Controlling wildlife reproduction:

Reversible suppression of reproductive function
or sex-related behaviour in wildlife species

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Reversible suppression of reproductive function or sex-related behaviour in wildlife species

Management van voortplanting bij dieren in het wild:

Reversibele beperking van voortplanting en geslachtsgebonden gedrag
(met een samenvatting in het Nederlands)

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General Introduction

Introduction

Population control for wildlife species was barely considered an issue some 50 years ago. On the contrary, many species were driven to the edge of extinction or became extinct through indiscriminate hunting and progressive loss of habitat (Kwagga; Smithers, 1983). Even today, some African countries have few or no elephants left, even though they had an abundance of this mega-herbivore as little as 100-200 years ago. One startling example of the scale of the decline in elephant populations is Botswana in which numbers decreased from around 400.000 (ca. 1790) to as few as 60 (1893). Another factor that had a tremendous impact on wildlife in southern Africa was the outbreak of Rinderpest during 1896 – 1897; this disease ravaged not only domestic ruminants but also buffalo and various antelope species (Gutsche, 1979). Although not investigated at the time, Rinderpest must have had a significant impact on predators as a result of the depletion of prey species. In addition, some wildlife species were culled because they were regarded as carriers of diseases that were a threat to domestic animals. For example, large numbers of rhino were destroyed in Natal because they were thought to be the carriers of Nagana (Trypanosomiasis; Meltzer, 1994). However, the founding of reserves like the Kruger National Park (KNP), signalled the development of a different attitude towards wildlife. Wildlife was suddenly seen as a valuable asset, instead of merely something to be hunted or destroyed because it could be a nuisance. It is interesting to note, however, that lions and wild dogs were still regarded as pest species during the early years of the KNP; as a result, many were shot. The concept of wildlife conservation started to gain momentum with the creation of the first private game ranches in the 1950's; although at that time they were mostly created for the purpose of trophy hunting. In time, however, ecotourism became the driving motivation. Satour's Winter Survey (1997) reported that more than 60% of foreign visitors came to South Africa for one of the following reasons; the scenic beauty (33% of arrivals), wildlife (30% of arrivals), and the climate (15% of arrivals).

As a result of the changes in attitude, wildlife is thriving in many southern African countries, in particular in protected areas, whether they be public or private reserves. In smaller fenced reserves (< 60 000 ha), it has become abundantly obvious that at least certain species need to be managed. The reasons for management include:

- Overabundance of large predators leading to unsustainable losses of prey species or breakouts into surrounding properties, many of which are developing communities, resulting in stock losses.
- Overabundance of herbivores leading to habitat degradation.

Overabundance of wildlife species is not restricted to range countries but may also occur in zoo's in the western world. Mammals and other animals have been housed in zoos for people to view for centuries, probably dating back to pre-Roman times. Until

fairly recently, zoos kept animals primarily as exhibits; during the last 15 years, however, the emphasis has shifted towards conservation and where possible, *in situ* conservation. Zoos seldom buy and sell animals – they usually swap their superfluous animals for others of the same or examples different species of which they have too few or have a need for new genetic lines. Many mammalian species breed well in zoo or captive environments and, with a limit to the number of institutions that can take excess animals, there is a need to control breeding. In species that breed well in captivity, inbreeding is also a very real danger. In short, for zoo and captive animals the main problems are:

- Limited market for excess animals and
- Risk of inbreeding and the associated complications.

This thesis investigates the possibilities of using contraception as a means of regulating the reproductive rate in elephants and a number of large African carnivore species. In elephants, a reversible contraceptive method is considered an ideal way of controlling reproduction and allowing parks to manage their elephant populations for optimal sustainable use of the habitat, without too much impact on the habitat or other species. Contraception of carnivores can be used to slow down the reproductive rate of carnivores which, if left unchecked in fenced reserves, could otherwise lead to a population explosion and problems like depletion of prey species, break-outs and inbreeding. Using contraception within a captive environment, should allow the rate of reproduction of various carnivores to be planned according to set requirements while avoiding irreversible sterilisation of animals, inbreeding and/or the need to euthanize unwanted offspring. In some countries, unwanted offspring find their way to backyard setups where they are neglected and end up as problem animals that have to be culled; the latter frequently through the despicable practice of 'canned hunting'. This thesis also investigates the possible use of a vaccine to down-regulate androgen-related aggressive behaviour in African elephant bulls. Androgen driven behaviour in bull elephants is primarily a feature of 'musth'; the annual period of dramatic behavioural and physiological changes induced by very high levels of testosterone that is seen in both African and Asian elephants over the age of approximately 35 years. The raised testosterone levels bring about a number of changes including heightened aggression and dominance. This is one of the reasons why bulls in musth sire about 75% of calves (Hollister-Smith *et al.*, 2007). Aggression during musth is, however, a problem of great concern in captive bulls. As bulls get older, testosterone production rises until it is sufficient to bring about musth and musth-related behaviour. Almost every year, someone is killed by a captive elephant bull in southern Africa, and in Asia the frequency of human deaths is even higher. Currently, there is no effective means of controlling aggressive behaviour in elephant bulls other than to wait until it has passed. The traditional control of musth bulls therefore involves the use of methods

now regarded as cruel, such as chaining, isolation and food deprivation, which may in fact exacerbate dangerous behaviour in subsequent years – elephants truly do not forget!

The large predator problem

The rationale for suppressing reproduction in large carnivores is somewhat different to that in herbivores, and also varies from species to species. It is a well known that, left unmanaged, free-ranging lions on fenced game reserves can reproduce at an alarming rate. This leads to rapid depletion of prey species, inbreeding and breakouts into neighbouring communities. A study of the effects of unchecked lion reproduction was carried out in Mabula Game Reserve, which has a single resident pride of lions consisting of two adult males, four adult females and cubs of various ages (Power, 2002). The lions are housed in a 1,500 ha camp and, for food, they hunt the prey species held on the property. When the lions were allowed to breed freely for a period of 5 years, prey species had to be replaced on a regular basis, which cost the reserve R 450 000 per annum. Many game reserves in South Africa also have species that are extremely valuable, such as Cape buffalo, sable and roan antelope. As the size of a lion pride increases, so does the size of the prey species targeted. Excessive loss of calves can be a problem for the targeted prey species, and at Thornybush Private Game Reserve no giraffe calves survived for a number of years as a result of an overabundance of lions on the property.

Large carnivores like lions and tigers also breed exceptionally well under zoo conditions and, since there are limited sites to house captive lions, reproduction needs to be managed. Free-ranging adult lionesses under extensive conditions, conceive when their previous litter of cubs are ~20 months old. In smaller fenced reserves, it appears that the interval between litters is shorter. Compared with extensive conditions like in the Kruger National (50%) and Etosha National (40%) Parks (Smithers, 1983), cub survival is also higher in smaller fenced reserves and is close to 100%. This is most likely due the absence of competition from other lions, and for example fewer or no pride take-overs, and less cub predation by hyenas. In zoos, it is common practice to remove cubs to be hand-raised or euthanized soon after birth. As a result, female lions and tigers come into heat and reconceive much sooner in captivity; sometimes within the first month after parturition.

In Namibia, the holding of wild carnivores on private property requires a permit stipulating that breeding of any such animals is not allowed (R E. Stander, personal communication). The main species involved are cheetahs, lions and leopards. In short, despite coming from an endangered species, cheetahs held in captivity in Namibia are not allowed to breed.

As mentioned previously, inbreeding in large carnivores is a very real problem, even under free-ranging conditions within fenced reserves. The most problematic species in this regard is the lion. Cheetahs, however, given the right conditions, can also thrive under such conditions. In order to protect or save free-ranging cheetahs in non-protected areas, the de Wildt Cheetah and Wildlife Trust established the de Wildt Wild Cheetah Project in 2000. One major goal of the project was to assist farmers in trapping cheetahs on farmlands where they were not wanted, and to relocate them to fenced game reserves. According to Marnewick et al. (2007), the first cheetahs were caught in 2000 and by December 2006, 137 had been removed from farmlands. Of these, 92 animals were finally released (58 males and 33 females) into areas ranging from 1500 to 70,000 ha in size. The first cubs were born in 2002 and by August 2007 94 cubs (average litter size 3.9 cubs) had been born to 23 females. Unless animals can be moved around, the dangers of inbreeding in these small isolated populations is clear.

Population control methods for large predators

So what options are there for population control of large carnivores given the limited availability of space for both captive and free-ranging animals? Hunting is certainly an option, but has come under severe criticism during the last few years. The main reason for this has been canned hunting, which is now banned in South Africa thanks to the promulgation of the 'National norms and standards relating to the management of large predators in South Africa', which controls the management and hunting of large predators in South Africa. Hunting is also a poor method for population control because it is both highly selective and does not follow the principles of natural genetic selection in free-ranging animals.

The only remaining option is to stop animals from breeding altogether, or to slow down the reproductive rate. In most cases, a reversible method is required and preferably one that is safe and does not interfere with key behaviours of the target species in question. This means that surgical methods are automatically excluded even though it is possible, if not practical, to reverse a vasectomy. Progestins in the form of long-acting implants have also been used extensively for contraception in large carnivores. But while progestin implants are extremely effective as contraceptives, they have largely gone out of use as a result of a number of serious side effects (Munson *et al.*, 2002; Munson *et al.*, 2005). Furthermore, the tailing-off period is long and, if reversal is required, the implants need to be removed well in advance.

There is, thus, a need to find better and, especially safer methods of contraception for carnivores. Depending on the reproductive behaviour of the species, there will also be differences in whether to treat males, females or even both sexes. First prize would be a single method that works in both sexes.

The elephant problem

Population density

Today, a number of game parks in southern African countries either have too many or believe they have too many elephants. The Kruger National Park (KNP) is one example, while Hwange and Chobe National Parks both have much higher elephant densities than the KNP. From 1967 to 1995, the elephant population in the KNP was 'managed' and maintained at a density of approximately 0.35 elephants/km^2. Recent estimates suggest it has now almost doubled to approximately 0.66 elephants/km^2. In northern Botswana, the population varies from 1.5 elephants/km^2 rising to 12 and even 20 elephants/km^2 during the wet season (Teren, 2008). Park managers are convinced that higher elephant densities, such as that found in the KNP, lead to habitat degradation and consequent reduction in biodiversity.

Elephants are the largest of the mega-herbivores and have a wide dietary range. Preferentially, they eat grass but they will also browse, during which they may break off branches, and consume the bark and roots of trees. It is the latter two practices which lead to the demise of trees. Trees such as Knob thorn (*Acacia nigrescens*) are particularly sensitive to debarking, whereas elephants seem to target them, perhaps because they are especially palatable, but certainly because their bark strips very easily. Trees damaged in this way are then more exposed to damage by insects or fire, while ring-barking leads to the death of the tree because of the removal of the system for transporting water and nutrients from the roots to the branches and leaves. The frequency of bark and root eating increases during the dry season, and is worst during periods of drought. Bulls are predominately responsible for the up-rooting of trees (J. Viljoen, personal communication). The overall effect of too many elephants is a changing landscape – from woodland to open grassland. Whether or not biodiversity, which consists of three components namely compositional, functional and structural diversity, is negatively affected is however debateable. There are indications that biodiversity may actually be improved, at least in large parks. Smaller parks are probably much more vulnerable to habitat degradation simply because the population is confined to a small area such that even during the wet season the elephants cannot move out and allow the habitat to recover. The range habitat types will also be smaller such that the more palatable species will be targeted more heavily than in large parks. The need to manage elephant population density is therefore more important in small than in large reserves.

Aggressive behaviour and musth in elephant bulls

In South Africa, a number of elephant bulls have been captured as calves, especially during culling operations. Elephants are easy to train in captivity, particularly in groups, and very quickly adopt their keepers as part of their family. Normally, the keepers would be regarded as the dominant animals within such a structure. However, bulls in particular, start to challenge this hierarchy as they get older and approach puberty. During puberty, testosterone concentrations start to increase which makes the bull more assertive, difficult to handle and even aggressive. Musth is the ultimate expression of testosterone-driven behaviour in elephant bulls, and bulls in musth are generally impossible to handle and very often dangerous. During the first two thirds of the 20th Century, when many zoos world-wide had adult bulls of either species, it was estimated that for every calf born in captivity one zoo keeper would be killed by a bull. In Asia, it is common practice to tie-up captive bulls with leg chains in the forest when they come into musth. The feed intake of these bulls is restricted because the mahouts believe that this will make them exit musth more rapidly. The use of leg chains and starvation are regarded as animal welfare issues, and probably also lead to increased aggression in the bulls. Free-ranging African elephant bulls are seldom a problem in larger reserves, but incidents have occurred with musth bulls trampling on tourists or smashing cars. More often than not, incidents like this are due to human error (ignorance or carelessness). In small reserves, however, the likelihood of encountering a bull in musth is probably increased. Elephants being highly intelligent, also quickly realise that 'vehicles' are scared of them and often terrorise people on game drives.

Population management methods for elephants

The elephant overpopulation problem, or prevention thereof, can be managed in three basic ways. The first is to expand existing game reserves, the second is to reduce numbers and the third is to slow down the rate of reproduction. The fourth option is the so-called 'laissez-faire' approach where nothing is done at all.

By far the best and most acceptable option is the establishment of transfrontier parks and corridors between elephant areas. In the medium to long-term, however, such areas will also fill up with elephants, particularly if artificial waterholes are not closed.

