thus management is necessary (Delsink 2006a; van Aarde and Jackson 2007). Following the theory of density-dependence, reproductive rate slows at higher population densities (van Aarde *et al*. 1999; van Aarde *et al*. 2008). However, if population densities are already at levels where destruction of habitat is evident, applying a laissez-faire approach at that late stage would do nothing to improve the situation. The uncertainty of the extent of vegetation damage at high elephant densities remains (van Aarde *et al*. 1999). It has also been speculated that density-dependence may only regulate elephant populations after severe degradation of their ecosystem (Kerley and Landman 2006), which would also have negative implications for other resident species. Due to the aforementioned consequences of large populations in many parks, it may actually *not* be morally acceptable for management to refrain from interfering.

2.5.2 Culling

The reduction of wildlife populations by lethal means is a commonly utilized method of population control throughout the world (Hall-Martin 1990). The idea of removing a target amount of animals in order to stem population growth is based on the concept of economic carrying capacity (van Aarde and Jackson 2007). Economic carrying capacity is described as the state of equilibrium achieved by the sustainable harvesting of a population (McLeod 1997). The reasoning behind the decided absolute number of takeoffs to achieve this sustainable harvest is not known and was based on a small amount of available information as well as untested assumptions regarding population growth rates and habitat impacts (Whyte *et al*. 1999; Slotow *et al*. 2005). Concerns were also raised about keeping a population at a stable number as this does not reflect natural ecosystem processes and could lead to distorted population structure and decreases in resilience of the population (Whyte *et al*. 1999; van Aarde and Jackson 2007).

At present, the KNP has moved from making culling decisions based on absolute elephant numbers to those based on a quantitative assessment of impacts on biodiversity, also known as

Thresholds of Potential Concern (TPCs) (Whyte *et al*. 1999). However, this method is also a trial-and-error approach as TPCs were initially based on what little available knowledge and experience was present at the time and is constantly being adjusted as more information is acquired by ongoing monitoring programs (Whyte *et al*. 1999).

Other shortcomings of this population control method have also been brought to light following careful scrutiny of the after-effects of culling practices. Following the cessation of culling operations brought about by the CITES ban in 1990, it was found that population growth rate radically increased (van Aarde *et al*. 1999; van Aarde and Jackson 2007). Sex and age ratios were seen to be negatively affected following selective culling practices which could explain in part the increase in growth rate (Hall-Martin 1990; Scholes *et al*. 2007; van Aarde and Jackson 2007). Disturbance due to the culling operations can lead to immigration and heightened impact by concentrating densities in smaller areas (van Aarde and Jackson 2007). Calves surviving culling operations are known to have heightened risk for future behavioural disorders, such as intensified aggression in later years (Bradshaw *et al*. 2005). Furthermore, the actual deleterious effects and persistence of stress potentially caused by the practice to remaining populations is still unknown as entire family units are rarely culled (Fayrer-Hosken *et al*. 1999; Scholes *et al*. 2007). Although it has been noted that revenue generated by culling exercises can be put toward management practices (Hall-Martin 1990) and the products can benefit rural communities which have little access to protein (Whyte 2003), income generated from tourism by those seeking to view elephants in game reserves has been shown to be greater than that created by selling culling products (Butler 1998).

While culling does effectively reduce population size in the short-term, it is not presently an ethically acceptable method of population control due to the aforementioned concerns and the need for further scientific study, and thus managers continue searching for better alternatives.

2.5.3 Translocation

Removal of portions of the population by means of translocation to another suitable habitat is considered to be the only other option, besides culling, to alleviate population pressures immediately (van Aarde and Jackson 2007). While avoiding lethal means of controlling numbers, translocation is still met with ethical issues. In the past, juvenile calves captured and sold to game reserves were noted to have severe behavioural problems as they matured (Hofmeyr 2003; Slotow *et al*. 2005; Millspaugh *et al*. 2007). Immature elephants were responsible for the killing of numerous white (*Ceratotherium simum*) and black (*Diceros bicornis*) rhino (Slotow and van Dyk 2001; Slotow and van Dyk 2004). Since this realization, only mature adult bulls and intact family groups are translocated (Slotow and van Dyk 2004; Slotow *et al*. 2005) though, similar to culling, there is still the uncertainty that part of the family unit may be left behind, disrupting social structure and potentially causing more problems (Whyte 2003; Delsink 2006a). Although relocation techniques have been much improved over time, mortalities have occurred, mostly due to stress-related diseases (Hofmeyr 2003; Slotow *et al*. 2005; Scholes *et al*. 2007). Stress levels determined via faecal glucocorticoid sampling was proven to be elevated both before and during translocation (Millspaugh *et al*. 2007; Viljoen *et al*. 2008) and concerns have been raised that the translocation process is stressful to individuals within sensory range who may not be directly involved (Scholes *et al*. 2007). There is also the need for precautions to be taken to prevent the spread of contagious diseases, such as Foot-and-Mouth and tuberculosis (Roberts and Travers 2004).

Investigating the effects of translocation on population dynamics, Slotow *et al*. (2005) determined that translocated populations tend to have a female bias, high population growth rates, high population densities, and a disturbingly high predicted population density. In addition, there is a risk of inbreeding over time in populations relocated to small, fenced reserves (Slotow *et al*. 2005; Bertschinger *et al*. 2008; Scholes *et al*. 2007). Thus, translocation only appears to result in a temporary solution for overpopulation and will require future management interference (Slotow *et al*. 2005; van Aarde and Jackson 2007). Furthermore, the number of areas willing and able to take more elephants is quickly dwindling (Butler 1998; Whyte *et al*. 1999; Foggin 2003;

Hofmeyr 2003; Whyte 2003; Slotow *et al*. 2005; Scholes *et al*. 2007; van Aarde and Jackson 2007). The expense alone at approximately ZAR 10,000 per elephant excluding transport cost (Hofmeyr 2003) or more than US $1,500 per elephant (Foggin 2003) may make the option unfeasible for most reserves.

2.5.4 Range Expansion

The concept of range expansion has also been brought forward as a possible solution to the overpopulation problem and also has the benefit of ensuring population persistence (van Aarde and Jackson 2007). By making more land available to large populations, overall impact is theoretically lessened by promoting dispersal (van Aarde *et al*. 2006). However, while this method does reduce densities in source areas, it does not lower the absolute growth rate (Scholes *et al*. 2007). In fact, population growth rate then remains high for a longer period of time permitting densities to eventually return to their original high levels and by that stage, further dispersal may not be possible (Scholes *et al*. 2007). At present, it is questioned if elephants will even utilize newly available habitats or if they would rather prefer to remain in their current familiar location and continue to concentrate their impacts (Slotow *et al*. 2005). Elephants are known to move short distances, avoid people, remain in close proximity to water, and choose highest vegetation cover (Harris *et al*. 2008). If all these preferred conditions are already met in their current locality, there would appear to be no incentive to migrate elsewhere until their impact becomes as great as to eliminate their food supply.

Translocation of portions of the population to initiate movement into new areas may offer a solution, but then runs into the same issues of feasibility and ethics that were previously mentioned. While some believe that given enough space, an elephant population may self-regulate, this theory remains to be proven (van Aarde *et al*. 2006; Scholes *et al*. 2007). This “wait-and-see” approach may be hampered by the reality that there are limited areas available for future expansion as competition over land for human settlement and agriculture is great (Scholes *et al*. 2007). Moreover, many local human populations are not in favour of allowing elephants to move back into areas that humans now inhabit (Hofmeyr 2003; van Aarde and Jackson 2007).

While range expansion is a more ethical method for dealing with large elephant populations, the aforementioned arguments raise considerable concerns regarding its true effectiveness.

2.5.5 Sterilization of Bull Elephants

Surgical vasectomy or castration of bulls in large populations is another possibility for population control but does not immediately alleviate dense population pressures on habitat and biodiversity (Foggin 2003). The original methods for sterilization were highly invasive and lengthy due to the abdominal location of the testes (Stout and Colenbrander 2004; Delsink 2006a). With the advent of laparoscopic surgery, the procedure became less invasive but still lengthy (approximately 5 hours) but the success rate was low at 33% and one mortality was recorded (Delsink 2006a; Bertschinger *et al*. 2008). As opposed to castration, vasectomies allow bulls to retain musth cycles and thus, social status (Bertschinger *et al*. 2008). However, several factors indicate that this option is fraught with ethical concerns. Behavioural problems have been reported including high levels of aggression and social abnormalities (Foggin 2003; Scholes *et al*. 2007). The procedure is extremely difficult and impractical to reverse (D'Occhio 1993; Bertschinger *et al*. 2008) thereby hampering the populations' ability to recover from possible future epidemics of disease or poaching (Whyte 2003; Stout and Colenbrander 2004). The cost to carry out a vasectomy on one adult bull equates to between ZAR 50,000 to ZAR 75,000 (Grobler 2008). A sizeable percentage of sexually mature bulls would need to be treated to ensure a reduction in population growth rate and thus, this cost would be significant (Bertschinger *et al*. 2008). This high cost coupled with the negative side-effects may make many wildlife managers opt for a more viable alternative to reduce population growth.

2.5.6 Hormonal Contraception

Another non-lethal method for population control lies with hormonal contraceptives. By lengthening inter-calving intervals or inducing late age of first conception, population growth rates can be effectively reduced (Stout and Colenbrander 2004; van Aarde and Jackson 2007). An ideal contraceptive needs to have high efficacy and reversibility, be able to be delivered remotely, have no negative effects on health, social behaviour or integrity, be safe when administered to pregnant individuals, must not pass through the food chain, have reasonable duration of effectiveness, and be economical (Delsink 2006a; Bertschinger *et al*. 2008; Perdok *et al*. 2007). Various types of hormonal contraceptives have been applied to elephants, such as synthetic or natural steroidal hormones (Rutberg 1998) and non-steroidal gonadotropin releasing hormone (GnRH) (Bertschinger *et al*. 2004).

Steroid hormones, such as estrogens, androgens, progestagens, and testosterone have proven to be effective as contraceptives and can be delivered orally, by injection or implant (Rutberg 1996). However, the deleterious side effects they induce include behavioural anomalies, prolonged state of sexual attractiveness, separation of cows from their herd group and from their calves due to harassment by bulls, impairment of lactation, calf mortality, tumours, abortions, difficult births and heightened levels of aggression (Butler 1998; Rutberg 1998; Whyte *et al*. 1998; Whyte *et al*. 1999; Foggin 2003; Whyte 2003; Stout and Colenbrander 2004; Delsink 2006a; Delsink *et al*. 2006b; Bertschinger *et al*. 2008; Kirkpatrick 2007; Scholes *et al*. 2007). Furthermore, steroids are known to pass through the food chain posing risk to other wildlife species as well as humans (Kirkpatrick 2005; Bertschinger *et al*. 2008). Costs range between US $50 to $500 per individual (Kirkpatrick 2007). Due to all of the overwhelming negatives associated with steroid contraceptives, they should not be considered for use in wildlife species (Bertschinger *et al*. 2008).

GnRH super-agonists are a new group of hormonal contraceptives that act centrally at the level of the pituitary gland without affecting to any great extent peripheral reproductive organs (Trigg *et al*. 2006; Ludwig *et al*. 2009). They down-regulate the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) with the downstream effects of blocking gonadal function and thereby suppressing fertility (Trigg *et al*. 2006; Ludwig *et al*. 2009). However, it still remains to be shown if GnRH can be passed through the food chain (Rutberg 1998; Stout and Colenbrander 2004). GnRH implants require immobilization to administer thus is not viable for use in cows associated with breeding herds (Bertschinger *et al*. 2008).

2.5.7 Immunocontraception

The use of immunocontraceptives has been explored as a potential alternative for population management. With immunocontraceptives, reproductive function is controlled by immunisation against either the hormone, receptors or surface antigens depending on whether suppression of sexual behaviour or prevention of conception is desired (D'Occhio 1993). GnRH and pZP vaccination are two such options that are currently under review.

2.5.7.1 GnRH

GnRH vaccines have been introduced as an option for managing population growth and management of sex-related behaviours in domestic as well as wildlife species (Corrada *et al*. 2006; Bertschinger *et al*. 2008; Botha *et al*. 2008). GnRH antibodies formed in response to the vaccine neutralizes endogenous GnRH released from the hypothalamus, thereby preventing the release of LH and FSH to suppress testicular steroid and sperm production as well as ovulation (Figure 1) (Turkstra *et al*. 2003; Bertschinger *et al*. 2004; Botha *et al*. 2008).

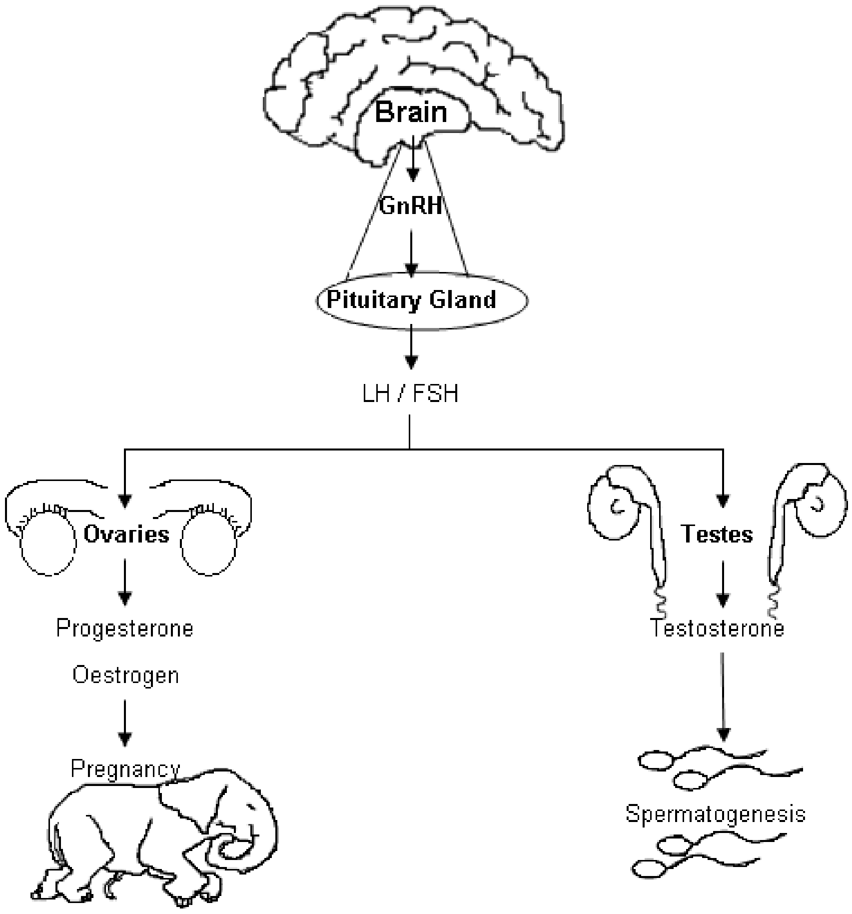


Figure 1. Endocrine control of GnRH on testicular and ovarian function modified from D'Occhio (1993).