Two further approaches can be used to reduce population size. In principle, translocation is a very good option, however it is a very expensive process and reserves that are willing and able to accommodate elephants in South Africa have space for only about 1000 animals. Since these reserves are already saturated, elephants are occasionally translocated to other southern African countries such as Mozambique and Angola. The other population control option is culling. Culling is

regarded by some South Africans as an acceptable solution to the overabundance of elephants in the KNP; indeed, an average of 300 elephants per annum were culled between 1967 and 1995 (Whyte, 2001). What makes it ethically acceptable, is that entire breeding herds are culled such that no family members remain; culling only parts of a group would almost certainly result in severe stress to the remaining individuals. The problem with this assumption is that, in the past at least, no one properly identified the breeding herds and all their members prior to the day of culling. It would in fact be almost impossible to achieve. Neither has anybody researched the effects of culling on the behaviour of the remaining population. A further disadvantage of culling is a reduction in density, to which the population responds by increasing its reproductive rate. So, the more you cull the more you need to cull.

The 'laissez-faire' approach may sound disastrous, but many conservationists are in favour thereof. Scientists have shown that the rate of reproduction in elephant populations is influenced greatly by population density. This was clearly shown in early studies by Laws (1969) and Laws *et al.* (1975). These studies revealed a distinct relationship between population density, age at first calving and calving interval. Mortality also contributes to population dynamics, and varies according to rainfall abundance. During the dry season and especially in the case of severe droughts, mortality amongst young calves and, less importantly, elderly elephants increases. The incidence of anthrax, a disease to which elephants are susceptible, also increases during droughts. This is why the provision of artificial waterholes is one of the major reasons for elephant population expansion in the KNP. An abundance of waterholes between perennial and annual rivers makes it possible for elephants to utilise much greater areas of habitat than would normally be the case during the dry season. This applies particularly to breeding herds in which migration is limited by old cows and young calves. During severe dry periods, these are the animals that would die first as they would be unable to make the daily journey from food source to water without the existence of these waterholes. The increased habitat availability thereby leads to an increased survival rate and maintenance of a larger population. Etosha National Park is the prime example of how the 'leis affairs' approach can work. The elephant population has been constant at about 300 elephants for the last 30 years without intervention (van Aarde and Jackson, 2007).

In recent years, the culling debate has once again reared its head – not only in the KNP but also in Zimbabwe, Namibia and Botswana. In South Africa, a massive debate was sparked, which resulted in a number of meetings to discuss the 'elephant problem', and if and how elephant populations should be controlled. The Great Elephant Indaba (Indaba is a Zulu word meaning group discussion, usually by tribal chiefs) held in the KNP in October 2004 was the first of these meetings. In 2006, the Minister for Environmental Affairs and Tourism convened a Scientific Round Table to

discuss and advise on the elephant issue. The most significant outcome of the meeting was that it emerged that the KNP had in fact never 'proved' that they had too many elephants. More to the point, the specific research to demonstrate that they had too many elephants had not been carried out. The Round Table recommended that a scientific assessment of elephant population management options should be undertaken and that a panel of scientists should be convened to write a book on all aspects of elephant management; this resulted in the publication of "Assessment of South African Elephant Management 2008". At about the same time, the South African Minister Marthinus van Schalkwyk commissioned the development of an extensive document called the "National norms and standards for the management of elephants in South Africa" on the basis of Section 9 of the National Environmental Management: Biodiversity Act, 2004 (Act No. 10 of 2004) These are detailed guidelines for elephant owners and park or game reserve managers on how to treat and manage elephants under a variety of conditions. The guidelines also state that a permit to cull may be issued provided that a reserve can a) prove that it has too many elephants and b) show that there are no alternatives other than lethal management.

One of the possible ways of preventing elephant populations from growing further is to control fertility by means of contraception. Any such contraceptive technique has to be effective, affordable, reversible, and, preferably, deliverable remotely by means of drop-out darts. The method would also need to be safe during pregnancy given that, at any one time, 30-50% of untreated cows under free-ranging conditions are probably pregnant. There is thus a need to test one or more contraceptive methods that have proven successful in other wildlife species in elephants. Examples are immunocontraception using the porcine zona pellucida (pZP) vaccine that had been used in wild horses and numerous ungulate species in zoos, and GnRH vaccines which have been developed for use in domestic animals. If the treatments proved effective, trials would need to be expanded to a number of private game reserves, to allow for more extensive monitoring of treated animals.

Control of aggressive behaviour and musth in elephant bulls

In the past, musth and aggressive behaviour in zoo elephants and captive Asian elephants has largely been controlled using managemental methods. In zoos, bulls of both species are either housed permanently 'hands-off' or are moved to hands-off facilities during periods of aggression and musth. Working Asian elephants are usually leg-chained in the forest and subjected to reduced feed intake during such periods, since starvation diets are thought to shorten the duration of musth. Occasionally, surgical castration has been performed in Asian elephants, and there is at least one African elephant bull in South Africa that has been castrated. This was done before the appearance of his first musth, and at 32 years of age the bull in question has never

shown musth or periods of aggressive behaviour. For free-ranging bulls, while it may be feasible, gonadectomy would be very expensive and, of course, irreversible. Similarly, while a number of free-ranging bulls in South Africa have been vasectomised as a contraceptive measure (Stetter *et al.*, 2007), this approach would not be expected to influence testosterone-related behaviour.

Anti-androgens (Niemuller, 1998) and GnRH agonists (de Oliveira, 2004) have previously been used to ameliorate aggression in elephant bulls but with limited or no success; further work is needed to determine their ability to control aggression. The GnRH agonist, Leuprolide, was used repeatedly in an attempt to down-regulate musth in an Asian elephant but with unconvincing results (de Oliveira, 2004). In this respect, GnRH agonists such as deslorelin are not able to down-regulate male reproductive function in cattle (D'Occhio, 1999), horse (Stout and Colenbrander, 2004) or donkey stallions (Bertschinger, unpublished).

In summary, there is currently no practical and affordable method of controlling testosterone-related behaviour in elephant bulls, particularly if the method is to be reversible. In domestic animals, there is one method that stands out because it has been so successful at down-regulating aspects of male reproduction function in a broad range of species. This method involves the use of GnRH vaccines that stimulate the production of GnRH specific antibodies. The antibodies neutralise endogenous GnRH and, in so doing, down-regulate the down-stream endocrine mechanisms that stimulate reproductive function in the male. One of the main end targets is testosterone, which is mainly responsible for aggressive behaviour and, in elephants, the phenomenon of musth. In theory, GnRH vaccination is reversible and remote delivery is possible. If such a vaccine was effective, there would be tremendous scope for using it to control aggressive behaviour and, if possible, musth in captive and even wild elephant bulls.

Contraception

Before discussing contraception in broader terms it is appropriate to look at certain aspects of reproductive endocrinology and sperm zona binding that have a bearing on the topic.

Endocrine control of reproduction

The hypothalamus is regarded as the coordinating centre for the control of most bodily endocrine systems subject to homeostasis. It controls the activities of endocrine organs elsewhere in the body either directly or via the pituitary gland (hypophysis) by means of liberins and statins. The processes by which the hypothalamus controls the target

endocrine and organ systems are complex and not completely understood. As the controlling centre, the hypothalamus receives feedback from the target tissues in the form of hormones and metabolites. These are integrated with messages from higher areas of the brain and the pineal gland, which modulate the response of the hypothalamus. Besides the nuclei or centres concerned with reproduction, there are areas that control metabolism, thirst and water balance, hunger and satiation, body temperature and the rate of corticosteroid secretion and response to stress. These functions are also integrated with the control of reproduction.

Central endocrine control of reproduction is organised via the hypothalamo-pituitary-gonadal axis. The hypothalamic liberin, GnRH, is central to this axis and is the primary controller of reproduction in both female and male mammals. GnRH is produced by neurosecretary cells in two main areas of the hypothalamus, namely the preoptic area (POA) and the arcuate nucleus (ARC). In females, the GnRH neurons of the POA are responsible for the preovulatory surge of GnRH which, in turn, stimulates the preovulatory LH surge. By contrast, the GnRH neurons of the ARC seem to be more involved in the slower pulsatile release of GnRH which controls FSH release from the adenohypophysis. During the luteal phase of the oestrous cycle, progesterone has a negative feedback effect on GnRH release resulting in slower pulses with lower amplitudes. In the absence of a viable embryo in species like the cow, prostaglandin $F_{2\alpha}$ is released by the endometrium and reaches the ipsilateral ovary via the utero-ovarian vein-ovarian artery counter-current mechanism where, if a corpus luteum is present, it induces luteolysis. As peripheral progesterone concentrations fall, GnRH pulse rates and amplitudes increase. This initial moderate increase provides the main stimulus for the release of FSH by gonadotrophs in the adenohypohysis. FSH stimulates follicle development including the synthesis of LH receptors and aromatase in the granulosa cells. This occurs during the phase of the cycle corresponding to pro-oestrus and up to early to mid-oestrus, when oestradiol levels peak. Oestradiol induces changes in the reproductive tract that optimise conditions for mating and sperm transport while simultaneously inducing behavioural changes at the level of the brain that make the female receptive to a male. Oestradiol is also intricately involved in follicular ripening and ovulation at the level of the POA. When oestradiol reaches threshold concentrations in spontaneous ovulators, the GnRH neurons respond with a prolonged surge of GnRH (up-regulation) and, distinct from the slow pulsing which stimulates FSH release, the surge leads to the preovulatory LH surge from adenohypophysial gonadotrophs. At the same time, oestradiol and inhibins suppress the release of FSH from the adenohypophysis. The LH surge stimulates ripening of the follicle, ovulation, and development of a progesterone-producing corpus luteum. Oestradiol plays a similar role in induced ovulators like lions and cheetahs, however the stimulus for the ovulatory GnRH surge is a neural reflex (hence they are also referred to as reflex ovulators), which arises from sensory nerve endings in the vagino-

clitoral area. In other words, the main stimulus for the GnRH surge is mating; this is the reason why cat species mate so frequently (Bertschinger *et al.*, 2008a).

Recently a new player in the field of GnRH control has been discovered (Caraty and Franceschini, 2008). The protein kisspeptin is the ligand for the receptor GPR54 which is found on GnRH neurons. The cells that secrete kisspeptin are found in the ARC and the antero-ventral periventricular nucleus (AVPV). Prolonged peripheral injection of kisspeptin to ovariectomised ewes is able to induce an LH peak while, in intact synchronised ewes, an acute injection of kisspeptin can induce an LH peak and ovulation much earlier than in control ewes. In addition, kisspeptin cells in the AVPV have oestradiol receptors and are thought to function as the link between oestradiol and the preovulatory GnRH surge. Just to complicate matters, however, kisspeptin has been shown to stimulate LH release by bovine and porcine pituitary cells and rat pituitary explants. In support of a function for kisspeptin at the pituitary level, GPR54 has now been shown to be expressed in human pituitary cells and, in the sheep, kisspeptin-immuno-reactive fibres have been identified in the external zone of the median eminence.

The endocrine control of reproduction of males is similar to that in females, with the major exception of a distinct cyclic pattern. Given the differences in hypothalamic control of reproduction, it is not surprising that several hypothalamic nuclei are sexually dimorphic, most notably a nucleus found in the POA which is only present in males. Another example of dimorphism is in the number of kisspeptin cells that are found in the AVPV of rats. Female rats have much greater numbers of these cells than male rats, which is logical given that the AVPV is primarily involved in the preovulatory surge of GnRH and ovulation. Such sex-related anatomical and physiological differences may have implications for hormonal manipulation of fertility. There are also differences between species that explain why the males of some species respond to a certain contraceptive method while those of another species do not.

The target cells that are down-stream of FSH and LH obviously also differ between males and females. FSH specifically binds to Sertoli cell membrane receptors, and is thus intricately involved in the regulation of spermatogenesis. Products of the Sertoli cells, such as oestrogens and inhibins, have a negative feed-back effect on FSH secretion at the levels of the hypothalamus and adenohypophysis. Leydig cells on the other hand are targeted by, and have membrane receptors for, LH which stimulates testosterone synthesis and secretion. Androgens also affect sperm production but more specifically the reduction division (spermatidogenesis) and spermiogenesis. The endocrine requirements for spermatogenesis do however show some species differences, which may explain why sperm production is easier to switch off in some species than in others. Examples are the boar (easy) and stallion (difficult), although some of the differences are in part also age-dependent. Further down-stream,

epididymal functions required for the acquisition of aspects of fertilising capacity by newly formed sperm are also androgen dependent. The roles of androgens in normal spermiogenesis and function of the epididymis require androgen concentrations 10 to 100-fold higher than usually encountered in the peripheral circulation. These concentrations are achieved by two important trapping systems. The one is the pampiniform plexus where, as a result of the counter-current flow of blood, androgens diffuse from high concentrations in the venous drainage to low concentrations in the arterial supply thus trapping androgens in the testis. The other is androgen binding protein (ABP) which has a high affinity for binding, and thus trapping, androgens within the seminiferous tubules. The flow of fluid and sperm products in the seminiferous tubules carries ABP-androgen into the epididymal ducts. Androgen sources originating outside the testis (e.g. exogenous sources), can never achieve the same concentrations within the testis-epididymal compartment because they would block the concentrating and secretory mechanisms. In this respect, circulating androgens regulate LH levels by negative feedback at the levels of the hypothalamus and, probably, adenohypophysis. For this reason, injected or oral androgens or anabolics with androgenic activity down-regulate sperm production. They affect sperm quality more profoundly than quantity.

Sperm-zona binding

Sperm-zona binding and the changes it triggers in the sperm are central to the process of fertilisation in eutherian mammals (Clark and Dell, 2006). The zona pellucida (ZP) surrounding the oocyte consists of a matrix of three (ZP_1, ZP_2 and ZP_3) and, in some species, four major glycoproteins. Where there are three, ZP_2 and ZP_3 form a network of fibres that are cross-linked by ZP_1 to build up a capsule surrounding the oocyte, which first appears in multi-laminar primary oocytes (Wassarman *et al.,* 1999*)*. ZP_3 is thought to provide receptor sites for sperm-binding since genetic deletion results in loss of ZP function accompanied by infertility in mice (Clark and Dell, 2006). While ZP_3 binds sperm, a process that is required to initiate the acrosome reaction, ZP_2 appears to be important for the penetration of the sperm through the zona. Moreover, it is the carbohydrate components of the ZPs that appear to be critical as far as their biological functions are concerned. Once bound to a ZP receptor site, the acrosome reaction is induced during which the outer acrosomal membrane fuses with the plasma membrane to release zonalytic enzymes (Fig. 1). This also exposes binding sites on the inner sperm acrosomal membrane which interacts with ZP_2 and allows the motile sperm to penetrate obliquely through the ZP towards the oolemma.

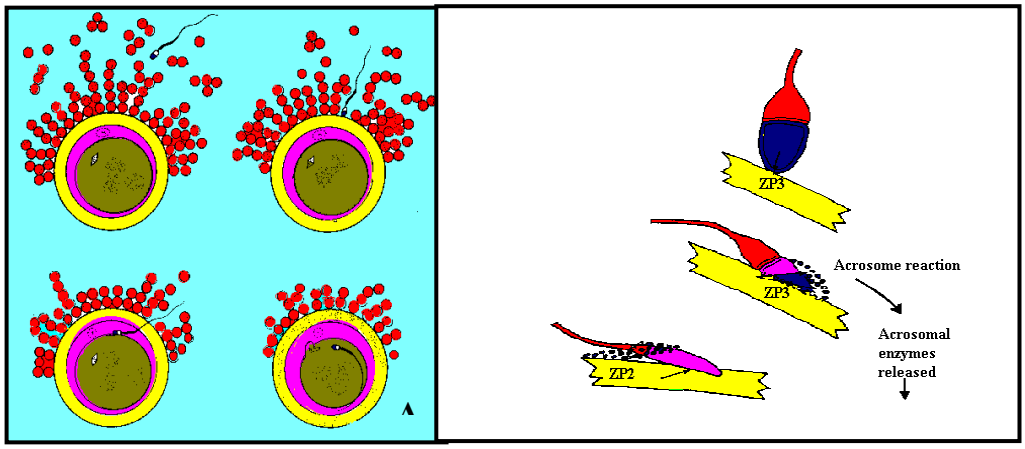

Figure 1: The process of fertilisation (A) and sperm-zona binding resulting in the acrosome reaction (B).

The carbohydrate components of the ZP proteins also constitute the most important immunological epitopes and are, as such, the preferred targets for infertility-inducing antibody production. The ZP proteins appear to be well conserved across a wide range of mammalian species and show very little homology with somatic proteins, such that autoimmune disease is unlikely to result if any or all three ZP proteins are used as targets for immunocontraception (Fayrer-Hosken, 2008).

Short history of contraception

According to Jöchle (2008), for thousands of years the only form of contraception practiced on domestic animals was surgical castration. Male castration dates back to 7-6000 BC. The early development of orchidectomy is not surprising, given that the male gonads of domestic species are situated extra-abdominally. After all, open castration without anaesthesia is still practiced today in male piglets. In the 1950s, rural Swiss veterinarians used the so-called 'gumboot method' for castrating tom-cats without anaesthesia (Zerobin, personal communication). More surprisingly, ovariectomy is quoted in Aristotle's writings as early as 384-322 BC (Jöchle, 2008). In Europe, it is interesting to note that from the 15th to the 19th century neutering of male and female domestic animals was performed by professionals with a special license, but not veterinarians. It was only in the 18th and 19th centuries that veterinarians slowly entered the castration business, and the last permit allowing a non-veterinarian to castrate in Austria was issued in 1929.

Non-surgical reversible contraception in women, in the form of contraceptive medications and abortifacients also date back some 3000 years (Finch and Green, 1963), although no information has been found for the use of similar products on domestic animals. The possible use of IUDs in animals may however date back as far as 3000 years ago, when nomads placed pebbles into the uteri of camels to prevent conception during their long treks through the desert (Museum of Contraception, University of Montpellier, France; Fig. 2). It was only really in the second half of the 20th century, however, that non-surgical methods for contraception of house pets were widely adopted (Jöchle, 2008). The first oral contraceptive for dogs, medroxy-progesterone acetate, became available in 1963. This was followed by a number of other progestins, such as megestrol acetate, norethisterone, chlormadinone acetate and proligestone in oral or injectable forms. Oestrogen compounds were the first abortifacients to be used in dogs (1936) and, despite serious side-effects that may result after treatment, are still commonly used in many countries. Jöchle *et al.* reported the first use of prostaglandin $F_{2\alpha}$ to abort pregnancy in bitches in 1973.

The development of 'modern' contraceptive methods such as the GnRH super-agonists and immunological tools such as porcine zona pellucida and GnRH vaccines did not begin until 20-30 years after the launch of the first progestins for fertility control in dogs.

Contraceptive methods used in wildlife

Contraception to control the rate of reproduction is an interesting and potentially practical means of population control in wildlife. Broadly speaking, the following methods can be used for contraception of animals

- Surgical (gonadectomy, vasectomy and salpingectomy)
- Hormonal (oral contraceptives, depot-injections or slow-release implants)
- Immunocontraception

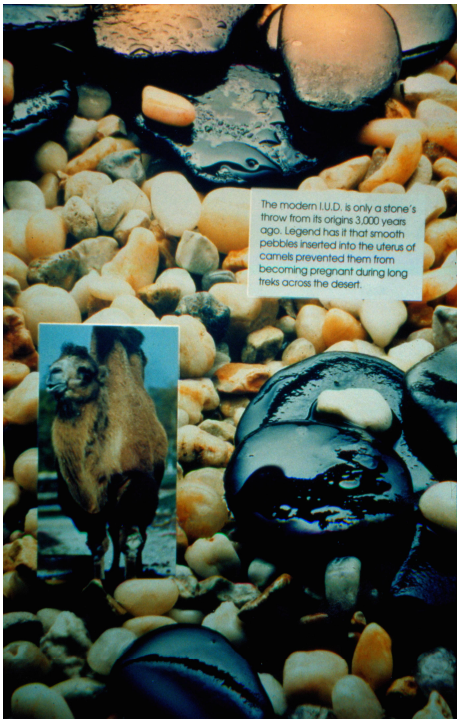

Figure 2: According to legend, the use of IUDs dates back 3000 years when travelling nomads placed pebbles into the uteri of camels to prevent them from getting pregnant during the long treks across the desert (Museum of Contraception, University of Montpellier)

The modern contraceptive era for domestic species started in the 1960s. Most of the early treatments were based on the use of progestins, which have a negative feedback effect on GnRH pulsatility in the hypothalamus (Fig 3B). Long-acting silicon implants impregnated with progestins, like MGA, have been extensively used to down-regulate female reproduction in wild felids, such as lions and tigers, and some wild canid species (Fig. 3B). Although highly successful as contraceptive agents, their prolonged use resulted in a number of side-effects, some of which were life-threatening (Munson 2002; Munson 2005). Provided no serious pathology had occurred, the effect of the progestins could be reversed by removing the implant – a process which required immobilisation.

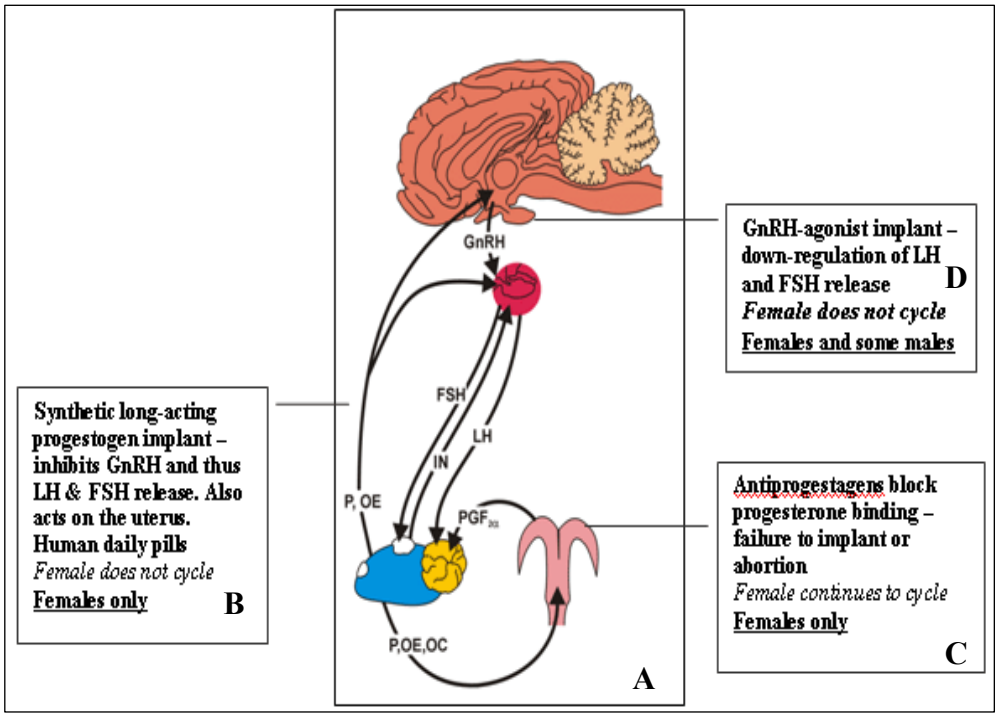

Figure 3: Endocrine control of ovarian function (A) and the mechanisms by which synthetic progestins (B), antiprogestagens (C) (Göritz *et al.*, 2001) and GnRH super agonists (D) exert their contraceptive effects.

A range of steroids in the form of depot injections and slow-release implants were tested for contraceptive efficacy in wild horses. In stallions, testosterone propionate and quinestrol resulted in oligospermia and decreased sperm motility, but treatment with large doses needed to be repeated at monthly intervals to maintain infertility (Kirkpatrick *et al.*, 1982). The use of microcapsules extended the duration of contraception, but each stallion had to be treated with 12 g of the preparation. In mares, success was achieved with ethinyl oestradiol (considered to block ovulation and/or implantation) delivered by means of impregnated silastic rods. Contraception lasted 48-60 months with a dose of 8 g (Plotka and Vevea, 1990). The delivery of such large amounts of steroid hormones to either sex and, in some cases, the need to adopt unusual depot sites (e.g. intra-peritoneal) render the depot administration of reproductive steroid hormones impractical for wildlife contraception. There were also concerns that the steroids used could result in a number of side effects and, more importantly, would pass through the food chain. Despite these concerns, implants

impregnated with oestradiol 17β were used to treat 10 elephant cows in the KNP in 1996. While effective as a contraceptive (Göritz *et al.*, 1999), oestradiol treatment resulted in almost continuous oestrus that lasted at least 12 months (Bartlett, 1997; Butler, 1998; Whyte and Grobler, 1998). No further trials were allowed with the drug.

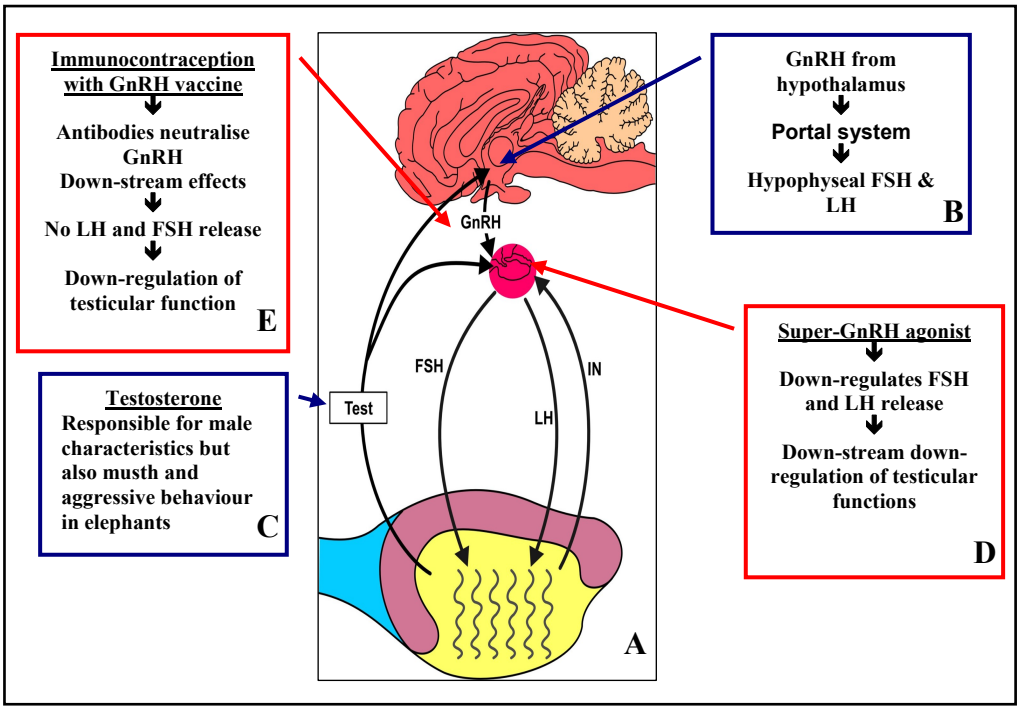

Figure 4: Endocrine control of testicular function (A-C), and the mechanisms by which GnRH super agonists (D) and GnRH vaccines (E) exert their contraceptive effects.