A vaccination of GnRH has been shown to be fully reversible, remotely deliverable, inexpensive and readily available (Bertschinger *et al*. 2008). When applied to elephant bulls, the GnRH vaccine notably decreased aggressive behaviour and reduced faecal epiandrosterone concentrations which are the main faecal metabolite of testosterone in elephant bulls (Ganswindt *et al*. 2002; Bertschinger *et al*. 2004; Bertschinger *et al*. 2008). However, if only dominant bulls are treated, the risk of less genetically desirable bulls mating with cows and producing a weakened gene pool exists (Stout and Colenbrander 2004). GnRH treated bulls have shown a reduction in libido which then results in the need to administer testosterone implants or injections should managers wish to retain dominant bull competitive control over subordinate, weaker bulls (Delsink 2006a). GnRH has been recently applied to elephant cows but as yet, there are no conclusive results (Bertschinger *et al*. 2008). Because GnRH vaccines are known to induce anoestrous (Bertschinger *et al*. 2008), it avoids the potential for attracting bulls through repeated oestrous events but the effects on behaviour are still unknown. Wildlife managers must take into consideration all of the aforementioned positives and negatives regarding GnRH use, but its potential use as a population control mechanism warrant further investigation.

2.5.7.2 Porcine Zona Pellucida Vaccine

The use of the porcine zona pellucida (pZP) vaccine on elephants has been considered to be a publicly acceptable method for elephant population management (Fayrer-Hosken *et al.* 1997; Delsink, *et al*. 2002). Zona pellucida glycoproteins isolated from pig ovaries have been shown to be homologous to those of the African elephant (Fayrer-Hosken *et al.* 1999; Fayrer-Hosken *et al.* 2000). Once the pZP vaccine is administered intramuscularly in the elephant cow, it triggers anti-zona pellucida antibodies thus preventing fertilization by blocking sperm receptor sites on the elephant ovum (Butler 1998; Fayrer-Hosken *et al.* 1999).

In keeping with the idea of an ideal contraceptive, field studies at KNP and Makalali Private Game Reserve (MPGR) have shown the pZP vaccine to be effective (Rutberg 1996; Fayrer-Hosken *et al.* 1999; Bertschinger *et al*. 2004; Delsink 2006a); be reversible in the short-term (Fayrer-Hosken *et al.* 2000; Delsink *et al*. 2006c); be safe when given to pregnant cows (Rutberg 1998; Delsink *et al.* 2002; Bertschinger *et al*. 2004; Scholes *et al*. 2007); have no effect on calf rearing (Bertschinger *et al*. 2004) and it does not pass through the food chain (Butler 1998; Rutberg 1998; Kirkpatrick 2005). Because the pZP vaccine only targets the zona pellucida of the cow, theoretically there should be no influence on behaviour or on the reproductive cycle as is the case with hormonal contraceptives (D’Occhio 1993; Butler 1998; Bertschinger *et al*. 2004). Following extensive behavioural monitoring of MPGR herds vaccinated with the pZP vaccine, Delsink (2006a) also found no significant change in core and total range use, matriarchal status, cow/calf interaction, herd fragmentation/isolation, musth occurrence, bull hierarchy, and no aberrant or unusual behaviour witnessed among the herds. Due to the small amount (micrograms) required, the vaccine can be successfully delivered remotely (Butler 1998; Rutberg 1998; Bertschinger *et al*. 2004; Delsink *et al*. 2006c). Notable increases were found in herd avoidance to helicopters and the darting team, as well as initial disruption to herd movements following darting disturbance and oestrous events (Delsink 2006a) but herds were observed to resume normal behaviour and activity within a day following treatment (Bertschinger *et al*. 2008; Bates, personal observation). Delsink (2006a) reports that there was a significant decrease in bull association with herds treated with the pZP vaccine, and thereby refutes any negatives associated with increases in oestrous cycling.

As has been the case for all forms of population control options, pZP vaccination has also been subjected to critical scrutiny from the public. The possibility of unintentional sterilization as a result of continuous treatment with the pZP vaccine has raised concerns (Rutberg 1998; Whyte *et al*. 1998; Whyte *et al*. 1999; Stout and Colenbrander 2004; van Aarde and Jackson 2007). Ovarian anomalies have been reported in some rodents, primates and other mammalian species, but have yet to be reported in feral horses and elephants (Powell and Monfort 2001; Stoops *et al*. 2006; Perdok *et al*. 2007; Bertschinger *et al*. 2008). Resumption of fertility has been demonstrated where cows have been taken off treatment for durations of 3 to 5 years (Bertschinger *et al*. 2008). Behavioural observations have confirmed oestrous behaviour in treated cows since the earliest implementation of the pZP vaccination program in MPGR in 2000 (Bertschinger *et al*. 2008) as well as in Thornybush Private Nature Reserve (TPNR) in 2005 (Bates personal observation).

As pZP treatment relies on an immunological reaction in the target cow, it is feared that there is a potential for only healthy cows to be effectively prevented from breeding and thus unhealthy, immunocompromised cows will be allowed to create populations of a weaker genetic strain (Kirkpatrick 2005; Perdok *et al*. 2007). This has been refuted by the knowledge that elephants, as are other species, are subject to natural selection processes and unhealthy individuals are highly unlikely to survive under African conditions (Bertschinger *et al*. 2008). Additionally, pZP vaccination has been tested on physiologically stressed and sick animals and normal antibody titers to pZP were established in these animals (Kirkpatrick 2005).

The concept of new diseases being introduced via the pZP vaccine, a biological product prepared from pig ovaries', has also been brought forward (Perdok *et al*. 2007). However, the production of the vaccine is subject to numerous safety tests, involving the use of ovaries from healthy pigs, subsequent washing with buffer solution and exposure to high heat before testing for bacterial presence (Bertschinger *et al*. 2008). Viral material surviving this rigorous process would still be specific to the pig species and as yet has not produced a new disease in over 80 different species that have been treated with the pZP vaccine to date (Bertschinger *et al*. 2008). Nevertheless, synthetic subunit zona pellucida vaccines are currently being examined to reduce the risk of micro-organism transmission as well as lower the risk of ovarian pathology (Perdok *et al*. 2007).

Altering reproductive rates also has the potential to drastically modify age, sex and social structures of breeding populations (Bertschinger *et al*. 2008; van Aarde and Jackson 2007). Over time with continuous contraceptive treatment, the population as a whole will experience an aging effect with a higher ratio of individuals represented in the oldest age groups (Bertschinger *et al*. 2008). Based on population modelling, it has been shown that bull mortality rates are higher than those for cows and thus sex ratios could also be affected (Bertschinger *et al*. 2008). The social, behavioural and ecological implications associated with these factors are unknown at present and requires further study (Bertschinger *et al*. 2008).

The only visible physical side effect of pZP treatment is the presence of slight swellings or abscesses at the injection site (Delsink *et al*. 2002). The reaction seen at the injection site is thought to be related to the adjuvant used (Rutberg 1998; Bertschinger *et al*. 2004; Kirkpatrick 2007) and since the switch to a less aggressive adjuvant, the number of swellings observed has decreased significantly (Bertschinger *et al*. 2008). The cause of the swellings are also thought to be more likely a result of mechanical introduction of bacteria, commonly present in water or dust, via dart needles (Bertschinger *et al*. 2008). In any case, all swellings visible after the inoculation date were reabsorbed over time with no obvious discomfort or other physical detriment being observed to the animal (Delsink *et al*. 2002; Bertschinger *et al*. 2008; Bates, personal observation).

The costs for implementing a pZP vaccination program range from ZAR 520 to ZAR 1,000 per animal inclusive of darts, vaccine, veterinary fees, and helicopter rental (Delsink *et al*. 2007). The biggest expenses are associated with helicopter rental, specifically the costs to ferry the helicopter to the project site, with a cost of approximately ZAR 3,800 per hour (Bertschinger *et al*. 2008). In game parks with smaller populations, the use of ground darting is more viable and would significantly reduce costs.

As with the aforementioned types of contraceptives, pZP treatment of a population results in longer inter-calving intervals and increases the age of first conception resulting in slower population growth rates (Stout and Colenbrander 2004; van Aarde and Jackson 2007). Vaccination with pZP has been shown to reduce population growth by as much as 33 per cent over a 10 year period (Delsink *et al*. 2006c). However, it can only be utilized as a mid- to long-term strategy to reduce population pressures as it depends on natural mortality and thus is ineffective at immediately curbing already prevalent ecological damage (Foggin 2003; Stout and Colenbrander 2004; Scholes *et al*. 2007; van Aarde and Jackson 2007). Thus, pZP treatment is more aptly used as a preventative measure for overpopulation rather than a quick fix (Bertschinger *et al*. 2008).

With all of these issues surrounding the ethics and safety of pZP vaccine use as a population management tool, it is clear that future study is required. However, the promising indications of its potential merit it being taken seriously as a possible solution to overpopulation.

2.6 Reproduction Monitoring Techniques

2.6.1 Oestrous Cycle Length and Behavioural Oestrous

In the female African elephant (*Loxodonta africana*), oestrous cycle length has been reported to last between 13 to 17 weeks, involving a 4 to 6 week follicular phase followed by a 8 to 11 week long luteal phase (Plotka *et al*. 1988; Wasser *et al*. 1996; Heistermann *et al*. 1997; Hodges *et al*.

1997; Hodges 1998; Fieβ *et al.* 1999; Brown 2000; Ortolani *et al*. 2005; Brown 2006). The end of the follicular phase is followed by ovulation and coincides with maximum male interest and female receptivity (Hodges 1998; Ortolani *et al*. 2005; Bagley *et al*. 2006). Behavioural oestrous is described as lasting from 2 to 6 days (range 2 to 10 days) (Moss 1983) and is characterized by loud vocalizations, wariness towards bulls, oestrous walk (head held high, back arched, and tail raised), olfactory and tactile interactions between bulls and cows, oestrous chase, mounting, and consort behaviour (Moss 1983; Ortolani *et al*. 2005).

2.6.2 Endocrinology of the Ovarian Cycle in the African Elephant

The endocrine profiles described in association with the ovarian cycle in the African elephant include: a) luteinizing hormone (LH), b) follicle-stimulating hormone (FSH), c) inhibin, d) prolactin, e) oestrogen and f) progesterone (Hodges 1998; Brown *et al*. 2004b; Brown 2006).

a) Luteinizing Hormone.

During the follicular phase, two types of LH surges, 3 weeks apart, have been reported in the African elephant, the second of which triggers ovulation and the formation of the corpora lutea (CL) (Plotka *et al*. 1988; Kapustin *et al*. 1996; Hodges 1998; Brown 2000; Rasmussen 2001; Brown *et al*. 2004b; Ortolani *et al*. 2005; Brown 2006). At present, there is no explanation for the repeated LH peaks though it has been postulated that it may be related to the presence of multiple CL found in the African elephant (Hodges 1998; Brown 2000). Potentially, the 1st surge initiates the formation of accessory CL which becomes active later in the cycle in releasing more reproductive hormones essential to perpetuating the ovarian cycle, such as a progesterone increase that triggers ovulation (Brown 2000; Brown 2006). Additionally, it has been shown that bull interest coincides with both LH surges (Ortolani *et al*. 2005) and this may indicate that the first LH surge may be a method for cows to attract bulls prior to ovulation in order to gain a greater selection in mates as well as to ensure bull presence when the cow becomes receptive (Brown 2000; Brown 2006).

b) *Follicle-Stimulating Hormone*.

FSH concentrations are highest at the beginning of the follicular phase/end of luteal phase, then drop 4 days prior to the 2nd LH surge, and begin increasing again at the commencement of the luteal phase (Brown *et al*. 2004b; Brown 2006). This drop in FSH is believed to play a role in dominant follicle selection and also triggers the onset of a series of follicular development waves (Hodges 1998; Brown 2000; Brown *et al*. 2004b; Brown 2006). FSH also serves to stimulate oestrogen production in a negative feedback mechanism whereby high concentrations of oestrogen, along with inhibin, then suppresses FSH in order to prevent maturation of under-developed follicles to allow for the selection of a dominant follicle (Brown 2006).

c) *Inhibin*

Inhibin is released from the ovaries and acts on the pituitary gland to suppress FSH release. The primary function of inhibin appears to be the suppression of FSH which is revealed by its inverse relationship in serum concentrations to FSH concentrations (Brown 2006).

d) *Prolactin.*

Prolactin concentrations are observed to increase during the follicular phase and are thought to also contribute to follicular development (Brown *et al*. 2004b). In other species, elevated prolactin concentrations during the follicular phase is a result of positive feedback of oestrogen, however, no such relationship has been confirmed in the African elephant (Brown *et al*. 2004b).

e) *Oestrogen.*

The activity and function of oestrogen in the ovarian cycle of the African elephant is poorly understood as concentrations remain low throughout the cycle and patterns are difficult to detect (Wasser *et al*. 1996; Hodges 1998; Fieβ *et al*. 1999; Brown 2000; Brown *et al*. 2004b; Brown 2006). However, oestrogen increases have been reported to precede each of the two LH surges in the follicular phase and thus likely play a role in triggering LH release (Hodges 1998; Brown 2000; Brown *et al*. 2004b).

f) Progesterone.

A progesterone increase released from the CL 3 to 4 days prior to the second LH surge is thought to be responsible for ovulation and marks the onset of the luteal phase (Plotka *et al*. 1988; Hodges *et al*. 1997; Hodges 1998; Fieβ *et al*. 1999). In Asian elephants, the slow decrease in progesterone concentrations is associated with a release of a pre-ovulatory pheromone that attracts bulls for mating (Rasmussen 2001). While such a specific pheromone has not been established in African elephants, evidence for pheromones has been established through field trials demonstrating heightened interest from both bulls and cows in urine sampled during the 2nd LH peak (Rasmussen 2001; Bagley *et al*. 2006; Meyer *et al*. 2008). Thus, progesterone may also function in releasing chemosensory signals by negative feedback methods to entice bulls at time of ovulation. Progesterone metabolites have been widely accepted as the major luteal and circulating progestins in the African elephant and are thus considered to be the leading method for monitoring the ovarian function (Heistermann *et al*. 1997; Hodges 1997; Fieß *et al*. 1999). Monitoring of progesterone concentrations throughout the ovarian cycle has proven difficult, albeit possible (Brown *et al*. 2004b), due to very low quantities in circulation, but the presence of 5α-reduced progestin metabolites in higher concentrations allows for more efficient monitoring (Hodges *et al*. 1997; Hodges 1998).