During the late 1990's, a new hormonal contraceptive method became available in the form of the GnRH analogue deslorelin, a so-called GnRH super-agonist. Peptech Animal Health (Sydney, Australia) designed a slow release formulation that released deslorelin for periods of months and even years. On the basis of results obtained in domestic dogs and cats (Munson *et al.*, 2001; Trigg *et al.*, 2001), deslorelin acetate released continuously from a subcutaneous biocompatible implant appeared to be an ideal agent for controlling reproduction in large predators. High continuous administration of deslorelin acts by down-regulating the release of FSH and LH at the

level of the anterior pituitary (Figs 3D and 4D). This in turn down-regulates endocrine stimulation of gonadal activity. As such, it has the potential to work in both males and females. Once all the deslorelin has been released from an implant, the target animal would revert to normal reproductive function. As a result of the successes achieved in male and female dogs and cats, preliminary trials were carried out in lionesses, and male and female cheetahs, wild dogs and leopards (Bertschinger *et al.*, 2001). With the exception of wild dog females, the contraceptive success rate achieved was 100%. Even in wild dog females, the failure rate was only 10%.

Immunocontraception relies on carefully selecting target proteins that are involved in critical steps of reproduction. Including such a protein as an antigen in a vaccine provokes the production of antibodies that neutralise the endogenous molecule or block a particular process. Examples are the native porcine zona pellucida (pZP) and GnRH vaccines. The antibodies produced to the pZP vaccine bind to zona proteins on the oocyte in vaccinated females. Binding to ZP_3 is thought to block sperm-zona binding and thereby prevent fertilisation from taking place (Fig. 5). The GnRH vaccines, which consist of modified GnRH peptides conjugated to a foreign protein to increase the antigenicity, induce antibodies that neutralise GnRH in the target animal. If the anti-GnRH antibody titre is sufficient, the antibodies neutralise hypothalamic GnRH and thereby block the ability of this releasing hormone to stimulate gonadotrophin release from the adenohypophysis (Fig. 4E). In theory, the vaccine should be effective as a contraceptive in both sexes.

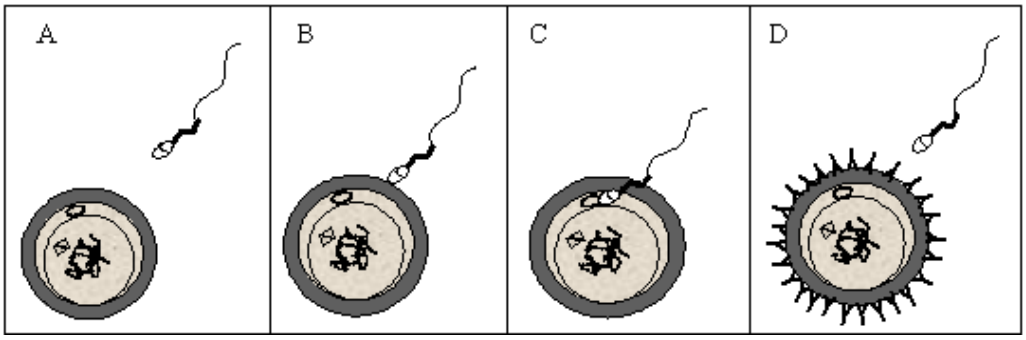

Figure 5: The proposed mechanism of PZP immunocontraception (Bertschinger *et al.* 2008b)
A - When the egg (oocyte) is ovulated into the Fallopian tube it is surrounded by a capsular layer known as the zona pellucida.
B - Before fertilisation can take place, the sperm must bind to one of thousands of receptor sites on one of the zona proteins. The sperm then undergoes the 'acrosome reaction'.
C - Only once the sperm has undergone the acrosome reaction can it penetrate the ZP and fertilize the egg.
D - The antibodies formed in response to the pZP vaccine recognise and coat all sperm receptors on the ovulated oocyte. Sperm-binding is blocked rendering fertilisation impossible.

The origins of the pZP contraceptive vaccine go back to 1973 when Sacco and Shivers (1973) demonstrated that antibodies to a pZP vaccine could inhibit sperm-zona binding. Further work demonstrated that the key to inhibition relied on the carbohydrate portions of the zona proteins, since recombinant ZP proteins that were not properly glycosylated were not able to stimulate a contraceptive effect. Liu *et al*. (1989) were the first to implement pZP vaccine successfully in domestic mares. Over the next 19 years, the pZP vaccine was used successfully as a contraceptive in feral mares and proved an effective means of limiting population growth. The success of the technique in wild horses led to its application to other wildlife species like white-tailed deer (Rutberg *et al.*, 2004), wapiti (Shidler *et al.*, 2002) and others (Frank *et al.*, 2005). The vaccine could be delivered remotely and was both safe and reversible.

GnRH immunocontraception was originally developed for the immunocastration of cattle (Hoskinson *et al.,* 1990). One of the main reasons for further development of a GnRH vaccine, however, was as an alternative to surgical castration to control the problem of boar taint in pork (D'Occhio, 1993; Oonk *et al*., 1998; Dunshea *et al.* 2001; Zeng *et al.* 2001). GnRH vaccines have also been used to suppress fertility or reproductive behaviour in male feral pigs (Killian *et al.* 2006), stallions (Dowsett *et al.* 1996; Turkstra *et al.* 2005; Burger *et al.* 2006; Janett *et al.,* 2009), rams (Janett *et al.* 2003) and bison bulls (Miller *et al.* 2004). GnRH vaccines have also been successfully used to down-regulate fertility and sex-related behaviour in domestic and wild mares (Goodloe *et al.,*1997; Killian *et al.,*2004; Botha *et al.,* 2008).

The ideal wildlife contraceptive

The ideal wildlife contraceptive should fulfil most of the following requirements:

- Effective
- Allow remote delivery
- Be reversible; although this depends on exact requirements for individual species and conditions
- Have little or no effect on social behaviours or organization of groups or herds
- Have no deleterious short or long-term health effects
- Should not pass through the food chain
- Be safe to use during pregnancy
- Have affordable production and application costs
- Use should be ethically acceptable

Table 1 lists contraceptive methods that could be used and the extent to which they comply with the properties of an ideal wildlife contraceptive.

Table 1: Extent to which potential wildlife contraceptive agents conform to the ideal properties (Bertschinger *et al*., 2008)

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

*Males can remain fertile for a number of weeks; **Only if given daily; ***Does not work in male ungulates; remote delivery system being developed (Herbert & Vogelnest, 2007); *****Requires microsurgery – not feasible under field conditions and especially not in mega-herbivores.
Gonadect = gonadectomy; Vasec = vasectomy; FallT = tying of of fallopian tubes; NA = not applicable

Scope of the thesis

Having decided that contraception could be useful for controlling the rates of reproduction in wildlife, different methods were sought that best suited the needs of carnivores on the one hand and elephant cows on the other. In carnivores, the aim was to slow-down the rate of reproduction and as such it was not considered necessary to treat all animals. In addition, knowing the reproductive status of most females would help to achieve this goal. The need for a remote delivery system was not therefore critical. For elephant cows, however, a remote delivery system was essential since immobilising cows for each treatment would have been prohibitively expensive, impractical and even dangerous if repeated too often. In the case of musth and control of aggression in elephant bulls, a method that could be delivered remotely is essential for free-ranging bulls and is also often necessary for captive bulls.

Chapter 2 describes extensive studies carried out to examine the efficacy of slow-release GnRH-implants for down-regulating reproductive function in cheetahs (males and females), lionesses, African wild dogs (males and females) and leopards (both sexes). The agonist, used at the correct dose was expected to inhibit the release of FSH and LH from the pituitary gland and thereby down-regulate down-stream reproductive functions. Previously, we had used the implants in pilot trials, but as yet had no clear data on the dose required or the duration of effect, i.e. the period before normal reproductive function returned. We tested doses of 3-6 mg in the smaller carnivores (all captive), while using 12 or 15 mg in captive and free-ranging lionesses.

Chapter 3 describes a more extensive study over a period of up to 10 years in free-ranging lionesses and captive lionesses and tigers. The aims of this study were to test various doses of deslorelin implants for their efficacy, interval to reversal and to examine whether there were cumulative effects of repeated use over a number of years. The 4.7 and 9.4 mg implants used in this study were new formulations that had not been tested previously. A further aim of the study was to monitor the faecal steroid profiles in two lionesses to generate more detailed information on the down-regulatory effects of the 9.4 mg implants and the endocrine changes underlying the return to cyclicity.

In Chapter 4 the repeated use of deslorelin in male cheetahs was investigated, with some males being treated annually for five consecutive years. Two different doses were tested, namely 6 mg and 4.7 mg implants. Efficacy was monitored in terms of fertility, and the effects on testicular size, penile spikes and serum testosterone concentrations. Testosterone concentrations are a more sensitive indicator of partial contraception or early reversal than reappearance of sperm in the ejaculate since down-regulation of spermatogenesis persists beyond the recovery of normal reproductive endocrine activity (~6-9 weeks).

In Chapter 5 the contraceptive potential of the native porcine zona pellucida (pZP) vaccine was assessed *in vitro* by testing for the existence of (immunological) homology between porcine and elephant zona pellucida proteins. This was carried out by examining the ability of antibodies raised against pZP in rabbits to bind to the zona pellucida in histological sections of elephant ovaries. An additional aim was to examine whether three zoo elephants would produce an effective antibody response to pZP vaccine injection. After confirming the potential of pZP to induce appropriate biological effects in elephants, the first field trials were carried out in the KNP. The aims of these preliminary trials were to test remote delivery, contraceptive efficacy, safety and short-term reversibility of the pZP vaccine.

In Chapter 6 the elephant pZP immunocontraception studies were extended to examine the effects of pZP immunocontraception on population growth in seven discrete elephant populations on separate reserves. The results were monitored in terms of the effect in the annual calving percentages for the various populations. A secondary, but no less important, aim was to determine whether pZP vaccination was safe if administered during pregnancy.

The aim of Chapter 7 was to test the ability of GnRH vaccines to down-regulate aggressive behaviour and/or musth in captive elephant bulls. The response was evaluated by monitoring behaviour, and where possible, blood testosterone or faecal epiandrosterone concentrations.

Finally the results of these studies and their implications for wildlife population control are discussed in Chapter 8. A number of areas where our knowledge of reproductive physiology is deficient, but which have a bearing on contraception of carnivores and elephants or the control of aggressive behaviour in male elephants, are identified. Areas requiring further research are listed and discussed briefly.

References

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

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

Bertschinger, H.J., D.G.A. Meltzer & A. van Dyk. 2008a. Captive breeding of cheetahs in South Africa – 30 years of data from the de Wildt Cheetah and Wildlife Centre. *Reproduction in Domestic Animals* 43 (Supplement 2): 66-73

Bertschinger, H., A. Delsink, J.J. van Altena, J. Kirkpatrick, H. Killian, A. Ganswindt, R. Slotow & G. Castley. 2008b. Reproductive control of elephants. In: Elephant management. A scientific assessment for South Africa. Eds RJ Scholes and KG Mennell, Wits University Press, 1Jan Smuts Avenue, Johannesburg: 357-328.

Botha, A.E., M.L. Schulman H.J. Bertschinger**,** A.J. Guthrie, C.H. Annandale & S.B. Hughes. 2008. The use of a GnRH vaccine to suppress mare ovarian activity in a large group of mares under field conditions. *Wildlife Research* 35: 548-554

Butler, B. 1998. Elephants: Trimming the herd. *BioScience* 48: 76-81.

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

Caraty A & I. Franceschini. 2008. Basic aspects of the control of GnRH and LH secretions by kisspeptin: potential applications for better control of fertility in females. *Reproduction in Domestic Animals* 43 (Supplement 2): 172-178.

Clark, G.F. & A. Dell. 2006. Molecular models for murine sperm-egg binding. *Journal of Biological Chemistry* 281: 13853-13856.

de Oliveira C.A., G.D. West, R. Houck & M. Leblanc. 2004. Control of musth in an Asian elephant bull (*Elephas maximus*) using leuprolide acetate. *Zoo and Wildlife Medicine* 35: 70-76.

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

D'Occhio, M.J. & W.J. Aspden. 1999. Endocrine and reproductive responses of male and female cattle to agonists of gonadotrophin-releasing hormone. *Journal of Reproduction and Fertility* Supplement 54: 101-114.

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

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

Fayrer-Hosken, R. 2008. Controlling animal populations using anti-fertility vaccines. *Reproduction in Domestic Animals* 43 (Supplement 2): 179-185.

Finch, B.E. & H. Green. 1963. Contraception through the ages. Peter Owen Ltd. London. Cited according to: Jöchle W 2008 History of non-surgical contraception in dogs and cats. In: Geschichte der Gynäkologie und Andrologie de Haustiere, Ed Johann Schäffer, 14. Jahrestagung der DVG-Fachgruppe, 2-3 November 2007: 250-259.