2.6.3 Quantitative Measurement of Progesterone Metabolites

As progesterone is metabolized to pregnanes prior to excretion in faeces (Schwarzenberger *et al*. 1995), the non-invasive detection of these pregnanes in faeces has proved to be a valuable tool for monitoring ovarian function and detecting pregnancy in the African elephant (Wasser *et al.* 1996; Fieβ *et al.* 1999) and correspond to results in studies of progestins circulating in the blood (Plotka *et al*. 1988; Hodges *et al.* 1997; Hodges 1998; Brown *et al*. 2004b). Previous studies on hormonal control of the ovarian cycle in the elephant have revealed that the 5α-reduced metabolites, 5α-pregnane-3,20-dione (5α-DHP) and 3α-hydroxy-5α-pregnan-20-one (5α-P-3α-OH) are the predominant progestins found in circulation in the African elephant: 5α-DHP dominant in the blood and 5α-P-3α-OH more prevalent in the urine and faeces (Wasser *et al*. 1996; Heistermann *et al*. 1997; Hodges *et al*. 1997; Hodges 1998; Fieβ *et al*. 1999; Brown 2000;

Brown 2006; Wittemyer *et al*. 2007). Faecal 5α-P-3α-OH was shown to provide the most reliable means of following cyclic patterns as it maintains a more stable baseline, reveals a greater luteal/follicular differential, has a more detectable initial luteal phase increment, as well as has better correlation with urine and serum results when compared to faecal 5α-DHP (Heistermann *et al*. 1997; Hodges 1998; Fieβ *et al*. 1999). Faecal sampling spaced out as widely as monthly intervals have allowed Wittemyer *et al.* (2007) to interpret progesterone profiles, leading to reliable information on ovarian cycles and avoids utilizing invasive methods that are difficult to employ in wild, free-ranging animals. Generally, determination of progestagen concentrations in the faeces is accomplished through enzyme immunoassay (EIA). The examination of ovarian function through faecal progestagen EIA rather than radioimmunoassay is used as it avoids the use of hazardous radioactive materials and cumbersome equipment and is less costly (Graham *et al*. 2001). EIA detects the amount of an antigen present in a sample by using an enzyme-bound antibody (Lequin 2005). Varying antibody cross-reactivities with circulating pregnanes allows for the examination of luteal activity (Brown 2006). The particular EIA utilized in this study uses a double-antibody technique where a microtitre plate is coated with a primary antibody and a second antibody is added which is recognized by and binds with the primary antibody (Graham *et al*. 2001; Ganswindt, personal communication). Competition for binding sites on the secondary antibody between the sample progestagen molecules and antigen-enzyme complexes follows and a resulting chemical reaction generates a coloured derivative. Following this, optical density measurement is used to determine the sample’s progestagen concentration (Ganswindt, personal communication).

Chapter 3: MATERIALS AND METHODS

3.1 Study Site

Thornybush Private Nature Reserve (24°23’ to 24°33’S, 31°05’ to 31°13’E) is situated in the Lowveld of the Northern Province of South Africa. The reserve in its entirety totals an area of 11,548 ha (Figure 2). The reserve is boarded in the north by a small private game reserve, in the east by the Timbavati Game Reserve and Sandringham, the south by the Orpen road, and in the west by the Guernsey road and Kapama Game Reserve. Two river systems penetrate the reserve, namely the Monwana and Timbavati. Trees that dominate the area include *Acacia gerardii*, *Albizia harveyii*, *Combretum* species, *Dichrostachys cinerea*, *Euclea* species, *Grewia* species, *Pterocarpus rotundifolius*, and *Terminalia* species (Bates personal observation). Annual rainfall fluctuates greatly from year to year with a mean of 601 mm falling within the months of October to April with the remainder of the year being dry (Peel 2005). Altitude varies between approximately 560 to 600 meters above sea level.

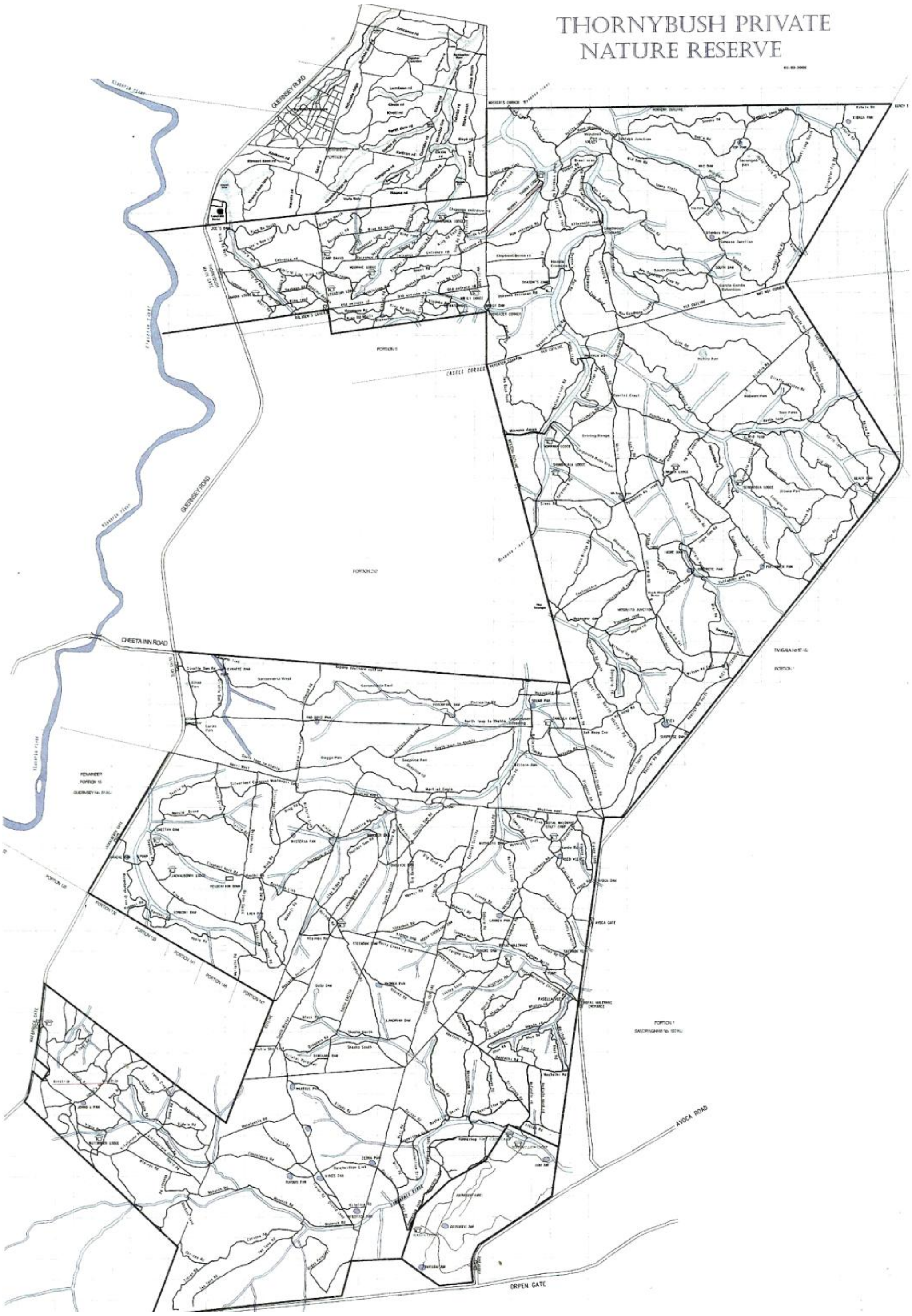


Figure 2: Map of Thornybush Private Nature Reserve (11,548 ha).

3.2 Study Population

In May 1994, eight elephants (all females) were relocated from the northern portion of KNP known as the Shingwedzi area. Four of these were of breeding age and were suspected to be pregnant at time of relocation. One nomadic bull, origin unknown, was sold to the Knysna Elephant Park in 1995 and in June 1998, one 40-year-old bull was added to the total population, also from KNP. To date, the Shingwedzi herd has increased to a total of 38 elephants plus 2 free-roaming bulls. The Thornybush population has been monitored every weekday from September 2005 to present by field researcher Melodie Bates. The herd splits into 3 family groups, reuniting approximately 50% of the time (Bates, unpublished data, Appendix A). The bulls consists of one 52 year old bull (introduced in 1998), and one approximately 20 year old bull. Complete identification kits, incorporating tusk shapes and sizes, ear markings and ear venation patterns, have been made for all individuals in the Thornybush population and for both sexes, which allows for individual recognition (Bates, unpublished data; Delsink 2006a). Age distribution was determined using a combination of known dates of birth, and rough estimates based on shoulder height and age correlation as compared to known adults in the population (Hanks 1979; Appendix B).

3.3 pZP Vaccine Treatment

As the lowest recorded breeding age in the African elephant is reported to be eight years (Garai *et al*. 1999), all cows of reproductive age at TPNR received three initial immunizations in order to build up antibody levels of pZP in 2005. These were delivered remotely from helicopter using Type P Pneu-darts fitted with 1 ½ inch needles and gelatine collars (inject and mark; Pneu-dart, Inc. 15223 Route 87 Highway, Williamsport, PA 17701). The darts were delivered from a Dan-Inject CO_2 dart gun (Dan-Inject ApS, Sellerup Skovvej 116, Børkop, Denmark) fitted with a modified barrel to accommodate the Pneu-darts. A primary dose of 400 µg pZP protein in 1 mL phosphate buffer saline (PBS) plus 0.5 mL of Freund’s Modified Complete Adjuvant was administered in May 2005. This was followed by two boosters of 200 µg pZP protein each in 1 mL PBS plus 0.5 mL of Freund’s Incomplete Adjuvant administered in June 2005 and August

2005 respectively. The Thornybush breeding population received its first annual booster of 200 μg pZP protein in 1 mL PBS plus 0.5 mL of Freund's Incomplete Adjuvant in September 2006 and subsequent equivalent boosters in September 2007, 2008, and 2009.

3.4 Experimental Procedures

3.4.1 Behavioural Data

Data collected from September 2005 to present with regards to herd composition, movements, oestrous and musth occurrences was incorporated into the study. Elephants were tracked daily by means of radio contact from field guides giving last reported location of herd, as well as fresh footprints and dung. Observation time was limited to hours between morning and afternoon game drives, i.e. between 9h00 to 15h30. Game drives occasionally occurred between this interval and these vehicles were given right of way as per agreement with TPNR. The use of a 4x4 research vehicle was issued by TPNR and has been used consistently to monitor the herds from 2005 to 2009, thus the vehicle remains a constant factor present at times of observation and was not considered to bias results.

Behavioural data was recorded by completing daily re-sightings records and completing focal sheets of 15-minute intervals of randomly chosen individuals beginning in March 2007 and continuing for one year duration (Appendix C & D). Oestrous or musth occurrences observed (as described in Moss, 1983 and Poole, 1987) in any individuals were marked on the daily re-sightings sheet. Additionally, video camera footage of behavioural activity for 2 days following darting event (conducted by two assistants) was taken and focal sheets generated.

The use of 15-minute interval focal sheets, where an animal's activity is recorded every minute for 15 minutes, is based on a method established by Pulliam and Caraco (1981) and Moss (1988) where time is used as a currency of the animal's behaviour. The specific method used is described as Focal-Animal Sampling (Altmann 1974) and codes representing specific activities commonly noted among elephant populations were used (Appendix E). This method enables the calculation of general daily activities (e.g. feeding, bathing, resting, drinking, walking), which can be used to examine changes in behaviour following the darting event and also serve as an alert to increases in stress.

3.4.2 Faecal Sample Collection

From March 2007 to February 2008, dung samples, not older than 30 minutes (Ganswindt, personal communication) were collected on a weekly basis, or as close to weekly as possible, whenever present, from known pZP-treated individuals following observed defecation once the individual moved to a safe distance. As precipitation is known to affect the accuracy of faecal steroid metabolite concentrations (Millspaugh and Washburn 2004), faecal boluses found in water or disturbed by heavy rainfall were not collected.

A homogenous sample was taken from the centre of the bolus or from the centres of various boluses if more than one was present, and stored in a 10 mL glass vial with lid (Wasser *et al.* 1996; Burke 2005). An example of the faecal sample record sheet is attached (Appendix F).

Samples were then stored in an insulated cooler box immediately after collection and then frozen at –20^{o}C at the end of the daily observation period. All samples were kept frozen in insulated cooler boxes with cold packs and transported to the University of Pretoria for analysis.

3.5 Sample Analysis

3.5.1 Faecal Sample Processing

Faecal samples were lyophilized for 48 hours at -54 degree Celsius in an Instruvac (Air and Vacuum Technologies; Model No. VFDT02.50) freeze-dryer with vacuum set at approximately 672 Torr. Following lyophilization, samples were pulverized by hand and sieved through a nylon mesh to separate faecal powder from any existing fibrous material (Wasser *et al.* 1996; Fieβ *et al.*

1999; Ganswindt *et al*. 2002; Ganswindt *et al.* 2003; Ganswindt *et al.* 2005a). A weighed amount (± 50 mg) of faecal powder was extracted with 3 mL of 80% ethanol in distilled water. The mixture of faecal powder and 80% ethanol was then placed in a shaker for 15 minutes prior to centrifugation at 3000 RPM for 10 minutes. The resulting supernatant fluid was then transferred to Eppendorf tubes for measurement of immunoreactive progesterone metabolites via microtiterplate enzyme immunoassay described in detail by Graham *et al*. (2001), Ganswindt *et al*. (2002), and Wittemyer *et al.* (2007).

3.5.2 Enzyme Immunoassay

Microtiter plates were coated with buffer solution (6.67 mg coating-IgG + 1.59 g Na_2CO_3 + 2.93 g $NaHCO_3$ ad 1 L H_2O; pH 9.6) plus an additional coating buffer of 150 µL per well (1 µg coating-IgG + 0.48 mg Na_2CO_3 + 0.88 mg $NaHCO_3$). Following the coating process, the plates were saturated with bovine serum albumin (BSA) (8.5 g NaCL + 3 g BSA + 5.96 g Na_2HPO_4 ad 1 L H_2O); pH 7.2; add 150 µL per well) and utilized antibodies raised in rabbits against 5α-pregnan-3β-ol-20-one-3-HS-BSA and had a 5α-pregnan-3β-ol-20-one-3-HS-peroxidase label. The major cross-reactivities for 5α-pregnan-3β-ol-20-one include: 5α-pregnan-3α-ol-20-one, 650%; 5α-pregnan-3β-ol-20-one, 100%; 4-pregnen-3,20-dione, 72%; 5α-pregnan-3,20-dione, 22%; and <0-1% for 5β-pregnan-3α,20α-diol, 4-pregnen-20α-ol-3-one, 5β-pregnan-3α-ol-20-one, 5α-pregnan-20α-ol-3-one, 5α-pregnan-3β,20α-diol, and 5α-pregnan-3α,20α-diol (Szdzuy *et al*. 2006). Dilutions of each sample ranged from 1:20 to 1:200 of extract to assay buffer solution (8.5 g NaCl + 1 g BSA + 5.96 g Na_2HPO_4 and 1 L H_2O; pH 7.2). Initially, 50 µL aliquots of the diluted faecal sample, standards (linear range 3.12 – 50 pg/well) and quality controls were pipetted in duplicate into each of the coated microtiter plate wells. The plates were incubated overnight at 4^oC, then washed four times with buffer solution (9.6 L ad 1 L H_2O + 0.05% Tween 20 + 400 mL PBS-solution (0.136 mol NaCl + 9.1 mmol $NaPO_4$ + 2.7 mmol KCl + 1.5 mmol KH_2PO_4 ad 2 L ad 1 L H_2O; pH 7.2). 150 µL of peroxidise substrate solution was added and subjected to another 30-60 minute incubation period. The addition of 50 µL of H_2SO_4 (2 mol/L) was added to irreversibly stop the enzyme reaction. Optical density was measured at 450 nm.

4-pregnen-3,20-dione, commonly known as progesterone, was used as a standard and serial dilutions of faecal extracts gave displacement curves that were parallel to the respective standard curves. The sensitivity of the assay at 90% binding was 3 pg per well. Inter- and intra-assay coefficients of variation ranged between 8.0% and 17.6% for the progestagen measurements. To adjust for water content variations, faecal hormone concentrations were expressed as mass/g dry weight.

3.6 Data Analysis

Progestagen concentrations were expressed as µg/g dried faeces and plotted against time (weeks) for each pZP-treated cow. Data were then compared descriptively and statistically for each individual as variability in progestagen concentrations between individual females has been well-documented (Wasser *et al*. 1996; Fieβ *et al.* 1999). Baseline values of progestagen concentrations were ascertained for each female using an iterative process where all values greater than the mean plus 2 standard deviations (SD) were removed (Brown *et al*. 1999; Moreira *et al*. 2001; Powell and Monfort 2001; Brown *et al*. 2004b; De Haas van Dorsser *et al*. 2007). The average was subsequently recalculated and the elimination process was repeated until there were no values greater than the mean plus 2 SD. The remaining values yielded the baseline progestagen concentration. Ovarian cycle length and periodicity was then determined by measuring weekly concentrations of progesterone metabolites where the first point rise in progestagen concentrations above baseline and remaining above baseline for at least two consecutive weeks marked the beginning of the luteal phase (LP) (Brown *et al*. 2001; De Haas van Dorsser *et al*. 2007). The commencement of the follicular phase (FP) was defined as the first of two consecutive progestagen concentrations falling below baseline concentrations. (Brown *et al*. 2001). The sum of the FP and LP yields the cycle length of the individual (Fieβ *et al.* 1999). Phase length estimates as well as ovarian cycle length estimates are given as mean ± standard deviation (SD). Females were categorized as having an irregular cycle when overall cycle length exceeded or fell short of the reported norm of 13 to 17 weeks (Plotka *et al*. 1988; Wasser *et al*. 1996; Heistermann *et al*. 1997; Hodges *et al*. 1997; Hodges 1998; Fieβ *et al.* 1999; Brown 2000;

Ortolani *et al*. 2005; Brown 2006; Bertschinger *et al*. 2008). The non-cycling category was reserved for females who had random fluctuations in progestagen concentrations throughout the luteal or follicular phases (Brown *et al*. 2004b). Due to small sample sizes, irregularly cycling females were combined with non-cycling females for statistical analysis. Individuals were categorized as demonstrating periods of anoestrous if they had a follicular phase lasting longer than twice the duration of an average normal follicular phase (5 weeks in the African elephant; range 4-6 weeks) (Brown *et al*. 2001; Brown 2006). Determination of pregnancy within treated cows was based on luteal phase lengths persisting longer than 3-5 months (Hodges 1998; Fieß *et al*. 1999). Increases in progestagen concentrations following the end of the FP indicate the ovulatory period which has been reported to coincide with maximum male interest and mating (Hodges 1998). As behavioural oestrous has been reported to last from 2 to 6 days (Bertschinger *et al*. 2008), behavioural oestrous was compared with the time of progesterone metabolite increase.

To determine the effect of age, dominance/rank, reproductive status, the darting event and seasonal influences on faecal progestagen concentration, individual averages were first tested for normality using Shapiro-Wilks where a probability value greater than 0.05 gave a distribution considered to be normal. Data that were normally distributed were analysed using Student's t-tests while data not meeting the normal distribution test were analysed using Wilcoxon Rank Sum Test (Table 1 and 2). Spearman Rank Correlation was used to assess the effects of seasonal rainfall on progestegen concentrations. A two-tailed Fisher's Exact Test was used to test correlation between cyclicity status and age, dominance, parity and lactational status (Bland and Altman 1994; Lowry 2000). Statistical significance was assumed when $P < 0.05$. Age groups were defined as adult (12+ years) (n=377 for 9 individuals), or non-adult (6-11 years) (n=219 for 5 individuals) (see also Appendix A). 1 immature female (*Ziggy*) was grouped in with the sub-adults to form the non-adult category to maximize sample size for a more accurate correlation test. Dominance/rank matrices were calculated using methods described by Archie *et al.* (2006) and Schulte *et al.* (2000) where aggressive interactions (charges, chases, displacements, poke, pushes, and supplants) captured from September 2005 to present were incorporated to determine dominance/rank between individuals. When no interactions were observed between females, rank was based on body size (Sikes 1971; Dublin 1983, Foley *et al.* 2001; Freeman *et al.* 2004).

Seasons were defined as wet (October-April) and dry (May-September) (Peel 2005). Statistical analyses were performed using the software programs OpenStat (Miller 2009) and KyPlot (Version 2.0 beta 13 1997). Data are presented as means ± SD.

Table 1. Statistical tests used for examining the correlation between individual average progestagen concentrations and age, dominance/rank, reproductive status, seasonal effects, and darting event.

Statistical Test Used	Age		Dominance/ Rank		Reproductive Status				Seasonal		Darting	
	Adult	Sub-Adult	Dominant	Subdominant	Parous	Nulliparous	Lactating	Non-Lactating	Wet	Dry	Before	After
t-Test							8 (n=330)	6 (n=266)				
Wilcoxon	9 (n=377)	5 (n=219)	9 (α-herd) (n = 419)	5 (β- & γ-herd) (n = 177)	9 (n=377)	5 (n=219)					11 (n=493)	11 (n=493)
			5 (n=227)	5 (n=219)								
Spearman									14 (n=596)	14 (n=596)		

Table 2. Statistical tests used for examining the correlation between cyclicity status of individuals and age, dominance/rank and reproductive status as well as the correlations between cyclicity status and individual progestagen concentrations in the wet and dry season

Statistical Test Used		Age		Dominance/ Rank		Reproductive Status				Seasonal	
		Adult	Sub-Adult	Dominant	Subdominant	Parous	Nulliparous	Lactating	Non-Lactating	Wet	Dry
Fisher's Exact	Cycling	5	1	4	1	5	1	4	2		
	Non-Cycling	4	4	1	4	4	4	4	4		
t-Test	Cycling									6 (n=263)	6 (n=263)
Wilcoxon	Non-Cycling									8 (n=333)	8 (n=333)

Chapter 4: RESULTS

4.1 Oestrous Cycle Length

Of the 19 elephant females sampled during the study period, 5 cows were removed from the data set on the basis of having faecal sample sizes less than 30. These low sample sizes contributed to large gaps in the weekly data preventing any inferences regarding oestrous cycles or luteal/follicular phase lengths.

All remaining females gave evidence of luteal activity as in all cases, progestagen concentrations exceeded baseline more than once during the year of study (Appendix G). In 42.9% of the sampled females (n=6), no cyclic pattern in progestagen concentrations could be detected (Figure 3). 14.3% (n=2) had an irregular cycle pattern lasting longer than the reported maximum of 17 weeks (range 17.43 – 20.43 weeks) (Figure 4), and 42.9% (n=6) showed at least one complete oestrous cycle within the year of study. Acyclic periods lasting 18.68 ± 5.94 weeks (range = 13.14 to 24.57 weeks) were detected in four females (28.6%), *Dana*, *Kombela*, *Madam M*, and *One Tusk* (Figure 5).

A total of 8 complete oestrous cycles were detected in the data set (Appendix G). Within cycling females, 66.7% (n=4) had one cycle falling within the normal 13-17 week oestrous cycle range, 16.7% (n=1) had 2 complete cycles during the study period, and 16.7% (n=1) had 3 consistent cycles throughout the study period (Figure 6). Mean oestrous cycle length was 14.72 ± 0.85 weeks with a luteal phase of 8.89 ± 1.38 weeks and a follicular phase of 5.82 ± 1.44 weeks (Table 3). 50.0% (n=3) of the cycling females showed longer than expected follicular phase lengths while 16.7% (n=1) had a shorter than expected luteal phase. Average baseline concentrations for all females were 1.21 ± 0.37 µg/g. Average progestagen concentrations were 1.61 ± 0.46 µg/g. Peak luteal phase concentrations ranged from 2.71 to 9.96 µg/g with the highest two concentrations found in the two females with irregular cycle lengths. There was no significant difference in baseline ($p > 0.05$) or average ($p > 0.05$) progestagen concentrations between cycling and non-cycling females.

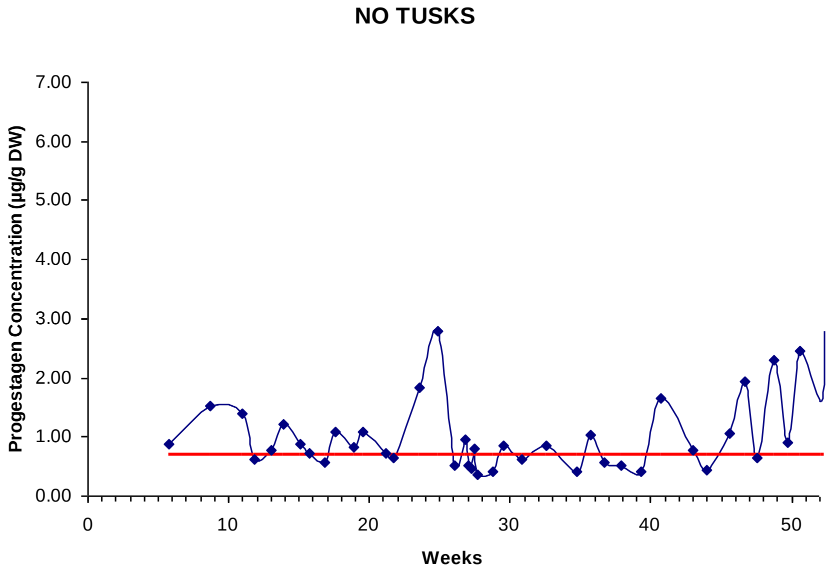


Figure 3. Faecal progestagen concentrations for a non-cycling adult African elephant female treated with the pZP vaccine. Red solid line represents baseline concentration.

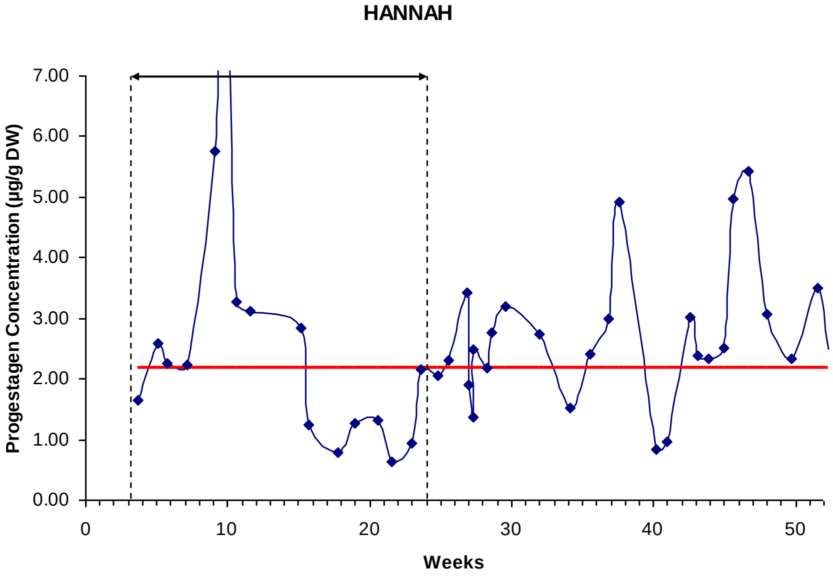


Figure 4. Faecal progestagen concentrations for a sub-adult African elephant female treated with the pZP vaccine demonstrating an irregular cycle of 23.43 week duration. Red patterned line represents baseline concentration and two-way arrows illustrate cycle length.

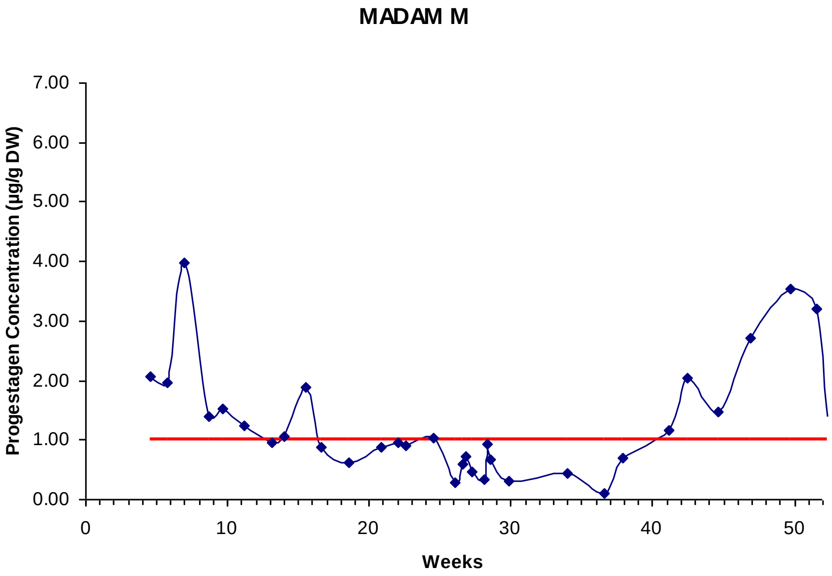
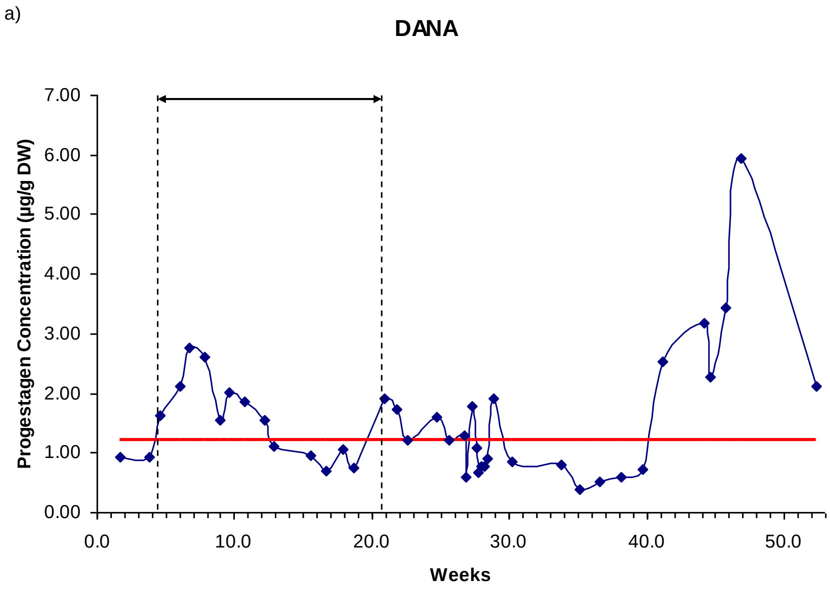


Figure 5. Faecal progestagen concentrations for an African elephant female treated with the pZP vaccine demonstrating an acyclic period lasting approximately 24.5 weeks. Red solid line represents baseline concentration.