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

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

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

Goritz, F., M. Quest, T.B. Hildebrandt, H.H.D. Meyer, L. Kolter, W. Elger & K. Jewgenow. 2001. Control of reproduction with anti-progestin and oestrogens in captive bears *Journal of Reproduction and Fertility* Supplement 57: 249-254.

Gutsche, T. 1979. There was a man. The life and times of Sir Arnold Theiler K.C.M.G. of Onderstepoort. Howard Timmins, 45 Shortmarket Street, Cape Town.

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

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

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

Janett F, R. Stump, D. Burger & R. Thun. 2009. Suppression of testicular function and sexual behaviour by vaccination with GnRH (Equity^{Tm}) in the adult stallion. *Animal Reproduction Science* 115: 88-102

Jöchle, W., R.V. Tomlinson & A.C. Anderson. 1973. Prostaglandin effects on plasma progesterone levels in the pregnant and cycling dog (Beagle). *Prostaglandins* 3: 209-217.

Jöchle, W. 2008 History of non-surgical contraception in dogs and cats. In: Geschichte der Gynäkologie und Andrologie de Haustiere, Ed Johann Schäffer, 14. Jahrestagung der DVG-Fachgruppe, 2-3 November 2007: 250-259

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

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

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

Laws, R.M. 1969. Aspects of reproduction in African elephants, *Loxodonta Africana*. *Journal of Reproduction and Fertility* Supplement 6: 193-217.

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

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

Marnewick, K., A. Beckhelling, D. Cilliers, E. Lane, G. Mills, K. Herring & P. Caldwell. 2007: Status of cheetahs in southern Africa. *Catnews,* Special Issue 3, Status and conservation needs of cheetahs in southern Africa: 22-31.

Meltzer, D.G.A. 1994. Diseases in free-ranging black and white rhinoceroses. In: B.L. Penzhorn and N.P.J. Kriek (eds) Proceedings of a symposium on rhinos as game ranch animals. Onderstepoort, Republic of South Africa, 9-10 September 1994, pp. i-iv, 1-242:176-179.

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

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

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

Munson, L., J.E. Bauman, C.S. Asa, W. Jöchle & T.E. Trigg. 2001. Efficacy of the GnRH analogue deslorelin for suppression of oestrous cycles in cats. *Journal of Reproduction and Fertility* Suppl. 57: 269-273.

Niemuller, C.A., J.L. Brown & J.K. Hodges. 1998. Reproduction in elephants. In Knobil E, Neill J D (eds) *Encyclopedia of reproduction*. New York Academic Press: 1018-1029.

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

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

Power, R.J. 2002. Prey selection of lions *Panthera leo* in a small enclosed reserve. *Koedoe* 45: 67-75.

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

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

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

Smithers R.H.N. (Ed.). 1983. *Panther leo*. In 'The mammals of the southern African region'. University of Pretoria: Pretoria: 374-381.

Stetter, M., D. Hendrickson, J.R. Zuba, M. Briggs, D. Grobler, L. Small & J.J. van Altena. 2007. Laparoscopic vasectomy as a potential population control method in free ranging African elephants (*Loxodonta Africana*). *Proceedings of the American Association of Zoo Veterinarians*, Annual Meeting, Knoxville, TN, USA: 185-188.

Stout, T.A. & B. Colenbrander. 2004. Suppressing reproductive activity in horses using GnRH vaccines, antagonists or agonists. *Animal Reproduction Science* 82-83:633-43.

Teren, G. 2009. Elephants and biodiversity. *Africa Geographic* January 2009

Trigg, T.E., P.J. Wright., A.F. Armour, P.E. Williamson, A. Junaidi, G.B. Martin, A.G. Doyle & J. Walsh. 2001. Use of a GnRH analogue implant to produce reversible long-term suppression of reproductive function of male and female domestic dogs. *Journal of Reproduction and Fertility* Suppl. 57: 255-261.

Turkstra, J.A., F. Van der Meer, J. Knaap, P. Rottier, K. Teerds, B. Colenbrander & R. Meloen. 2005. Effects of GnRH immunization in sexually mature pony stallions. *Animal Reproduction Science* 3-4: 247-259.

van Aarde, R.J. & T.P. Jackson. 2007. Megaparks for metapopulations: Addressing the causes of locally high elephant numbers in southern Africa. *Biological Conservation* 134: 289-297.

Wassarman, P., J. Chen J, N. Cohen, E. Litscher, C. Liu, H. Qi & Z. Williams. 1999**.** Structure and function of the mammalian egg zona pellucida. *Journal of Experimental Zoology* 285: 251-258.

Whyte, I.J. 2001. Conservation management of the Kruger National Park elephant population. PhD thesis, University of Pretoria: pp 235.

Whyte, I.J. & D.G. Grobler. 1998. Elephant contraception in the Kruger National Park. *Pachyderm* No. 25, 45-52.

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

Chapter 2

Induction of contraception in some African wild carnivores by downregulation of LH and FSH secretion using the GnRH analogue deslorelin

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Abstract

The GnRH analogue deslorelin, in long-acting biocompatible implants, was used as a contraceptive in 31 cheetahs (13 females and 18 males), 21 African wild dogs (15 females and 6 males), 10 lionesses and four leopards (three females and one male). A dose of 12 or 15 mg deslorelin was administered to lions, whereas 6 mg deslorelin was administered to the other species. Monitoring consisted of observations, measurement of plasma progesterone and testosterone concentrations, vaginal cytology and evaluation of semen and sex organs. Deslorelin induced contraception in lionesses for 12-18 months, and in female cheetahs and leopards for a minimum of 12 months after treatment. Two male cheetahs had no viable spermatozoa or detectable plasma testosterone 21 months after treatment with deslorelin. Female wild dogs responded less consistently and one bitch conceived 4 weeks after implantation. However, in nine bitches, mating could be postponed until the next breeding season. Male dogs responded consistently and the contraception was effective for approximately 12 months. Although lionesses and cheetahs may become attractive to males for a few days after treatment, mating was not observed. No side-effects or behavioural changes were noted, indicating that deslorelin is a safe drug to use for the contraception of the species described. Males remain fertile for the first 6 weeks after the insertion of implants and should be separated from cyclic females during this period.

Introduction

Contraception has become a useful tool in population management of wild carnivores in zoos, wildlife sanctuaries and smaller conservancies. The choice of reversible or irreversible methods of fertility control depends on requirements. The main reason for carnivore contraception in southern Africa is to slow down the rate of breeding rather than to produce permanent sterilization. This applies particularly to endangered species, such as African wild dogs and cheetahs. In Namibia, the holding of wild carnivores on private property requires a permit stipulating that breeding of any such animals is not allowed (P. E. Stander, personal communication). The main species involved are cheetahs, lions and leopards.

In South Africa, lions are kept on a number of smaller conservancies (1 000-10 000 ha) where they are allowed to range freely with prey species. Under such conditions, the lack of competition from other lions and large predators results in an increased cub survival rate. The increased survival rate of young leads to a population explosion and consequently to a depletion of the prey species, which are expensive to replace. For example, at Mabula Nature Reserve it costs R 400-500 000 annually to restock the park with prey species for a pride of lions consisting of four adults, four sub-adults and some suckling cubs. Another example is Thorny Bush where giraffes have raised very few calves successfully over the past few years because of predation by lions. Some of these reserves house valuable species such as sable antelope and disease-free buffalo, which large prides tend to prey on to obtain sufficient bulk. The potential for inbreeding of carnivores on smaller reserves is also increased. Therefore, in an attempt to ameliorate the problem, the rate of reproduction should be slowed down and, for genetic reasons, lionesses should be allowed to breed on a rotational basis.

Selection of contraceptive method

Other than obvious criteria, such as safety to the animal, safety during pregnancy and within the food chain, the main requirement of a contraceptive for carnivores under southern African conditions is reversibility. Remote delivery, although an advantage, is not essential because animals are usually captured to determine their reproductive status or for other management purposes. Further considerations are hormone-dependent characteristics, such as a mane and dominance. For this reason, castration or downregulation of LH release resulting in basal concentrations of testosterone and loss of the mane is not acceptable for male lions. From results obtained in domestic dogs and cats, the GnRH analogue deslorelin acetate released long-term from a biocompatible implant (Peptech Animal Health, Sydney) appears to be an ideal agent for controlling reproduction (Munson *et al.*, 2001; Trigg *et al.*, 2001). The implants

are manufactured by a proprietary method that involves extrusion of deslorelin with matrix consisting principally of low-melting point lipids and biological surfactant (Trigg *et al.*, 2001). Bertschinger *et al*. (2001) reported on the preliminary results of the use of deslorelin in wild carnivores.

Experience with deslorelin

Including animals described in the first report, contraceptives have been administered to 31 cheetahs (13 females and 18 males), 21 African wild dogs (15 females and 6 males), 10 lionesses and four leopards (three females and one male) in southern Africa. Monitoring before and after treatment consisted of observations (mostly daily except for leopards), measurements of plasma progesterone and testosterone concentrations, vaginal cytology and evaluation of semen and sex organs. Blood plasma hormone concentrations were measured by radioimmunoassay using commercial kits (Coat-A-Count total progesterone and testosterone kits; Diagnostic Products Corporation, Los Angeles, CA). Plasma progesterone concentrations are typically low (< 1.5 nmol l^{-1}) during anoestrus in wild carnivores, although wild dogs and lionesses with values of up to 5 nmol l^{-1} and 6.73 nmol l^{-1}, respectively, were considered to be in anoestrus in the present study. The majority of the plasma progesterone content in such animals is likely to originate from the adrenal cortex as a result of ACTH stimulation from the stress of capture (H. J. Bertschinger, unpublished). Vaginal cytology (smears stained with Cam's Quick-Stain; Milch, Krugersdorp, SA) was also used to confirm low ovarian steroid activity. Semen was collected by electro-stimulation of anaesthetized males and evaluated as described by Bertschinger and Meltzer (1998) and Meltzer *et al.* (1998). The presence of viable spermatozoa was the only parameter used to assess fertility in males treated with deslorelin. The results for each species are described below.

Cheetahs

This work was out carried in northern Namibia at the Africat Foundation. Male and female cheetahs were housed in mixed groups in camps ranging from 10 to 50 ha. Both male and female cheetahs were treated with a 6 mg deslorelin implant and were observed each day thereafter. Although four of the implanted females attracted males for 5-14 days after treatment (Bertschinger *et al.*, 2001), none of the 13 females was mated and no pregnancies occurred (Table 1). Only one of the 11 females examined within the first 3 months after deslorelin implantation appears to have ovulated (AJ84, plasma progesterone concentration of 10.29 nmol l^{-1}). Eight of the females were treated in two consecutive years without significant changes in body weight or other side-effects. Two of these females attracted males sporadically during this period but,

once again, would not allow mating. Plasma progesterone assays and vaginal cytology indicated an anoestrous status for each of the 13 females at the end of each year.

The results exhibited by six males treated with deslorelin and one male implanted with 50 mg vehicle as a placebo are summarized (Table 2). Spermatozoa were still present in the ejaculate 6 weeks after deslorelin treatment, whereas plasma testosterone concentrations were already undetectable. One year after and, in the case of two animals, 21 months after treatment, viable spermatozoa were still undetectable in the ejaculates and plasma testosterone concentrations were also minimal. Ejaculate volume was either substantially decreased or no fluid could be recovered despite prolonged electro-stimulation. The testes were small and hard, and penile barbs were barely visible at 1 and 2 years after treatment, respectively. In untreated adult cheetahs, penile barbs are particularly well-developed compared with those of lions and leopards. Another 12 males were implanted in February 2001 but, other than the observation that they have not mated, no further results are available.

Hierarchy within groups containing treated male and female cheetahs was unaffected by deslorelin treatment.

African wild dogs

The wild dogs were housed in 0.5 ha camps in pairs or threes of mixed sex at the de Wildt Cheetah and Wildlife Centre. One female was isolated throughout the observation period. The wild dogs are highly fertile, monoestrus and breed once a year from February to mid-April. The dose of deslorelin administered was 6 mg.

The results for 15 females are shown (Table 3). Deslorelin treatment induced oestrus in one bitch after 4 weeks and this bitch gave birth to seven live pups 2 months later (Bertschinger *et al.*, 2001) and conceived again during the 2001 breeding season. The remaining 13 bitches, excluding the isolated bitch, showed signs of oestrus between 3 and 21 months after deslorelin implantation. The mating season was bypassed in nine bitches. Three females, two of which were in pro-oestrus at the time, received only 3 mg deslorelin. Both of the pro-oestrous animals ovulated after treatment but only one of them allowed mating and became pregnant. The other bitch also ovulated.

The six deslorelin-treated males responded more consistently than the females (Table 4). One month after deslorelin treatment, spermatozoa were still present in the ejaculate but the plasma testosterone concentration was already basal (n = 1). Azoospermia and basal plasma testosterone concentrations were observed in the same dog for 14 months after the deslorelin implant. Reversal of contraception occurred in two dogs, one of which mated successfully 16 months after deslorelin treatment. The other dog had good semen quality and normal plasma testosterone concentrations 12 months after deslorelin administration. Six months after a second implant the testes had atrophied; no spermatozoa were present in the ejaculate and plasma testosterone

concentration was basal. The size and structure of the prostate gland is similar to that in domestic dogs and the prostate gland is readily palpable per rectum. Six months after deslorelin treatment the prostate gland was difficult to palpate and measured only 7 mm across each lobe in one wild dog. The placebo-treated dog had a normal plasma testosterone concentration 23 days after treatment.