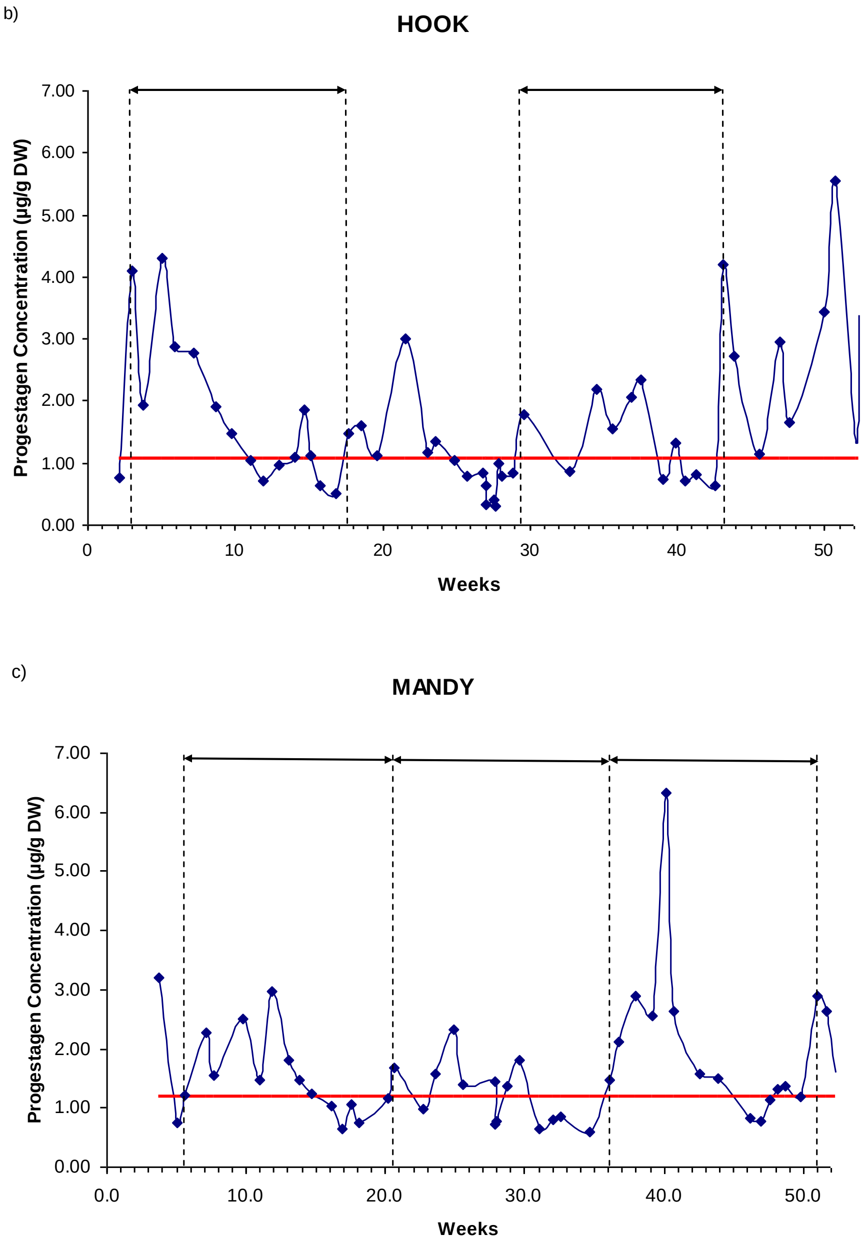


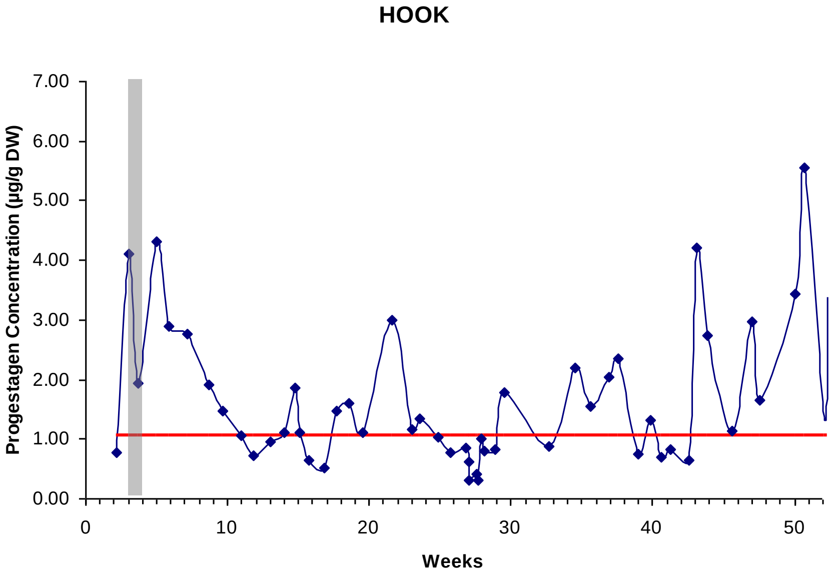
Figure 6. Faecal progestagen concentrations for cycling adult African elephant females treated with the pZP vaccine depicting a) one complete cycle, b) two complete cycles, and c) three complete cycles. Dotted black lines represent onset of successive luteal phases and two-way arrows represent the length of one complete oestrous cycle. Solid red line illustrates baseline concentrations. *Hook's* two complete cycles (b) were separated by a shorter cycle of 11.86 weeks and was thus not considered to be another full oestrous cycle.

Table 3. Baseline, mean, and phase lengths ± SD of faecal progestagen concentrations in cycling pZP-treated African elephant females at Thornybush Private Nature Reserve, South Africa

Name	n	Baseline (µg/g DW)	Mean (µg/g DW)	Luteal phase (weeks)	Follicular phase (weeks)	Cycle length (weeks)
Dana	41	1.22 ± 0.54	1.54 ± 1.03	8.29	8.00	16.29
Hook	52	1.06 ± 0.44	1.66 ± 1.17	8.72 ± 1.01	5.43 ± 1.82	14.14 ± 0.81
Mandy	47	1.19 ± 0.36	1.64 ± 1.00	10.33 ± 0.30	4.81 ± 0.36	15.14 ± 0.38
One Tusk	51	1.04 ± 0.40	1.39 ± 0.88	8.14	5.71	13.86
Rex	37	1.14 ± 0.45	1.29 ± 0.61	8.86	5.43	14.29
Thembisa	35	1.43 ± 0.52	1.53 ± 0.64	6.29	8.00	14.29
TOTAL	263					
Mean ± SD		1.18 ± 0.14	1.51 ± 0.14	8.89 ± 1.38	5.82 ± 1.44	14.72 ± 0.85

4.2 Oestrous Behaviour

Oestrous behaviour was noted on 3 occasions during the study and coincided with the onset of the luteal phase and a subsequent rise in progestagen concentrations above baseline (Figure 7). In all instances, oestrous chase and wariness were recorded although actual mating was not observed as the oestrous chase ended out of line of sight.



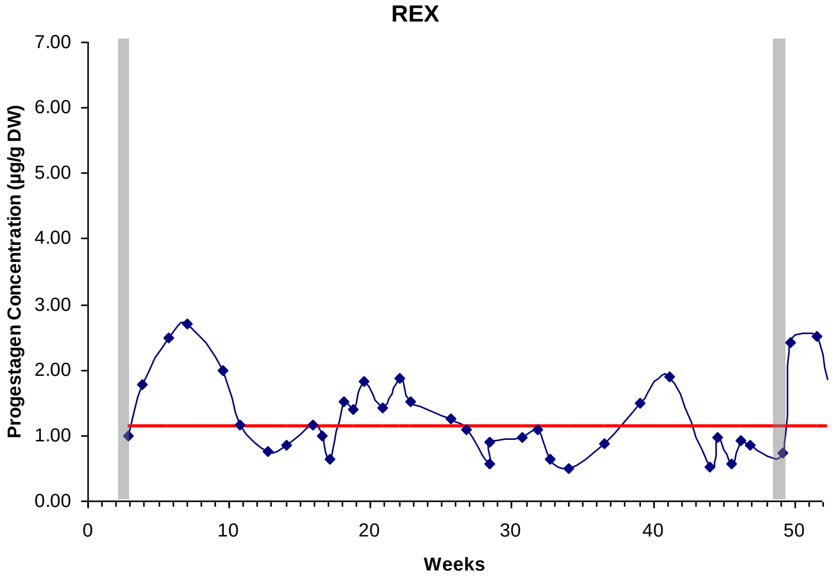


Figure 7. Faecal progestagen concentrations in pZP-treated African elephant females measured for one year duration in one adult (*Hook*) and one sub-adult (*Rex*) showing behavioural oestrous. Progestagen concentrations are shown in blue and baseline concentration is shown as a red solid line. Periods of behavioural oestrous are shown as vertical grey shaded bars.

4.3 Age

While adults had slightly higher average progestagen concentrations than non-adults, there was no statistically significant difference ($p > 0.05$) between adult (1.61 ± 0.46 µg/g) and non-adult (1.54 ± 0.65 µg/g) categories (Figure 8).

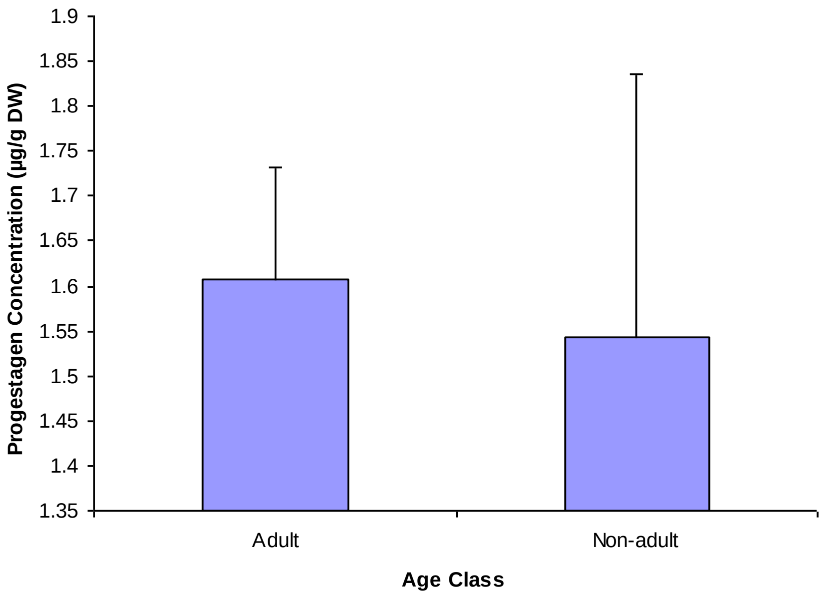


Figure 8. Average progestagen concentrations for adult and non-adult pZP-treated African elephant females from March 2007 to February 2008 at Thornybush Private Nature Reserve, South Africa.

Within those females who demonstrated evidence of oestrous cycles, 5 (83.3%) were adults and only 1 (16.7%) fell into the non-adult category. Females that had no pattern of oestrous cycles were divided evenly (50%, n=4) between adult and non-adult categories. No statistical association ($p > 0.05$) between age and cycling and non-cycling females was detected.

4.4 Dominance/Rank

Within the Thornybush elephant population, behavioural observations have revealed that the three herd groups are dominated by three matriarchs, namely *Flo* (α-herd), *Kombela* (β-herd), and *Thembisa* (γ-herd). On rare occasions (witnessed a total of 8 times during the study period), the α-herd splits into two groups in which case *Flo* led a portion of the herd and *One Tusk* presided over the remaining. A dominance matrix revealed that *Flo*, *One Tusk*, and *Thembisa* were the most dominant females of the population while *Rex* and *Ziggy* ranked the most subdominant (Table 4). *Ziggy* was also the youngest female of the study females examined.

Table 4. Dominance matrix determined for number of agonistic interactions among African elephant females in Thornybush Private Nature Reserve from September 2005 to February 2008. Aggressors are represented in the rows while the recipients are seen in the columns. Rank was ascertained from most (*Flo*) to least dominant (*Ziggy*) based on number of agonistic interactions and body size.

Cow	FL	OT	TH	MN	HO	KH	KO	MM	DA	HA	SU	NT	RE	ZI
FL	■			3		1			1		3	2		2
OT		■		4		1			1	4	1	2		5
TH			■										4	
MN				■		2	3	1	2	4	2			1
HO					■	1					5		1	
KH						■				2	2			1
KO							■		6	1				
MM								■	2	1				
DA									■					
HA										■	1	1		
SU						1					■	3	1	2
NT												■	1	2
RE													■	1
ZI														■

When all herd groups coalesced, the α-herd regularly dominated the β- & γ-herds. The β- & γ-herds were more often found united (20.3%) than in their respective herd groups (β – 4.0%, γ – 1.6%) and were thus grouped together for the purpose of generating a large enough sample size for comparison. Statistical analysis revealed that individual average progestagen concentrations were significantly different ($p<0.05$) in the α-herd when compared to β- & γ-herds combined. The α-herd females had higher progestagen concentrations (1.73 ± 0.54 µg/g) than those in the β- & γ-herds (1.38 ± 0.14 µg/g).

All of the non-adult, nulliparous females fell into the last 5 placements in the dominance matrix while all of the adult, parous females ranked above them. However, there was no significant difference between average progestagen concentrations in adult parous females of higher rank and non-adult nulliparous females.

Within the top 5 higher ranking females, 4 were cyclic and 1 had an irregular cycle in contrast with the 5 lowest ranking where only 1 female was cyclic and 1 had an irregular cycle. Female falling in the middle of the dominance hierarchy tended to be non-cyclic. Statistical analysis comparing the top 5 dominant versus the 5 most subdominant females revealed that dominance rank had no bearing on cyclicity status ($p>0.05$).

4.5 Seasonality

There was a strong correlation ($p < 0.01$) between average progestagen concentrations and season with concentrations being significantly higher in the wet season (1.98 ± 0.58 µg/g) than the dry (1.25 ± 0.44 µg/g). Progestagen concentrations were also significantly correlated with mean monthly rainfall ($p < 0.05$) (Figure 9).

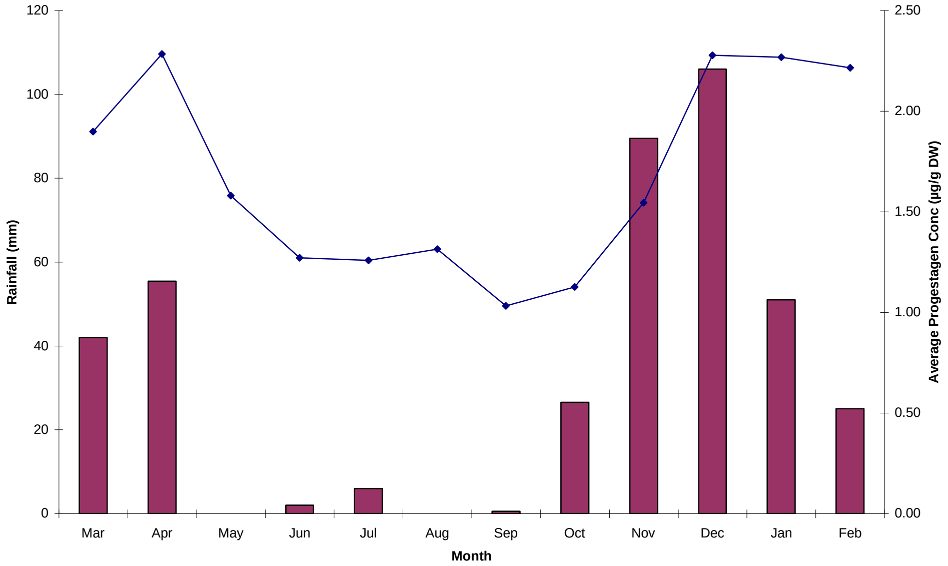


Figure 9. Mean monthly rainfall (horizontal bars) and progestagen concentrations (line) for 14 pZP-treated African elephant females from March 2007 to February 2008.

Average progestagen concentrations between cycling and non-cycling females did not vary significantly with either the dry season ($p > 0.05$) or the wet season ($p > 0.05$) (Figure 10).

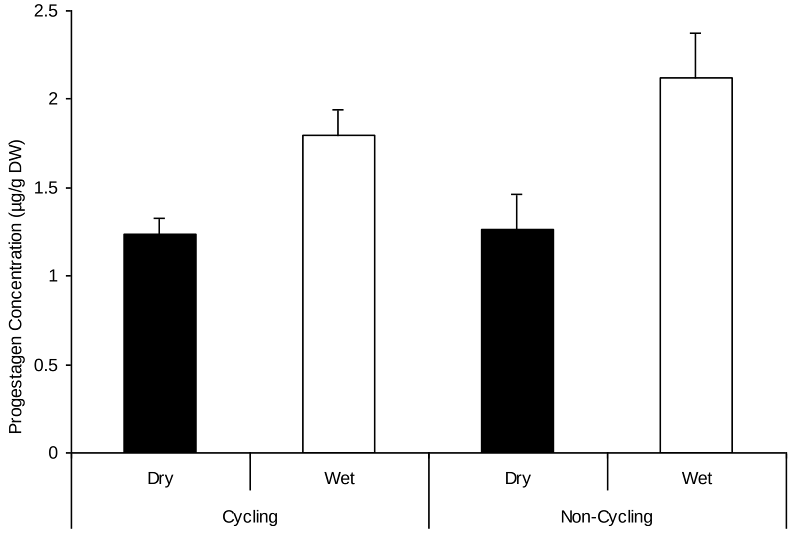


Figure 10. Dry and wet season average progestagen concentrations for cycling and non-cycling pZP-treated African elephant females from March 2007 to February 2008.

4.6 Reproductive Status

All of the adults considered in the study had all conceived prior to treatment with the pZP vaccine with the most recent dates of parturiton occurring in August 2006 (Table 5). Longitudinal progestagen profiles revealed that no females were pregnant at the time of the study. All adult females except *Mandy* were lactating for the duration of the study. Of the three females who had given birth most recently in August 2006, two (66.7%, *One Tusk* and *Thembisa*) exhibited an oestrous cycle at 32 weeks and 56 week post-partum respectively and one demonstrated irregular progestagen fluctuations with no cyclic pattern and a lengthened follicular phase indicative of anoestrous (*Madam M*, Figure 5).

Table 5. Age classes and records of most recent births among the Thornybush elephant herd since pZP treatment in May 2005. Birth dates denoted with an asterisk represent approximate ages based on ranger reports and/or comparative body size (Hanks 1979, Appendix B).

Name of Cow	Age of Cow	Date of Birth of Youngest Calf
Dana	Adult	*May 2005
Flo	Adult	* 2004
Hannah	Sub-adult	
Hook	Adult	* 2004
Khala	Adult	04 January 2006
Kombela	Adult	12 July 2006
Madam M	Adult	29 August 2006
Mandy	Adult	27 February 2006
No Tusks	Sub-adult	
One Tusk	Adult	01 August 2006
Rex	Sub-adult	
Suka	Sub-adult	
Thembisa	Adult	22 August 2006
Ziggy	Immature	

There was no statistically significant difference in progestagen concentrations between nulliparous and parous females ($p>0.05$) or between lactating and non-lactating females ($p>0.05$). Parity and lactational status also had no bearing on cyclic activity ($p>0.05$).

4.7 Darting Event

Focal samples of randomly chosen females for a total of 160 minutes prior to and after the darting event were examined. The resulting activity budget is presented in Table 6. There was no significant difference ($p < 0.05$) in behavioural activities before or after the darting event.

Table 6. Percentage of time spent by the Thornybush elephant herd participating in various behavioural activities for 160 minutes prior to and following the pZP vaccine darting event in September 2007. All dust-bathing, mud-bathing and water-bathing activities were grouped together into the "bathing" category.

	Pre-Darting Event	Post-Darting Event
Feeding	39.2%	47.4%
Bathing	1.3%	0.0%
Drinking	4.1%	3.9%
Walking	6.6%	8.1%
Resting	37.5%	21.9%
Standing	10.5%	18.2%
Interacting	0.9%	0.6%

A total of 37 faecal samples were collected in the week following the darting event from 11 individuals. Analysis revealed a significant drop (p<0.01) in progestagen concentrations in the week following immunization (0.94 ± 0.57 µg/g) when compared to their yearly averages (1.67 ± 0.50 µg/g) (Figure 11).

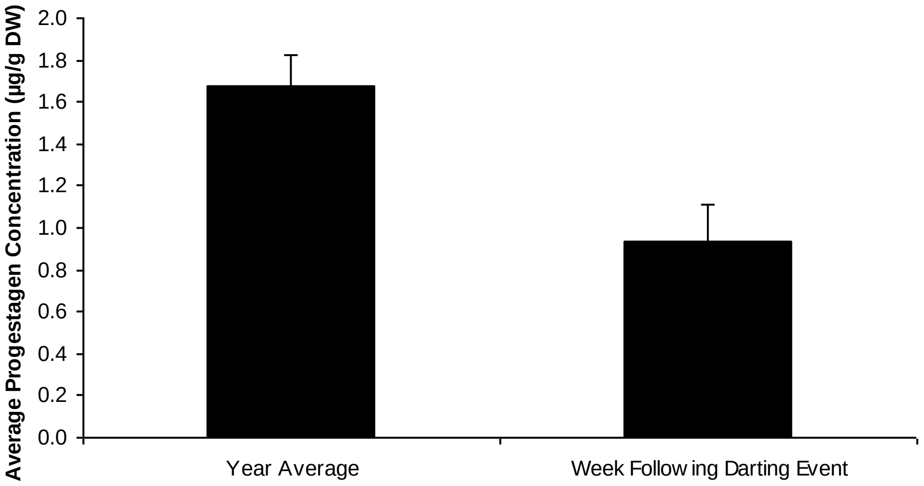


Figure 11. Average progestagen concentrations for 11 female African elephants for the year of study (March 2007 to February 2008) and in the week following the darting event.

Chapter 5: DISCUSSION

5.1 Oestrous Cycle Length

All of the pZP-treated individuals examined in the study showed signs of luteal activity and none gave evidence of interminable periods of flat-line ovarian inactivity as reported by Stoops *et al.* (2006) in domestic ewes (*Ovis aries)* during the breeding season. Only one female demonstrated continuous oestrous cycles throughout the study period, and another showed two full cycles interrupted by a shortened luteal phase length mid-study. Earlier studies have revealed a high incidence of oestrous cycling abnormalities in untreated populations of captive populations of African elephants (Wasser *et al.* 1996; Brown 2000; Schulte *et al.* 2000; Brown *et al.* 2004a; Brown *et al.* 2004b). Most data on wild populations have been unable to determine continuous cyclicity status as in the majority of cases, the subjects already are or become pregnant during the study (Foley *et al.* 2001; Brown *et al.* 2004a; Freeman *et al.* 2004). Due to this lack of information on non-pregnant, wild African elephants, it is difficult to ascertain if those females showing erratic patterns of progestagen concentration in this study are a result of pZP treatment or a common reproduction irregularity inherent in African elephants. Episodic ovulatory failure amongst pZP-treated feral horses has also been reported but could not be irrefutably linked to pZP treatment alone and estrous cycle characteristics remained consistent with untreated mares (Powell and Monfort 2001). More frequent sampling over a longer period is needed in order to ascertain ovulatory failure rate amongst these pZP-treated elephants and compared against untreated populations to determine if the low incidence of oestrous cycles are related to pZP treatment.

5.2 Oestrous Behaviour

During the course of the one-year study period, only three incidences of behavioural oestrous were observed. Due to the density of vegetation, it is likely that there were periods of oestrous behaviour that were missed as attaining clear visibility of the herds proved difficult. While visibility would likely be best in the mid to late dry season due to sparseness of vegetation, the oestrous behaviour that was observed occurred in the mid to late rainy season and thus it is unlikely that season played a role in contributing to frequency of oestrus behaviour. Oestrous behaviour that was witnessed in two of the pZP-treated females was associated with the initiation of the luteal phase as well as a rise in progestagen concentration as confirmed by Hodges (1998) and Brown (2000) in untreated African elephant females.

5.3 Age

No relationship between average progestagen concentration and age was detected in the statistical analysis. However, the possibility that the small sample size contributed to a Type II error must be taken into consideration.

In regards to cyclicity status being influenced by age, it does appear that there is a low percentage of cycling females in the under 12 years of age category. While African elephant females have been reported to reach puberty as young as 10 years of age, the age of first ovulation can be influenced by population density due to density-driven nutritional, physiological and social stresses (Laws 1969). High population densities can result in a delay of the onset of first ovulation as late as 20 years of age (Laws 1969). The recommended maximum carrying capacity for elephants in the Thornybush Private Nature Reserve has been reported to be 26 (Peel 2002) and the current population nearly doubles this figure. Population density may therefore explain the low number of cycling females in the Thornybush elephant population. Regardless, no statistical relationship between age and cyclicity status was found. Acyclicity was once reported to be independent of age class (Brown 2000). However, more recent literature on reproductive status of captive elephants (Brown *et al.* 2004a) reports a greater likelihood of non-cycling in older, nulliparous females and that females showing irregular cycles were more likely to be younger than either cycling or non-cycling females. Brown *et al.* (2004a) put forth a recommendation that females under the age of 25 years should be bred, after which the risk of reproductive abnormalities, such as reduced fecundity, endometrial hyperplasia, increased likelihood of stillbirths, become greatly heightened. This should be taken into consideration in pZP-treated populations to prevent local extinction as the population ages should young, nulliparous females be kept on treatment past the age of 25. In populations where individuals have been identified, pZP treatment can be discontinued for a period to allow conception or alternatively, pZP treatment could only be administered to those females who have already conceived at least one offspring in order to avoid this risk.

5.4 Dominance/Rank

Complex interactions between female African elephants within a herd define their place in the hierarchy and dictate which individuals will have increased access to resources such as food, shelter, and reproductive mates (Dublin 1983; Archie *et al.* 2006). The higher the rank a female holds in the herd, the greater access she will have to these resources and thus improve her chances of reproductive success (Dublin 1983; Schulte *et al.* 2000).

Over the course of the study period, the more dominant α-herd was seen regularly chasing off or supplanting the less dominant β- & γ-herds from preferred resources. Average faecal progestagen concentrations in the α-herd were higher than those of the β- & γ-herds. Females with hindered access to resources would be expected to have reduced body condition and a positive link between poor body condition and lowered progestagen concentrations has been reported (Foley *et al.* 2001). Stress measures also indicate that lower-ranking females have higher cortisol than their more dominant counterparts (Foley *et al.* 2001) which leads to poor health and lowered reproductive success (Romero 2004; Gobush *et al.* 2008).

A study in captive African elephants revealed a higher likelihood of ovarian inactivity in dominant females (Freeman *et al.* 2004). However, the study does not report on parity status and accordingly the susceptibility of older, nulliparous females in captivity having reproductive anomalies described previously could be a factor. This study found that 4 out of 5 of the more dominant females exhibited oestrous cycles in contrast to only 1 out of the lower 5 subdominant females but no statistical link between cyclicity and dominance rank was found. No literature on cyclicity status and dominance in free-ranging populations currently exists for comparison.

5.5 Seasonality

Seasonal influences on reproductive steroid hormones have been widely reported in a variety of species (Ziegler *et al.* 2000; Moreira *et al.* 2001; Cerda-Molina *et al.* 2006) as well as African elephants (Foley *et al.* 2001; Wittemyer *et al.* 2007). As a consequence of low rainfall experienced during the dry season, quality and availability of food and water decline (Foley *et al.* 2001). These dry season conditions result in a decline in body condition and have been linked to lowered progestagen concentrations, periods of anoestrous, silent heats, and subsequently reduced reproductive function (Foley *et al* 2001; Wittemyer *et al*. 2007). The period of study took place during a drought where rainfall in both 2007 (405.4 mm) and 2008 (333 mm) fell below the average 601 mm reported for the study area (Peel 2005). The sub-optimal conditions arising from the poor rainfall during the study period likely resulted in nutritional stress to some extent amongst the Thornybush elephant population which in turn could explain the low frequency of cyclic patterns in their progestagen concentrations. While season did not appear to affect the cycling status of the population, longitudinal monitoring of progestagen concentrations in the Thornybush pZP-treated elephant population revealed seasonal effects with higher concentrations found in the wet season. Overall average monthly progestagen concentrations closely followed rainfall patterns and further verified past studies that indicated availability of water, food and body condition greatly influence reproduction (Foley *et al.* 2001; Wittemyer *et al*. 2007).

5.6 Reproductive Status

Periods of anoestrous or acyclicity following parturition have been reported in cattle, sheep, and buffalo (Roche *et al*. 1992; Peclaris 1998; Yavas & Walton 2000; Singh *et al*. 2005). Following parturition, increased oestrogen concentrations brought about during late pregnancy results in a negative feedback mechanism that decreases the LH and FSH concentrations required for ovulation (Roche *et al*. 1992). The duration of the post-partum period of anoestrous has been linked to lactation, parity, nutrition, age, season, body condition and stress in cattle (Roche *et al.* 1992; Peclaris 1998; Yavas & Walton 2000; Singh *et al*. 2005). Lactation, in particular, is thought to result in higher levels of prolactin which also suppresses ovarian activity (Peclaris 1998).

Lactational anoestrous in African elephants has been reported to last for an 8-12 month period (Brown 2000). However, one female in the study (*One Tusk*) revealed an oestrous cycle occurring within a seven month period following parturition while she was still lactating. As this cycle appeared in the first week of study, it is possible she may have had more oestrous cycles prior to the study period, thus no information regarding the exact duration of anoestrous following parturition could be determined. One other female who had a recent birth (*Thembisa*) had her first detectable luteal cycle in the study period approximately 13 months post-partum while another (*Madam M*) had no detectable oestrous cycles within 6 to 18 months post-partum. The remaining parous females were only studied after the minimum 8 month reported lactational anoestrous period preventing any further insight.

Madam M was the last to give birth of all the adult females and although a cyclic pattern was not detected, evidence of luteal activity was present. Her parturition date came one week after *Thembisa* and less than a month after *One Tusk*, both of whom had one complete oestrous cycle. *One Tusk's* oestrous cycle began in the 1st week of the study while *Thembisa's* occurred in the 30th week. Since *Madam M* was only a week behind *Thembisa* and no cyclic pattern was detected from the 30th week onward, it is unlikely that *Madam M's* irregular progestagen concentration fluctuations have to do with time since parturition. One possible explanation is that the duration of the post-partum period of anoestrous is also reliant on ecological conditions (Wittemyer *et al*. 2007). Being a lower-ranking female, *Madam M* may not have had access to the same resources as the more dominant *One Tusk* and *Thembisa*, consequently delaying resumption of cyclicity.