Lionesses

The results from ten lionesses treated with 12 or 15 mg deslorelin managed under various conditions are shown (Table 5). Deslorelin treatment was able to suppress cyclicity for 12 months in two females treated during dioestrus and for 18 months in two animals treated in anoestrus. Both the females treated in anoestrus were attractive to the male 2 days after deslorelin treatment but did not allow mating (Bertschinger *et al.*, 2001). Recovery of fertility has not yet been proven as two lionesses at Mossel Bay are with vasectomized males and the Mabula pride male is arthritic and azoospermic. The first lioness treated at Mabula has shown signs of oestrus every 2-3 weeks on a regular basis after returning to cyclicity 18 months after treatment, indicating that she has not responded with an induced ovulation. The other six lionesses have not yet undergone oestrus but the period since deslorelin implantation has been only 8 and 10 months, respectively.

Leopards

At the Africat Foundation, two females, each housed with a male in a 20 ha camp, were treated with 6mg deslorelin. Neither female became pregnant within the subsequent 12 months. A third female was implanted with the same dose of deslorelin in the Lowveld of South Africa and has not become pregnant within 18 months. A single male housed with a female in a 10 ha camp was also implanted at Africat. Only 4 months have passed since then and so far no mating has been observed.

Table 1. Adult female cheetahs at Africat housed in mixed sexual groups in 10-50 ha camps and implanted with 6 mg deslorelin

	Deslorelin implant			Examinations/observations after treatment			
ID	Date	Plasma progesterone (nmol l^{-1})	Treatment-induced oestrus	Interval since first implant (months)	Plasma progesterone (nmol l^{-1})	Interpretation	Return to heat
AJ128[a]	May 99	0.67	None observed	3	0.61	Anoestrus	None after 21 months
				9	0.61	Anoestrus	
AJ240[a]	May 99	0.83	9 days later lasting 2 days	3	0.01	Anoestrus	None after 21 months
				21	0.68	Anoestrus	
AJ5[a]	May 99	0.27	None observed	3	0.21	Anoestrus	None after 21 months
				9	0.09	Anoestrus	
				21	0.81	Anoestrus	
AJ4[a]	May 99	0.62	5 days later lasting 2 days	3	1.11	Anoestrus	None after 21 months
				9	1.01	Anoestrus	
				21	0.25	Anoestrus	
AJ260[a]	May 99	0.23	14 days later lasting 5 days	3	0.48	Anoestrus	None after 21 months
				9	0.01	Anoestrus	
				21	0.49	Anoestrus	
AJ177[a]	May 99	0.54	None observed	3	0.22	Anoestrus	None after 21 months
				9	0.01	Anoestrus	
				21	0.45	Anoestrus	
AJ19[a]	May 99	0.11	5 days later lasting 4 days	3	0.01	Anoestrus	None after 21 months
				9	2.08	Anoestrus	
				21	0.58	Anoestrus	
AJ81[a]	May 99	0.52	None observed	3	0.00	Anoestrus	None after 21 months
				9	0.14	Anoestrus	
				21	0.01	Anoestrus	
AJ84	Feb 00	0.00	None observed	1.5	10.29	Dioestrus	None after 16 months
				12	0.16	Anoestrus	
AJ74	Feb 00	0.07	None observed	1.5	0.39	Anoestrus	None after 16 months
				12	0.64	Anoestrus	
AJ228	Feb 00	0.39	None observed	1.5	0.78	Anoestrus	None after 16 months
				12	1.01	Anoestrus	
AJ82	Feb 00	0.03	None observed	12	0.22	Anoestrus	None after 16 months
AJ244	Feb 00	0.21	None observed	12	0.27	Anoestrus	None after 16 months

[a]These 8 females were given a second 6 mg deslorelin implant 9 months after the first one.

Table 2. Adult male cheetahs at Africat housed in mixed sexual groups in 10-50 ha camps and implanted with 6 mg deslorelin

	Deslorelin implant			Examinations/observations after treatment			
ID	Date	Plasma testosterone (nmol l^{-1})	Semen	Interval since implant (months)	Plasma testosterone (nmol l^{-1})	Semen	Mating and result
AJ70	May 99	1.41	Spermatozoa present	3	ND	No spermatozoa	None after 24 months
				9	0	No spermatozoa	
				21	0	Few dead spermatozoa	
AJ79	May 99	0.31	Spermatozoa present	3	ND	No spermatozoa	None after 24 months
				9	0	No spermatozoa	
				21	0	No spermatozoa	
AJ302	Feb 00	0.79	Spermatozoa present	1.5	0	Spermatozoa present[a]	None after 12 months
				12	0	No sperm	
AJ303	Feb 00	11.33	Spermatozoa present	1.5	0	Spermatozoa present[a]	None after 12 months
				12	0	No spermatozoa	
AJ9	Feb 00	2.05	Spermatozoa present	12	0	No spermatozoa	None after 12 months
AJ18	Feb 00	4.85	Spermatozoa present	12	0	Few dead spermatozoa	None after 12 months

[a]Small ejaculate volume with a high concentration of spermatozoa.
ND: no data collected.

Table 3. Adult African wild dog females at de Wildt Cheetah and Wildlife Centre housed in mixed pairs or threes and treated with deslorelin implants

	Deslorelin implant					Examinations after treatment			First oestrus after treatment	
ID	Date	Dose (mg)	Plasma progesterone (nmol l^{-1})	Stage of oestrus cycle	Treatment induced heat	Interval since implant	Plasma progesterone (nmol l^{-1})	Stage of oestrous cycle	Interval since implant	Result 2 months after mating
F139	Feb 99	6	3.74	Anoestrus	None	80 days	3.77	Anoestrus	12 months	6 pups
F144	Feb 99	6	3.79	Anoestrus	None	1 month 3 months 9 months	3.94 2.83 4.62	Anoestrus Anoestrus Anoestrus	13 months	Whelped but number unknown
F59	Feb 99	6[a]	4.75	Anoestrus	None	3 months 9 months 21 months	16.31 3.57 1.45	Dioestrus Anoestrus Anoestrus	Contracepted male after 21 months	No pups to date
F143	Feb 99	6[a]	2.34	Anoestrus	None	1 month 3 months 9 months	54.52 3.08 2.71	Dioestrus Anoestrus Anoestrus	Sold 12 months after implant - NP	No further information available
F142	Feb 99	6	2.72	Anoestrus	None	1 month 3 months 9 months	7.91 2.81 2.87	Dioestrus Anoestrus Anoestrus	Sold 12 months after implant - NP	No further information available
F177	Nov 99	6	1.96	Anoestrus	None	12 months	1.96	Anoestrus	Contracepted male after 12 months	No pups to date
F166	Nov 99	6	4.05	Anoestrus	None	None	ND	ND	16 months	10 pups
F104	Nov 99	6	3.56	Anoestrus	None	None	ND	ND	Died 18 months after implant	No pups before she died
F178	Nov 99	6	3.60	Anoestrus	None	None	ND	ND	3 months 15 months	7 pups 8 pups (second litter)
F72	Nov 99	6	2.74	Anoestrus	4 weeks later	None	ND	ND	4 weeks 14 months	7 pups Pregnant (second litter)

Table 3. Adult African wild dog females at de Wildt Cheetah and Wildlife Centre housed in mixed pairs or threes and treated with deslorelin implants

	Deslorelin implant					Examinations after treatment			First oestrus after treatment	
ID	Date	Dose (mg)	Plasma Progesterone (nmol l^{-1})	Stage of oestrus cycle	Treatment induced heat	Interval since implant	Plasma progesterone (nmol l^{-1})	Stage of oestrous cycle	Interval since implant	Result 2 months after mating
F211	Nov 99	6	ND	ND	None	None	ND	ND	No male present	No further information available
F75	Jan 00	6	3.33	Anoestrus	None	None	ND	ND	7 months	6 pups
F157	March 00	3	8.43	Pro-oestrus	Not mated	1 month	19.96	Dioestrus NP	11 months	Whelped but number of pups unknown
F161	March 00	3	8.06	Pro-oestrus	Mated	1 month	77.71	Pregnant 2.5 weeks	± 2 weeks	Whelped but number of pups unknown
F162	March 00	3	2.72	Anoestrus	None	1 month	34.95	Dioestrus NP	None after 15 months	

[a]Treated simultaneously with 150 mg proligestone (Bertschinger *et al.*, 2001). ND: no data collected ; NP: not pregnant.

Table 4. Adult African wild dog males at de Wildt Cheetah and Wildlife Centre housed in mixed pairs or threes and treated with 6 mg deslorelin implants *(n* = 6) or 50 mg placebo (*n* = 1)

	Deslorelin treatment				Examinations/observations after treatment				
ID	Date	Plasma testosterone (nmol l^{-1})	Semen	Testis size (mm)	Interval since first implant	Plasma testosterone (nmol l^{-1})	Semen	Testis size/ prostatic lobe (mm)	Mating and result
M141	Feb 99	1.87	ND	ND	1 month 3 months 9 months 14 months	0.27 0 0 0.02	ND ND ND ND	ND ND ND Small and hard	One bitch mated 3 weeks after implant – no other bitches mated for at least 14 months[a]
M54	Nov 99	0.32	ND	ND	ND	ND	ND	ND	Had not bred by 13 months when he died
M173	Nov 99	3.39	Spermatozoa present	ND	19 months	10.69	Spermatozoa present	L: 37x19 R: 38x20	Bitch mated after 16 months - pregnant
M130	Nov 99 Nov 00	1.63	Spermatozoa present	ND	12months 18 months	5.40 0	Spermatozoa present No spermatozoa	L: 46x29 R: 49x29 L: 31x19 R: 28x18	No mating occurred
M 56	Nov 00	5.2	ND	L: 52x23 R: 50x23	6 months	0	ND	L: 33x17 R: 34x16	No mating occurred
M176	Nov 00	5.7	Spermatozoa present	ND	6 months 7 months	0 0	No spermatozoa ND	L: 28x17 R: 29x16 Prostate: 7	No mating occurred
M107 Placebo	May 01	5.26	Spermatozoa present	L: 44x23 R: 43x27	23 days	7.25	ND	Prostate: 22	Not with cycling female

[a]Dog with three untreated bitches (Bertschinger *et al.*, 2001).
ND: no data collected; L: left testis; R: right testis.

Table 5. Adult lionesses treated with deslorelin implants at various locations

	Location		Deslorelin implant					Post-treatment observations	
ID	Name	Management	Date	Dose (mg)	Plasma progesterone (nmol l^{-1})	Stage of oestrous cycle	Treatment-induced oestrus	Interval since implant	Results of mating
40	Mabula	1 400 ha[a]	Dec 98	12[b]	5.47	Anoestrus	2 days later lasting 2 days	18 months	After first oestrus, 12 heats 2-3 weeks apart. Dominant male examined and found to be infertile
26	Mabula	1 400 ha[a]	Nov 99	12	ND	Unknown	2 days later lasting 2 days	18 months	Mating observed; too early for result but with same male as no. 40
38T	Mossel Bay	65 ha[c]	Nov 99	12	170.8	Dioestrus	None	12 months	Mated by vasectomised male. Second oestrus after 1.5 months; then at 3 week intervals
E6T	Mossel Bay	65 ha[c]	Nov 99	12	125.1	Dioestrus	None	12 months	Mated by vasectomised male. Second oestrus after 1.5 months; then at 3 week intervals
C6T	Thornybush	10 000 ha[a]	Aug 00	15	4.11	Anoestrus	None	10 months	Oestrus not observed
33T	Thornybush	10 000 ha[a]	Aug 00	15	3.13	Anoestrus	None	10 months	Oestrus not observed
46T	Thornybush	10 000 ha[a]	Aug 00	15	1.02	Anoestrus	None	10 months	Oestrus not observed
5CB	Pretoria Zoo	0.75 ha[d]	Oct 00	12	2.51	Anoestrus	Separated from male for 3 months	8 months	Oestrus not observed
2B3	Pretoria Zoo	0.75 ha[d]	Oct 00	12	14.44	Late dioestrus	Separated from male for 3 months	8 months	Oestrus not observed
Elsa	Doman Namibia	10 ha[e]	Jan 01	15	6.73	Anoestrus	Separated from male for 3 weeks	5 months	Oestrus not observed

[a]Free-ranging lions kept with prey species.
[b]Treated simultaneously with 3mg norgestomate implant – examination 3 months later revealed late dioestrus (Bertschinger *et al.*, 2001).
[c]Two lionesses with 2 adult vasectomised males in a camp where they are fed.
[d]Two adult lionesses with one male and two sub-adults in a camp where they are fed.
[e]Lioness with male and 6-month-old cubs in camp where they are fed.
ND: no data collected.

Discussion

For wild carnivores, the selection of a contraceptive that is both safe and suits the specific requirements of the region is extremely important. In the present scenario, the most important requirement was reversibility with accompanying good fertility. From the point of view of safety, long-term use of progestagens, which have been associated with complications, such as cystic endometrial hyperplasia (CEH), pyometra, mammary and endometrial cancers are inappropriate (Munson and Mason, 1991; Munson, 2001). The reversibility of the pig zona pellucida (PZP) contraception in wild carnivores remains questionable. In domestic dogs PZP has been shown to produce permanent infertility (immuno-sterilization) as a result of destruction of ovarian follicles (Mahi-Brown *et al.*, 1985; Fayrer-Hosken *et al.*, 2000). Munson (2001) described hypercalcaemia leading to renal failure and cardiomyopathy in felids vaccinated with PZP in Freund's adjuvant. In addition, immunocontraception of 27 felids and four canids with PZP yielded variable and sometimes disappointing results (J. F. Kirkpatrick and K. M. Frank, personal communication). Continued cyclicity and the resulting management problems were also deemed undesirable. For this and other reasons, consideration was not given to the use of anti-progestins, which have been used successfully in captive bears (Göritz *et al.*, 2001). The efficacy of immunocontraception using peptide hormones or their receptors as antigens (Meloen *et al*., 1994; Remy *et al.*, 1996; Thompson, 2000) must be tested properly in domestic carnivores before attempting to use them in valuable wild carnivore species.