One adult female (*Mandy*) gave birth in February 2006 but the calf developed an umbilical hernia and died soon afterwards. She ceased lactating prior to the study and was the only female demonstrating a consistent cyclic pattern. Brown (2000) reports that early weaning, retained placentas, or as in *Mandy's* case, death of a calf, can shorten the postpartum anoestrous to 8 weeks. Although no statistical correlation between lactational status and cyclicity was found, small sample size could once again have been a factor. *Mandy* was the only parous, non-lactating female while the rest of the non-lactating females (n=5) were all nulliparous, non-adults. Nevertheless, *Mandy's* pattern of continuous oestrous cycling demonstrates that normal reproductive function is possible under treatment with the pZP vaccine.

5.7 Darting Event

Previous study on the effects of pZP vaccination on the behaviour of African elephants have reported a period of one or two day post-darting disruption before herd behaviour patterns returned to those witnessed prior to darting via helicopter (Delsink *et al.* 2003; Bertschinger *et al.* 2004; Delsink *et al.* 2004). Through focal sampling, the current study has also shown no change in behavioural activities in the two days following the darting event, further supporting these previous findings. Additionally, game viewing of the pZP-treated herds resumed the following day post-treatment and no reports of aggression or aberrant behaviour was given.

Further studies have also reported no unusual behaviour exhibited in female elephants treated with the pZP vaccine in the medium-term (Delsink *et al.* 2004; Delsink *et al.* 2006c) and the present study has found no evidence to the contrary. Pooling all focal sampling data for females and males in the study population from September 2005 to present revealed activity budgets within the ranges found in untreated populations in various reserves across South Africa (Shannon *et al.* 2008) indicating the pZP-treated population behaves similarly to untreated populations.

The decrease in individual progestagen concentrations following the darting event indicates that stress may play a role in influencing reproduction in African elephants. A slight negative correlation between cortisol, a hormone typically indicative of stress (Mostl and Palme 2002), and progesterone has been reported in African elephants (Bechert *et al*. 1999). Chronic stress is known to negatively affect reproductive function in humans, rodents, and non-human primates (Rivier and Rivest 1991; Mostl and Palme 2002). Studies on domestic species, humans, primates, and rodents have revealed that stress can block or delay the pre-ovulatory surge; suppress follicular growth; hinder hormone release within the follicular phase; reduce oestradiol production; and increase inter-calving intervals (Rivier and Rivest 1991; Norman *et al*. 1994; Dobson and Smith 2000; Macfarlane *et al*. 2000). As herd behaviour resumed within a short period following the darting event, it is unlikely that the darting event results in the negative connotations associated with chronic stress. Nevertheless, studies are currently underway to ascertain the effectiveness of a single dose primary pZP vaccine as well as a longer lasting-pZP vaccine to reduce the number of darting events (Turner *et al*. 2008). While it is too early to assess fertility, preliminary results show a 62% greater titre response in elephants treated with a single controlled-release dose over the standard 2-injection pZP vaccine previously required for fertility control (Turner *et al*. 2008).

While it is clear that the magnitude, duration, and the individual's ability to respond to stress determines the overall impact, the actual mechanisms by which stress influences reproductive potential is still poorly understood (Rivier and Rivest 1991). Progesterone concentrations in the pZP-treated study population were frequently erratic and stress associated with darting, social pressures, and/or lack of resources in the particularly dry study period, could have played a role in altering hormone patterns. Whether pZP-treated elephant populations experience higher levels of stress or have poorer coping mechanisms is beyond the scope of the present study but is worthwhile examining in future.

Chapter 6: CONCLUSIONS

This study is the first to reveal underlying physiological effects of pZP treatment in female African elephants while further demonstrating the usefulness of non-invasive faecal endocrine monitoring in assessing reproductive function in wild populations of African elephants (Wasser *et al.* 1996; Fieβ *et al.* 1999; Foley *et al.* 2001; Wittemyer *et al.* 2007). Earlier efficacy of pZP vaccination was established via faecal analysis with no pregnancies detected 22 months after treatment. The efficacy of pZP vaccination is undeniable with birth rates falling to zero by the fourth year of treatment in 4 private game reserves using the pZP vaccine as a population control method (Bertschinger *et al.* 2008). The results generated in the study concerning behaviour, dominance and seasonality in pZP-treated individuals all remained in agreement with behavioural findings in literature regarding untreated populations.

While it is clear that ongoing investigation of cycling patterns in both free-ranging untreated and treated populations of African elephant is needed, these results demonstrate that in two years following pZP treatment, oestrous cycles are present amongst 42.9% of treated individuals, indicating ovarian functionality. Speculation that pZP treatment may interfere with developing oocyte and follicular cell communication or antibodies against pZP alter ovarian function by attacking oocytes or follicular cells, both resulting in oocyte death has been proposed in dogs (Mahi-Brown *et al*. 1985). However, the prevalence of progestagen increases above baseline amongst all treated females in the study also demonstrates ongoing luteal activity and thus little probability that pZP vaccination has caused follicular damage within these females. Interpreting causes for irregular or non-cyclic patterns of progestagen secretion in African elephants is fraught with complexity given the vast variety of potential influences discussed. Alternation between cyclic and non-cyclic periods as well as erratic progestagen secretion has been documented in untreated African elephant populations (Schulte *et al*. 2000; Brown *et al.* 2004a). Known seasonal and social influences on hormone activity also complicate analysis (Schulte *et al*. 2000; Wittemyer *et al*. 2007). This truth makes it difficult to conclude the true influence that the pZP vaccine has on reproductive function.

The absence of an indefinite period of anoestrous within the study population is encouraging. Future study should be geared towards monitoring pZP-treated females alongside a comparable untreated, free-ranging, control group and for a longer duration to minimize external influences on reproduction as well as ascertain the long-term effects of pZP vaccination on free-ranging African elephant populations.

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Appendix A: Thornybush elephant herd composition.

THORNYBUSH
HERD COMPOSITION – September 2007

where Class A = 12 + / adult
Class B = 9-12 / sub-adult
Class C = 6-9 / immature
Class D = 2-6 / juvenile
Class E = 0-2 / infant

α-Herd:

Class A	5	Flo, Hook, Khala, Mandy, One Tusk
Class B	3	Hannah, No Tusks, Suka
Class C	3	Ziggy (Dabuka male, Fabien male)
Class D	4	Hook's female, Khala's male, Mandy's female, One Tusk's female
Class E	4	Flo's male, Hook's female, Khala's male, One Tusk's male
TOTAL	**19**	**[13 F + 6 M]**

β-Herd:

Class A	4	Dana, Kombela, Madam M, Umkhonto
Class B	1	Skew
Class C	3	Nkanu, Zula, Ulwazi
Class D	2	Kombela's female, Madam M's female
Class E	3	Dana's female, Kombela's female, Madam M’s male, Umkhonto's female
TOTAL	**14**	**[13 F + 1 M]**

γ-Herd:

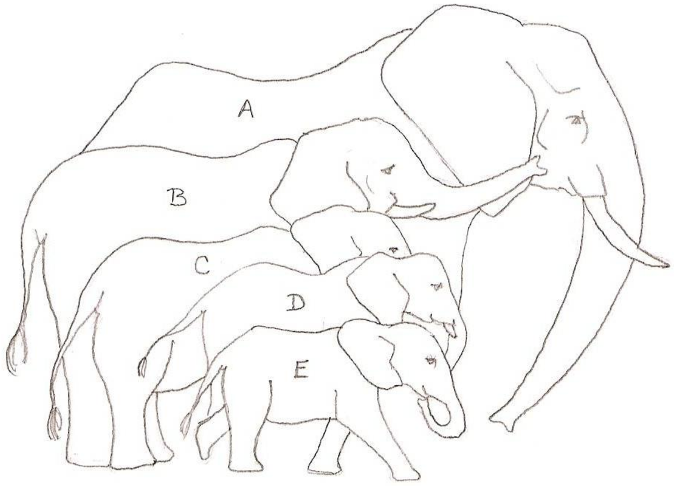
Class A	1	Thembisa
Class B	1	Rex
Class C	1	(Elliot male)
Class D	1	(Ephraim male)
Class E	1	Thembisa’s female
TOTAL	**5**	**[3 F + 2 M]**

2 x Free-roaming bulls = Xibala (52 yr old) + Iqhawe (20 yrs)

TOTAL POPULATION = 40

[29 Females + 11 Males]

Appendix B: Age distribution as determined from the relative sizes of elephants in a family group compared with that of an average adult cow modified from Hanks (1979).



A	Adult female (12 + years)
B	Sub-adult (9-12 years)
C	Immature (6-9 years)
D	Juvenile (2-6 years)
E	Infant (0-2 years)

Appendix C: Daily re-sighting record sheet.

Herd Resightings Summary Sheet

Date							
Time							
Location (GPS Coordinates)							
Direction of Travel							
Weather							
Group Size: Total							
# Infants							
# Juvenile							
# Immature							
# Subadults							
# Adults							
Accuracy Index							
COWS:							
Dana	☐	☐	☐	☐	☐	☐	☐
Flo	☐	☐	☐	☐	☐	☐	☐
Hannah	☐	☐	☐	☐	☐	☐	☐
Hook	☐	☐	☐	☐	☐	☐	☐
Khala	☐	☐	☐	☐	☐	☐	☐
Kombela	☐	☐	☐	☐	☐	☐	☐
Madam M	☐	☐	☐	☐	☐	☐	☐
Mandy	☐	☐	☐	☐	☐	☐	☐
Nkanu	☐	☐	☐	☐	☐	☐	☐
No Tusks	☐	☐	☐	☐	☐	☐	☐
One Tusk	☐	☐	☐	☐	☐	☐	☐
Rex	☐	☐	☐	☐	☐	☐	☐
Skew	☐	☐	☐	☐	☐	☐	☐
Suka	☐	☐	☐	☐	☐	☐	☐
Thembisa	☐	☐	☐	☐	☐	☐	☐
Ulwazi	☐	☐	☐	☐	☐	☐	☐
Umkhonto	☐	☐	☐	☐	☐	☐	☐
Ziggy	☐	☐	☐	☐	☐	☐	☐
Zula	☐	☐	☐	☐	☐	☐	☐
BULLS:	☐	☐	☐	☐	☐	☐	☐
Dabuka	☐	☐	☐	☐	☐	☐	☐
Elliot	☐	☐	☐	☐	☐	☐	☐
Ephraim	☐	☐	☐	☐	☐	☐	☐
Fabien	☐	☐	☐	☐	☐	☐	☐
Iqhawe	☐	☐	☐	☐	☐	☐	☐
Xibala	☐	☐	☐	☐	☐	☐	☐
Comments:	☐	☐	☐	☐	☐	☐	☐

Accuracy Index: Total count Accurate count of Cows Best Guess

Individuals Identified: (L) = lactating (M) = Musth (O) = Oestrous Behaviour

Appendix D: Focal sample data sheet.

FOCAL DATA SHEET

F/S code		Time start		GPS start	S	E	Observer	
Location		Time end		GPS end	S	E	Date	
Group size		Mood			Herd data/s #		Habitat	
Weather								

	Individual			Time start		Time end	
	Behav	Species	Code	Comments (including final % damage to trees)			
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							

Behaviour: F - feeding; W - walking; M - moving; S - standing; I - interacting (social - describe in comments);
D - drinking; DB - dustbathing; MB - mudbathing; WB - waterbathing; O - other; OOS - out of sight

Codes: L - leaves; F - fruit; B - bark; R - roots

Appendix E: Ethogram utilized for focal-animal sampling.

Behaviour	**Code**	**Definition**
Drinking	D	Sucking in water with trunk, placing trunk to mouth, and draining water into mouth.
Dust-bathing	DB	Grasping sand/dust with trunk and throwing over body, or rolling in sand/dust.
Feeding	F	Consumption of any tree, grass, or succulent species as well as soil. Tree species as well as portion of tree consumed (i.e. bark (B), branch (Br), flower (Fl), fruit (Fr), leaves (L), or roots (R)) will also be specified.
Interacting	I	Includes calves suckling, play-fighting (where 2 or more individuals engage in head-to-head, head-to-side pushing, chasing, or climbing on top of one another), oestrous chase (as described by Moss 1983), mounting, and rumbling/vocalizing in unison with other herd member. Exact behaviour specified under comments section.
Mud-bathing	MB	Grasping mud clumps in trunk and throwing over body, or rolling/lying in mud wallow.
Other	O	Any other behaviour not covered in the other categories of behaviour. Behaviour observed in this category will be further specified under comments section.
Resting	R	Eyes closed, in lying or standing position (no motion). Herd activity as a whole is resting.
Standing	S	Eyes open, remaining stationary but can include trunk-reaching, trunk-smelling, leg rubbing against the other. Herd activity (i.e. all behaviour categories except resting) continues around individual.
Walking	W	Consistent movement in forward direction. Individuals walking whilst feeding on grass will be classified under feeding behaviour.
Water-bathing	WB	Sucking in water with trunk and splashing/spraying over body or swimming/submerging body in water.

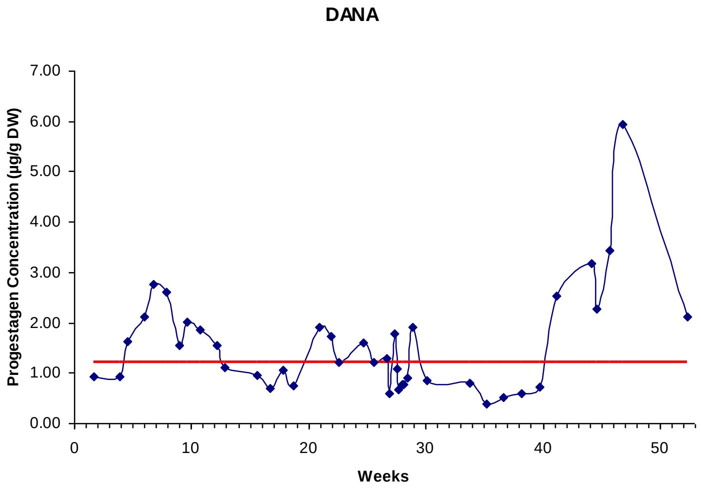
Appendix F: Faecal sample record sheet.

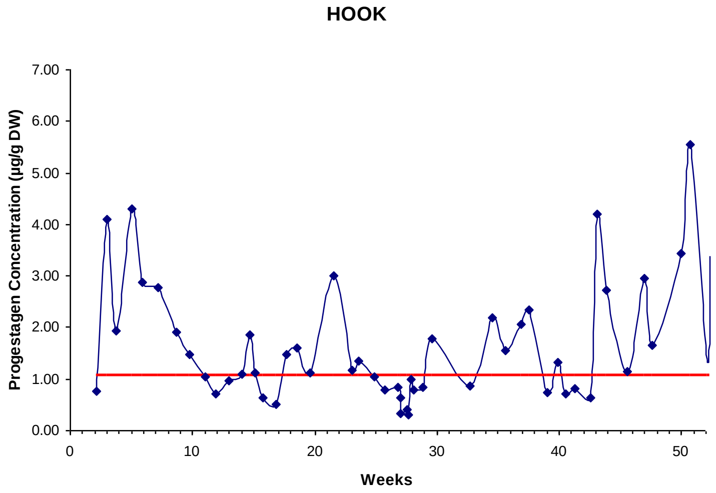
FAECAL SAMPLE RECORD SHEET

Sample No.	Sample Code	Name of Individual	Date	Time of Defecation	Time Collected	Location	Weather/ Temp.	Appearance/ Consistency	Bull Present	Oestrous Behaviour

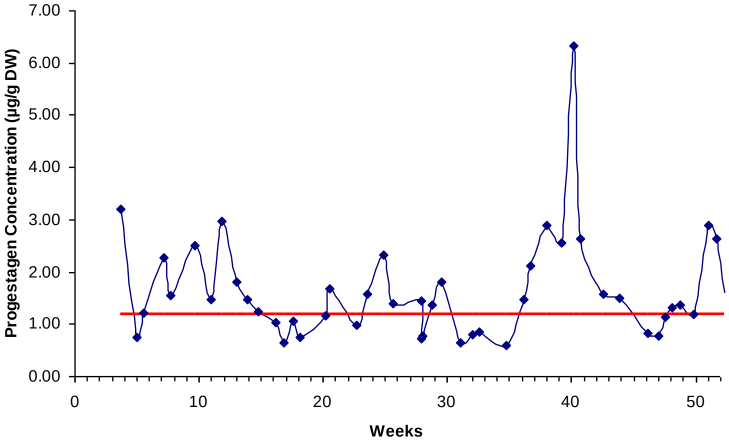
Appendix G: Individual progestagen concentrations (µg/g DW) for pZP-treated African elephant females at Thornybush Private Nature Reserve from March 2007 to February 2008.

a) Cycling Females

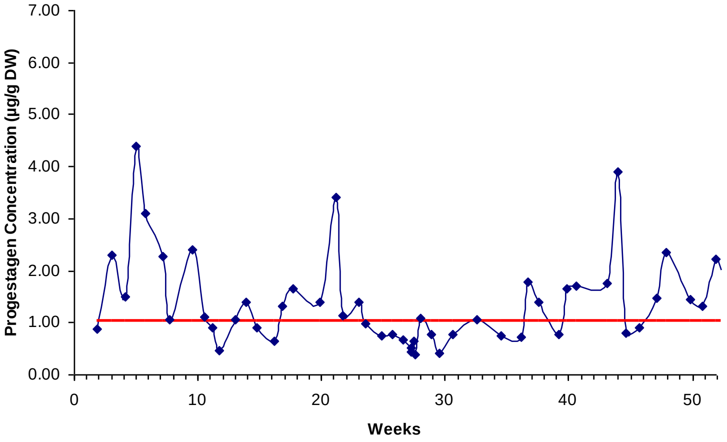




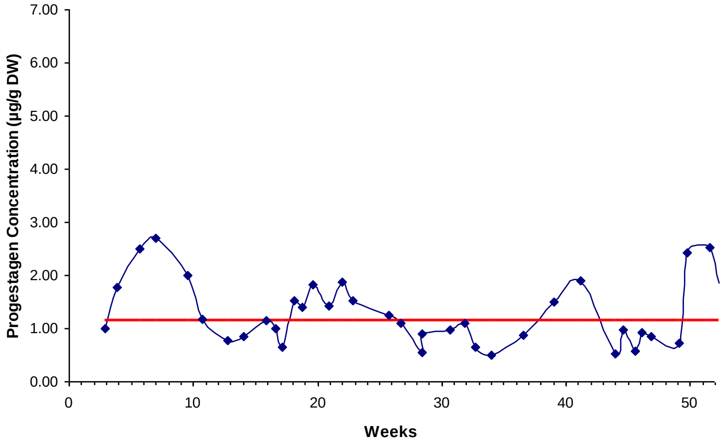
MANDY

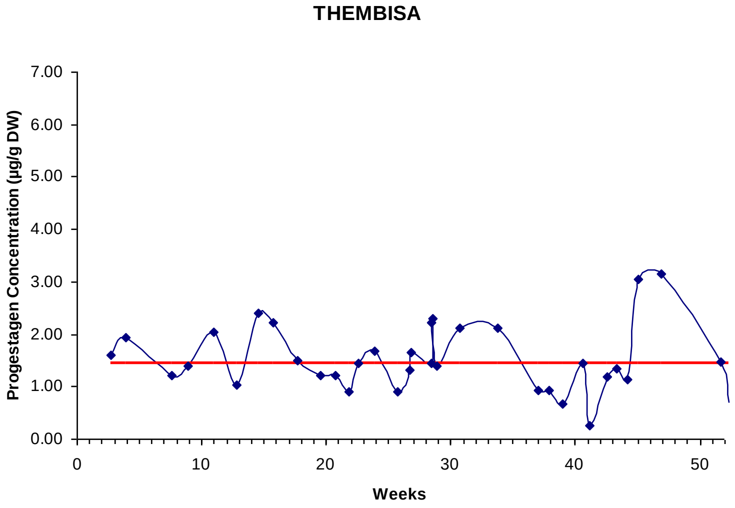


ONE TUSK

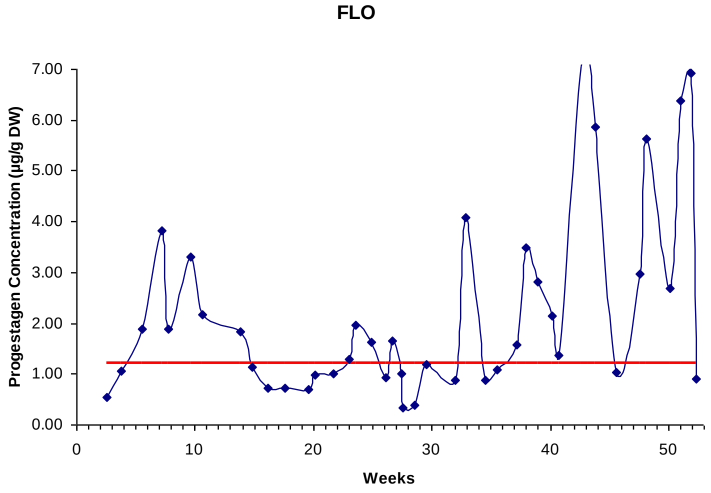


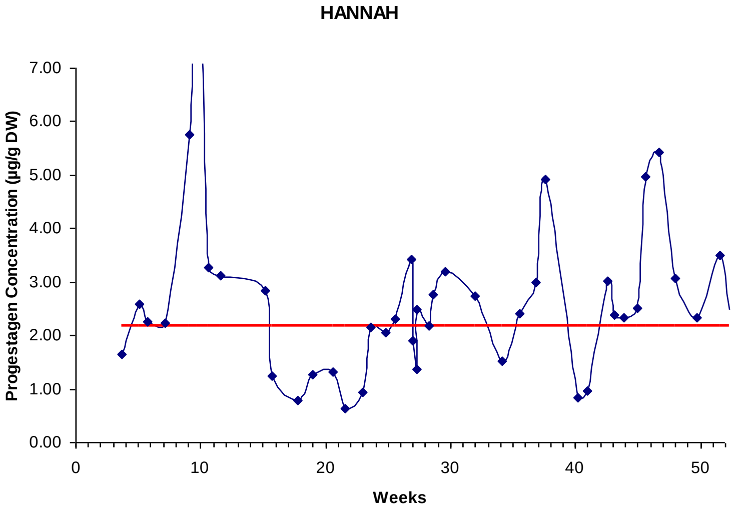
REX



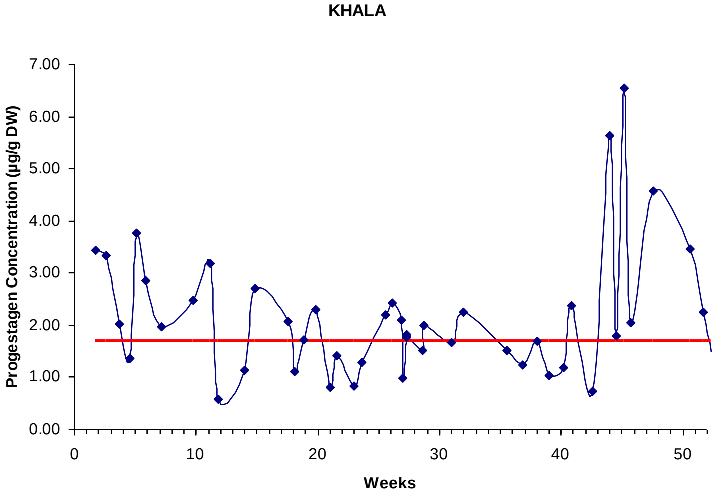


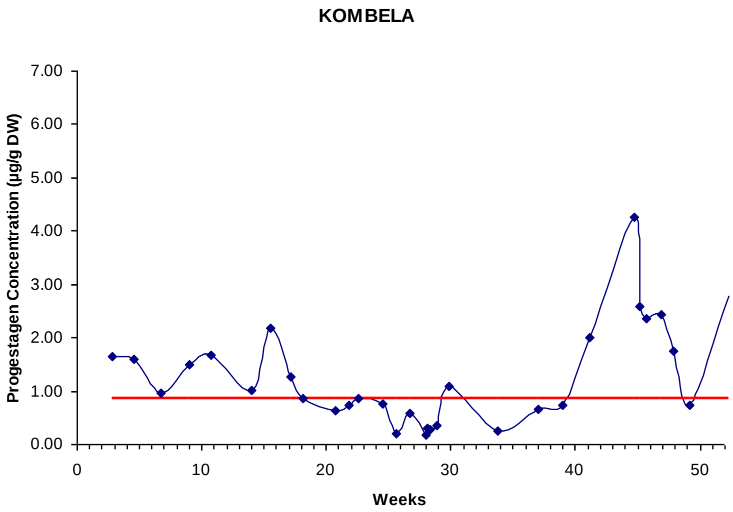
b) Irregular cycling females

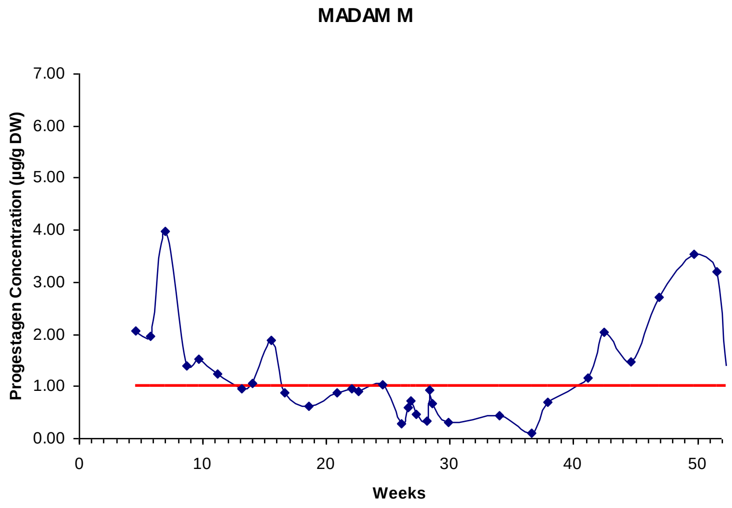


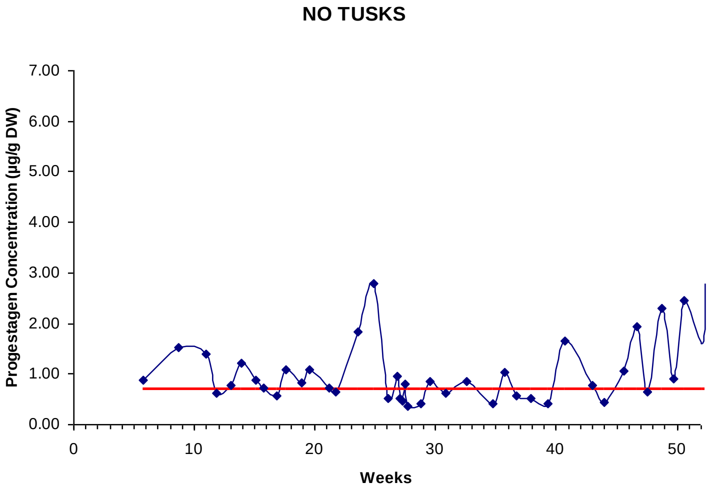


c) Non-cycling females

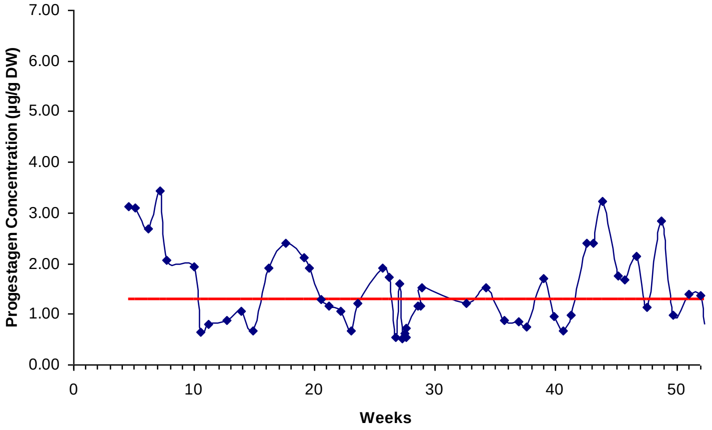



KOMBELA
Progestagen Concentration (µg/g DW)
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0
10
20
30
40
50
Weeks

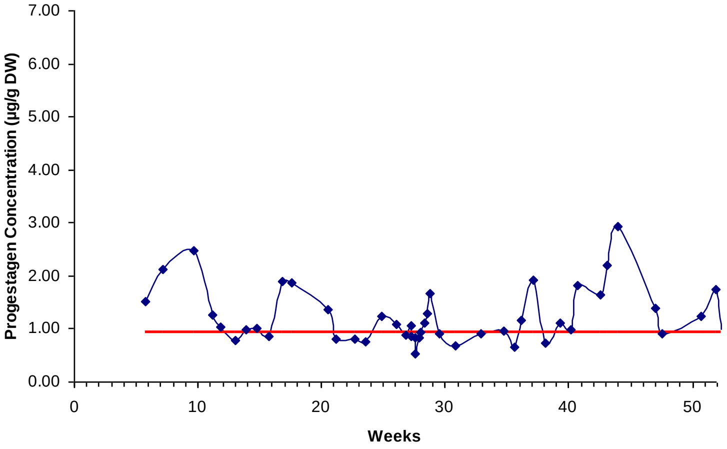

MADAM M
Progestagen Concentration (µg/g DW)
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0
10
20
30
40
50
Weeks


NO TUSKS
Progestagen Concentration (µg/g DW)
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0
10
20
30
40
50
Weeks

SUKA


Progestagen Concentration (µg/g DW)
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0
10
20
30
40
50
Weeks

ZIGGY


Progestagen Concentration (µg/g DW)
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0
10
20
30
40
50
Weeks