In the present study, deslorelin was found to be an effective contraceptive for lionesses and female leopards, and for male and female wild dogs and cheetahs. Apart from the females attracting males for a short period after treatment, no side-effects were observed. The use of progestagens to suppress such behaviour in a lioness or ovulation in two wild dogs after deslorelin treatment was unsuccessful (Bertschinger *et al.*, 2001). These results are not comparable with the work of Wright *et al.* (2001) in domestic dogs, as these workers used the oral progestagen megestrol acetate, and started treatment before deslorelin implantation. As pre-treatment of wild carnivores with progestagens in whatever form is highly impractical and may be dangerous with regard to induction of CEH, the practice was not pursued in the present trials. The possibility of mating during the first 3 weeks after implantation can be avoided by separating the female from the untreated males if possible.

Although acceptable, the results were more variable in female wild dogs. This finding may have been due to the social interaction, which is totally different from that of the other species treated in the present study. Previously, it has been observed that, when new packs of dogs are formed by mixing animals from different sources, oestrus may be induced in all the females of the new pack (H. J. Bertschinger, unpublished; M. Hofmeyr, personal communication). The other difference observed in dogs (body

weight 23-26 kg) is a much shorter contraceptive period compared with that in cheetahs (34-45 kg) when the same dose of deslorelin was used. It is possible that the contraceptive period can be attributed to different sensitivities of species like the domestic dog and cat to the same dose of deslorelin (Munson *et al.*, 2001; Trigg *et al.*, 2001). However, African wild dogs are extremely hyperactive compared with cheetahs, indicating a much higher metabolic rate. The daily energy requirement of 15.3 MJ in free- ranging wild dogs is almost double the requirement for working border collies (Gorman e*t al.*, 1998). A dose of 3 mg deslorelin was ineffective in suppressing oestrus and mating in a bitch that was already in pro-oestrus at the time of treatment. Two other bitches, one in pro-oestrus and one in anoestrus, also ovulated after treatment with this low dose; however, ovulation may also have occurred with treatment with 6 mg deslorelin. Safety during pregnancy was demonstrated in one wild dog that conceived 4 weeks after deslorelin treatment and later delivered seven live pups (Bertschinger *et al.*, 2001).

An added advantage of long-term contraception in carnivores using deslorelin appears to be a decreased risk of developing CEH and pyometra. The relationship between advancing age and CEH-pyometra is well established in domestic dogs, particularly if they have not produced a litter (Dow, 1957).

As a contraceptive and in the formulation used, deslorelin is highly effective in male wild dogs and cheetahs once spermatozoa are no longer present in the ejaculate. In addition, the duration of contraception appears to be longer in males than in females. As in females, male dogs recover fertility much earlier than cheetahs after they have received the same dose of deslorelin. Male lions on display (free-ranging or in zoos) should not be treated with deslorelin, as they are likely to lose their testosterone-dependent manes. In cheetahs, decreased semen volume was a constant feature of deslorelin contraception, probably as a result of reduced formation of dihydrotestosterone required for the androgen-dependent secondary sex glands (Shasin, 1998). Androgen dependency of the secondary sex glands could be verified by the fact that the prostate gland of a wild dog showed marked atrophy after deslorelin treatment.

Another encouraging aspect of the present trial using deslorelin was the absence of side- effects, including behavioural changes. Although there was no objective measurement of behavioural interaction, all animals, with the exception of the leopards, were observed each day. No hierarchical alterations were noted and scent marking in male wild dogs continued despite undetectable plasma testosterone concentrations. Testosterone is converted to oestradiol in the brain, which, according to Bahsin (1998), is responsible for this behaviour in dogs. Perhaps the behaviour becomes imprinted earlier, in fetal life or at about the time of puberty.

Conclusions

In conclusion, the deslorelin implant offers a safe and reversible method of contraception for small numbers of captive and free-ranging wild carnivores. Continued cyclicity of females, as observed with PZP vaccine (J. F. Kirkpatrick and K. M. Frank, personal communication), weight gain and increased incidence of uterine and mammary tumours, or endometrial hyperplasia as observed with progestagen implants (Munson and Mason, 1991; Munson, 2001), seem unlikely sequelae to deslorelin treatment. The contraceptive results in males are more reliable if they are not exposed to oestrous females for the first 6 weeks after deslorelin treatment. In wild dogs treated with 6 mg deslorelin, contraception is effective for 9-14 months. In cheetahs treated with the same dose, lionesses treated with 12 mg deslorelin and in leopards treated with 6 mg deslorelin, contraception was effective for approximately 2 years, 12-18 months and > 12 months, respectively.

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References

Bertschinger HI and Meltzer VGA (1998) Reproduction in male cheetahs. 2: Sperm morphology. *In Proceedings of a Symposium on Cheetahs as Game Ranch Animals* Onderstepoort, Republic of South Africa, 23-24 October 1998. pp 153-158 Ed. 31 Penzhorn Wildlife Group, South African Veterinary Association, Onderstepoort

Bertschinger HJ, Asa CS, Calle PP. Long JA, Bauman K, DeMatteo K, Jochie W, Trigg TE and Human A (2001) Control of reproduction and sex related behaviour in exotic wild carnivores with the GnRH analogue deslorelin: preliminary observations *Journal of Reproduction and Fertility Supplement* 57 275-283

Bhasin S (1998) Androgens, effects in mammals. In *Encyclopedia of Reproduction* pp 197-206 Eds E Knobil and JD Neill. Academic Press, San Diego

Dow C (1957) The cystic hyperplasia-pyometra complex in the bitch *Veterinary Record* 69 1409-1415

Fayrer-Hosken RA, Dookwah HD and Brandon CI (2000) Immunocontrol in dogs *Animal Reproduction Science* 60-61 365-373

Goritz F, Quest M, Hildebrandt TB, Meyer HHD, Kolter L, Elger W and Jewgenow K (2001) Control of reproduction with anti-progestin and oestrogens in captive bears *Journal of Reproduction and Fertility* Supplement 57 249-254

Gorman ML, Mills MG, Raath JR and Speakman JR (1998) High hunting costs make African wild dogs vulnerable to kleptoparasitism by hyaenas *Nature* 391 479-481

Mahi-Brown CA, Yanagimachi R, Hoffman JC and Huang TTF (1985) Fertility control in the bitch by active immunization with porcine zona pellucidae: use of different adjuvants and patterns of estradiol and progesterone levels in estrous cycles *Biology of Reproduction* 32 761-772

Meloen RH, Turkstra JA, Lankhof H, Puijk WC, Schaaper WMM, Dijkstar G, Wensing CJG and Oonk RB (1994) Efficient immunocastration of male piglets by immunoneutralization of GnRH using a new GnRH-like peptide *Vaccine* 12 741-745

Meltzer DGA, Bertschinger HJ and van-Dyk Ann (1998) Reproduction in male cheetahs: 1. Breeding management and semen evaluation. In *Proceedings of a Symposium on Cheetahs as Game Ranch Animals.* Onderstepoort, Republic of South Africa, 23-24 October 1998. pp 145-152, Ed. BL Penzhorn wildlife Group, South African Veterinary Association, Onderstepoort

Munson L (2001) Health risks of contraceptives in wildlife. In *Proceedings of the 5th International Symposium on Fertility Control in Wildlife* Skukuza, 19-22 August pp 12-13 Eds HJ Bertschinger and JF Kirkpatrick. University of Pretoria Academic Press

Munson I and Mason R (1991) Pathological findings in 52 the uteri of progestogen-implanted exotic feuds. In *Proceedings of the American Association of Zoo Veterinarians*, Calgary 28 Septeniber-3 October pp 311-312, Ed. EJ Randal. American Association of Zoo Veterinarians

Munson L, human JE, Asa CS, Jochle W and Trigg TE (2001) Efficacy of the GnRH analogue deslorelin for suppression of oestrous cycles in cats Journal *of Reproduction and Fertility Supplement* 57 269-273

Remy J-J, Couture L, Rabensona H, Haertle T and Salesse R (1996) Immunization against exon 1 decapeptides from the lutropin/choriogonadotropin receptor or the follitropin receptor as potential male contraceptive *Journal of Reproductive Immunology* 32 37-54

Thompson DL (2000) Immunization against GnRH in male species (comparative aspects) *Animal Reproduction Science* 60-61 459-469

Trigg TE, Wright PJ, Armour AF, Williamson PE, Junaidi A, Martin GB, Doyle AG and Walsh J (2001) Use of a GnRH analogue implant to produce reversible long- term suppression of reproductive function of male and female domestic dogs *Journal of Reproduction and Fertility Supplement* 57 255-261

Wright PJ, Verstegen JP, Onclin K, Jochle W, Armour AF, Martin GB and Trigg TE (2001) Suppression of the oestrous responses of bitches to the GnRH analogue deslorelin by progestin *Journal of Reproduction and Fertility Supplement* 57 263-268

Chapter 3

The use of deslorelin implants for the long-term contraception of lionesses and tigers

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Abstract

Contraception is an essential tool for controlling reproduction in captive and free-ranging lions. This paper describes the treatment and contraception of 23 captive and 40 free-ranging lionesses (*Panthera leo*) and four tigers (*Panthera tigris*) in South Africa using 3x4.7 mg, 2x4.7 mg, 9.4 mg or 4.7+9.4 mg deslorelin implants. Thirty one lionesses were treated more than once at 11 to 60 month intervals. In Brazil two lionesses were treated with 9.4-mg implants and faecal progesterone and oestradiol concentrations monitored for 920 days. All combinations of deslorelin showed the length of contraception to be around 30 months with one 3x4.7 mg treatment lasting 40 months in one captive lioness. The mean time taken to reconception was 30.1 months for the 3x4.7 mg combination. The faecal analyses of the lionesses in Brazil reflected quiescent ovarian activity for periods of 17 and 30 months, respectively, when small oestradiol peaks but no progesterone peaks started to appear. This confirmed the field observations in South Africa. No side effects occurred several lionesses were treated repeatedly for up to 8 years. Deslorelin (Suprelorin®) is a safe and effective means of controlling reproduction in captive or free-ranging populations of lions. Where contraception is to be maintained, the implementation of implants at 24-month intervals is recommended.

Introduction

Left unmanaged, discrete free-ranging lions on fenced game reserves reproduce at an alarming rate. This leads to rapid depletion of the prey species, inbreeding and breakouts into neighbouring communities. Large carnivores like lions and tigers also breed exceptionally well under zoo conditions and, with limited possibilities to place captive lions, reproduction needs to be managed. Adult free-ranging lionesses under extensive conditions reconceive when the cubs are ~20 months old. Our experience in smaller fenced reserves is that litter intervals tend to be shorter. Compared to extensive conditions like in the Kruger National (50%) and Etosha National (40%) Parks, cub survival is close to 100% in smaller fenced reserves (Smithers 1983). This is most likely due to less competition from other lions with fewer or no pride take-overs and less cub predation by hyenas. In zoos it is common practice to remove cubs for hand-raising or euthanasia soon after birth. As a result, female lions and tigers come on heat and reconceive much sooner and sometimes within the first month after parturition.

Previously we reported the use of long-acting biocompatible deslorelin implants to down-regulate reproduction in a variety of carnivores, including African lionesses (Bertschinger *et al.* 2001 and 2002). At the time a limited number of lions had been treated but the data had shown that the implants were both safe and effective at controlling reproduction of the species. The deslorelin implants, marketed as Suprelorin (Peptech Animal Health, Sydney), have been specifically formulated to deliver long-term release of deslorelin, which is a gonadotrophin-releasing hormone (GnRH) agonist. Following initial stimulation, the release of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) is downregulated. The overall result is downregulation of ovarian and testicular functions, although in some species it is not effective in males (Munson *et al.* 2001; Wright *et al.* 2001; Trigg *et al.* 2001; Junaidi *et al.* 2003).

The present paper describes the extensive use of deslorelin at various doses and intervals to manage reproduction in captive and free-ranging African lionesses and a few captive tigers during the period 1999 to April 2007. During this period, over 200 treatments on at least 80 lionesses and four female tigers were carried out. The present paper, however, only reports on 67 females where follow-up examinations or observations were possible. It also reports on the faecal steroid profiles of two captive African lionesses housed with a vasectomised male in a zoo in Brazil.

Materials and methods

The Suprelorin and Suprelorin12 are imported from Peptech Animal Health, Sydney, and used with permission from The Medicines Control Council, Republic of South Africa, under Section 21 of Act 101 of 1965 (authorisation number: SP/14/2006).

Animals and behavioural observations
In South Africa captive lionesses (n = 23; ages 18 months to 8.5 years) and female tigers (n = 4; ages 2 to 4 years) were housed in typical zoo enclosures, mostly with night rooms and ample space to exercise during the day in the presence of males. All these animals were fed meat. Keepers saw the animals daily when any interactions between sexes would have been noted. Another 40 (ages 18 months to 13 years) free-ranging lionesses were treated on private fenced game reserves ranging from 1 500 to 22 000 ha. These lions ranged the reserves with males and were seen on a regular basis (almost daily) by rangers on game drives or conservation business. For food they were reliant on hunting.

In Brazil, two captive African lionesses were housed together with a vasectomised male at the São Paulo Zoo for the past 3 years. The oestrous cycles of the females were monitored by means of observation (signs of oestrus and mating) and faecal oestradiol and progesterone profiling.

Immobilisation
Captive lionesses and tigers were immobilised in their enclosures or night rooms by means of darting using 2.5 to 3 mg/kg^{-1} Zoletil 100 (Virbac, Halfway House, South Africa) for treatments and collection of blood samples and vaginal smears. Spontaneous recovery was allowed. Free-ranging lionesses were darted opportunistically but most were immobilised using bait. Immobilising drugs used were Zoletil, a combination of ketamine and medetomidine or a combination of Zoletil (60 mg total dose) and medetomidine (6 mg total dose). With the latter two combinations the medetomidine was reversed with Antisedan (atipamizole HCl, Pfizer Animal Health, Sandton, South Africa), which is a major advantage when working with free-ranging lions.

Sample collections and examinations
Blood samples for serum progesterone concentration (SPC) were collected from all immobilised South African lionesses. Vaginal smears for cytology were then taken and most females were subjected to transrectal ultrasound examination using a 7.5 mHz linear probe (Aloka 900, Tokyo, Japan) fitted to a custom-made handle. This handle allowed the probe to be positioned over and follow the uterine horns.

In Brazil faecal samples were collected from each female from the night room every one to seven days and an aliquot stored at – 20 °C until extracted for assay. The period of observation started on Days –72 (Lioness Salita) and –45 (Lioness Zomba), respectively (Day 0 = day of deslorelin treatment), and continued until Day 920 (≈ 30 months).

Deslorelin treatment

Previously we reported on the use of 12-mg deslorelin (2 x 6 mg implants) to down-regulate reproduction in African lionesses and where females (n = 2) were not retreated reconception took place 29 months later (Bertschinger *et al*. 2002). However, these implants were discontinued, meaning that, at the beginning of this trial, there were no guidelines available on dose and frequency of treatment required with the newly manufactured 4.7-mg implants (Suprelorin, Peptech Animal Health, Sydney). As a result the treatment regime has varied quite a bit. Initially the dose of deslorelin used per treatment was 14.1 mg (3 x 4.7 mg; number of treatments = 43). In 2004, the 9.4-mg implants became available. At first we treated each female with 14.1 mg (1 x 9.4 plus 1 x 4.7; number of treatments = 23) but in 2004 and again in 2006 we treated a number of females (number of treatments = 50) with a single 9.4-mg implant (Suprelorin12, Peptech Animal Health, Sydney). Owing to unavailability of the 9.4-mg implants in 2006, some animals were treated with 2 x 4.7 mg (number of treatments = 10) implants. All females were implanted subcutaneously on the left or right side of the neck, which was noted. Thirty-six females (including two tigers) were treated once, 12 (including two tigers) twice (intervals of 14-60 months), 11 three times (intervals of 11-33), two four times (intervals of 17-49 months) and six females five times (intervals of 11-30 months) Lionesses previously treated once with 12 mg (2 x 6 mg; number of treatments = 5) were part of the group treated five times. The four longest periods during which individual lionesses have been subjected to continuous treatment with deslorelin was five (n = 3), six (n = 2), seven (n = 2) and eight (n = 1) years. The lionesses in Brazil were each given a single 9.4-mg implant on Day 0.

Abortion procedure

Females found to be pregnant were either left untreated, treated with deslorelin or aborted and treated with deslorelin. The prostaglandin dinoprost (Lutalyse, 7.5 mg; Pfizer Animal Health) was used on three consecutive or alternate days as an abortifacient. The second and third doses were administered by means of darting. Abortifacient treatment was delayed by two weeks in lionesses and tigers that were mated less than two weeks earlier provided SPC were raised above anoestrus or inter-oestrus levels.

Hormone assays

Faecal extractions (Brazil lions) were done according to Brown *et al*. (1993, 1994, 1996) and Brown and Wildt (1997). Samples were lyophilised and 0.2 g was extracted with in 5 mL of 90% ethanol in water. Samples were vortex-mixed for one minute and then placed in a boiling water bath for 25 min. Samples were then centrifuged for 15 minutes at 500*g* and the supernatant was recovered. The pellet was re-suspended in 5 mL of ethanol 90% and the process repeated. The supernatants were combined and then dried under a flow of air. Samples were taken up in 1 mL of absolute methanol, vortex-mixed for a minute and transferred to an ultrasonic cleaner for 15 min. They were then diluted 1 :40 with phosphate-buffered saline (PBS)-gelatine buffer for radioimmunoassay (RIA). Progesterone was assayed using a progesterone RIA (Progesterone DSL 3900, Diagnostics Systems Laboratories, Webster, TX, USA) that has been validated for the determination of feline faecal progesterone. The intra-assay and inter-assay coefficients of variation (CVs) of the progesterone assay were 7.43% and 3.11%, respectively. Faecal oestradiol was performed using RIA (Oestradiol Coat-a-Count, Diagnostic Products, Los Angeles, CA, USA). The intra-assay and inter-assay CVs of oestradiol assay were 7.43% and 3.60%, respectively. Simple linear regression between the standard curve of the kit and the curve obtained by serial dilutions of the standard hormone in faecal matrix with very low values were: Progesterone R^2=0.98 and Oestradiol R^2= 0.99 (Viau *et al*. 2005).

SPC was determined on thawed serum samples using progesterone RIA kits (Progesterone Coat-a-Count, Diagnostic Products) previously described (Bertschinger *et al.* 2001 and 2002).

Results

In South Africa the use of deslorelin implants at various doses was 100% successful at preventing pregnancy 63 lions and four tigers. With the 2 x 4.7 implants, however, we have incomplete data, as the maximum interval from treatment in the nine females treated so far is only 9 months. During this period no animals have shown heat. In Table 1 the anoestrus periods (≥20 months) following treatment are shown.

Table 1:Summary of 30 lionesses and one female tiger treated with different doses of deslorelin**.** Only females that have reached anoestrus periods of 20 months or more are tabled

Dose of deslorelin	ID of female c = captive w = free-ranging	Anoestrous periods of females: (months) No heat Not retreated	No heat Retreated	1st heat Retreated	Interval until conception (months)
3 x 4.7 mg (14.1 mg)	Nischila (w)				15
	Dharma (w)	30			
	Tabby (w)				40
	Gertrud (w)	35			
	Elsa (w)				30
	Midget (w)				31
	One-Eye (w)				31
	Cora (w)				37
	Doris (w)		33		
	Nweti (w)			30	
	Dyason (w)				27
	Simone (c)		24		
	Kiara (c)		24		
	Shumba (c)		24		
	#60 (w)		23		
	#26 (w)		23		
	Mean				*Mean = 30.1*
9.4 mg	Sabre (c)		20		
	Amber (c)		30		
	IDA7 (w)	27			
	Begera (c)	36			
	Elsa (c)	36			
	Subadult (w)			30	
	Cayla (c)		30		
	Emma (c)	30			
	[A]Laya (c)	33			
4.7 + 9.4 mg (14.1 mg)	Niobe (c)		32		
	Jesse (w)	23			
	Scar (w)	23			
	7E34 (w)		27		
	CBD7 (w)		21		
	IDF3 (w)		21		

[A]Female tiger

The thirty six remaining females had been treated at intervals of 11 to <20 months and were not included in Table 1. Although they were successfully down-regulated until the next treatment it provided insufficient time in our experience for reversal to take place. Reversibility could be shown in 8 of 14 lionesses treated with 3 x 4.7-mg deslorelin implants. Seven of the eight lionesses conceived and produced live cubs. The eighth female was re-implanted during her first post-treatment oestrus. The mean period until reconception in this group was 30.1 months. Reversibility was shown in

one of the nine females given 9.4-mg deslorelin. Four (including the last-mentioned) of these were re-implanted while the others were in anoestrus with durations varying from 27 to 36 months. Four of the 6 females treated with the 4.7 + 9.4 mg combination were re-treated with the two remaining being 23 months post treatment and in anoestrus. The mean SPC concentrations during anoestrus were 3.11 nmol L^{-1} (± s.d. 1.72; *n* = 109) whereas during oestrus it was 6.95 nmol L^{-1} (± s.d. 5.68; *n* = 6). During pregnancy SPC ranged from 63.7 to 212.8 nmol L^{-1} (mean 135.38 ± s.d. 42.68 nmol L^{-1}).

Three captive lionesses and two tiger females were treated with dinoprost during various stages of pregnancy (Table 2). Each one of these females was also implanted with deslorelin on the day of pregnancy diagnosis. No cubs were seen as a result of these pregnancies.

Table 2: Summary of lionesses and tiger females treated with the abortifacient dinoprost

Species	Female ID	SPC nmol/l	Approximate stage of gestation	Dinoprost treatment	Dose of deslorelin
Tiger	Olga	177.92	<3 weeks	7.5 mg on 3 alternate days 2 weeks later	9.4 mg
Tiger	Orcha	63.70	70 days	7.5 mg on 3 alternate days from PD	9.4 mg
Lion	Happy	212.80	80 days	7.5 mg on 3 consecutive days from PD	4.7+9.4 mg
Lion	Senanga	194.08	3 weeks	7.5 mg on 3 consecutive days from PD	4.7+9.4 mg
Lion	Diana	107.29	3 weeks	7.5 mg on 3 consecutive days from PD	4.7+9.4 mg

No side effects as a result of deslorelin treatment were observed in any females, with some having been treated four to five times over 5-8-year periods. Two captive lionesses were treated with 9.4-mg deslorelin during pregnancy (70 and 90 days of gestation, respectively) but cubs were never seen. It is not clear whether they aborted or lost the cubs postnatally.

The faecal progesterone and oestradiol concentrations of the two captive lionesses (Zomba and Salita) in Brazil are shown in Fig. 1. As can be seen from the profiles, both females had been cycling shortly before the treatment with deslorelin. In Zomba there was an oestradiol peak on Day 4 following treatment and a progesterone peak from Days 37 to43. Salita showed an oestradiol peak on Day 7 and progesterone on Days 16 to 17. Behavioural signs of heat and mating were not observed and the duration of progesterone peaks were short (~7 and 2 days respectively). By Day 920 post treatment, Zomba had shown no signs of oestrus despite minor oestradiol peaks towards the end of the observation period. Salita showed oestrus with mating on Days530 to 536 post implantation and her faecal oestradiol concentrations were marginally higher during this period. However, no rise in faecal progesterone was seen. Following that she remained in behavioural anoestrus until Day 920 but with periodic small peaks of oestradiol.

Discussion

The traditional approach to contraception of large felids has been the use of slow-release silicon implants impregnated with progestins like melengestrol acetate (MGA). Although highly effective as a contraceptive, the prolonged use of MGA is associated with serious side effects like cystic endometrial hyperplasia-pyometra and uterine, mammary and hepatic tumours. Another side effect is the development of virulism, particularly in lionesses. Obesity also seems to be a sequel to MGA treatment (Munson, 2001; Munson *et al.* 2001; Munson *et al.* 2002; Munson *et al.* 2005).

Deslorelin as a long-acting implants (Suprelorin, 4.7 and Suprelorin 12, 9.4 mg implants) were developed to down-regulate reproduction and reproduction-related behaviour in female domestic dogs (Wright *et al.* 2001; Trigg *et al.* 2001), male domestic dogs (Trigg *et al.* 2001; Junaidi *et al.* 2003) and domestic female and male cats (Munson *et al.* 2001). The implants have been shown to be safe and downregulatory effects are completely reversible. Their use as contraceptives in wild carnivores dates back to 1998 (Bertschinger *et al.* 2001). The implants have also been used as a contraceptive in male carnivores, particularly cheetahs, many of which have been treated yearly for up to 7 years (Bertschinger *et al.* 2006).

This study demonstrated that deslorelin (Suprelorin/Suprelorin12) is a safe and effective means of controlling captive and free-ranging populations of lions as well as captive female tigers. The doses tested were 3 x 4.7 mg, 9.4 mg, 4.7 + 9.4 mg and 2 x 4.7 mg. With the exception of the 2 x 4.7 mg dose all other regimens appear to be effective for ~30 months but possibly longer when the 9.4-mg implants are used.

The pay-out of the 9.4-mg implants was designed to release deslorelin for a longer period than the 4.7-mg implants (T.E. Trigg, unpubl. data). We could show complete reversal in eight of 14 females treated with 3 x 4.7-mg implants, the mean time required until reconception being 30.1 months. In a previous study two out of two lionesses conceived 29 months after treatment with 12 mg (2 x 6 mg) deslorelin (Bertschinger *et al*. 2002). The data for the 9.4 mg and 4.7 mg plus 9.4 mg are as yet incomplete, meaning that time taken to complete reversal is not available yet. The data in Table 1, however, provides useful information with regard to these two doses. At 27, 30, 33 and 36 months, one, three, one (tiger) and two lionesses were still in anoestrus respectively. One lioness treated with 9.4 mg showed her first heat 30 months after treatment. In the case of the combination treatment (4.7 + 9.4 mg) one female each were still in anoestrus after 27 and 32 months respectively. One female treated with the 3 x 4.7-mg dose recovered fertility much sooner at 15 months than the rest of the group. It is possible that this outlier may have been due to the earlier 4.7-mg implants as she was treated in 2000.

C

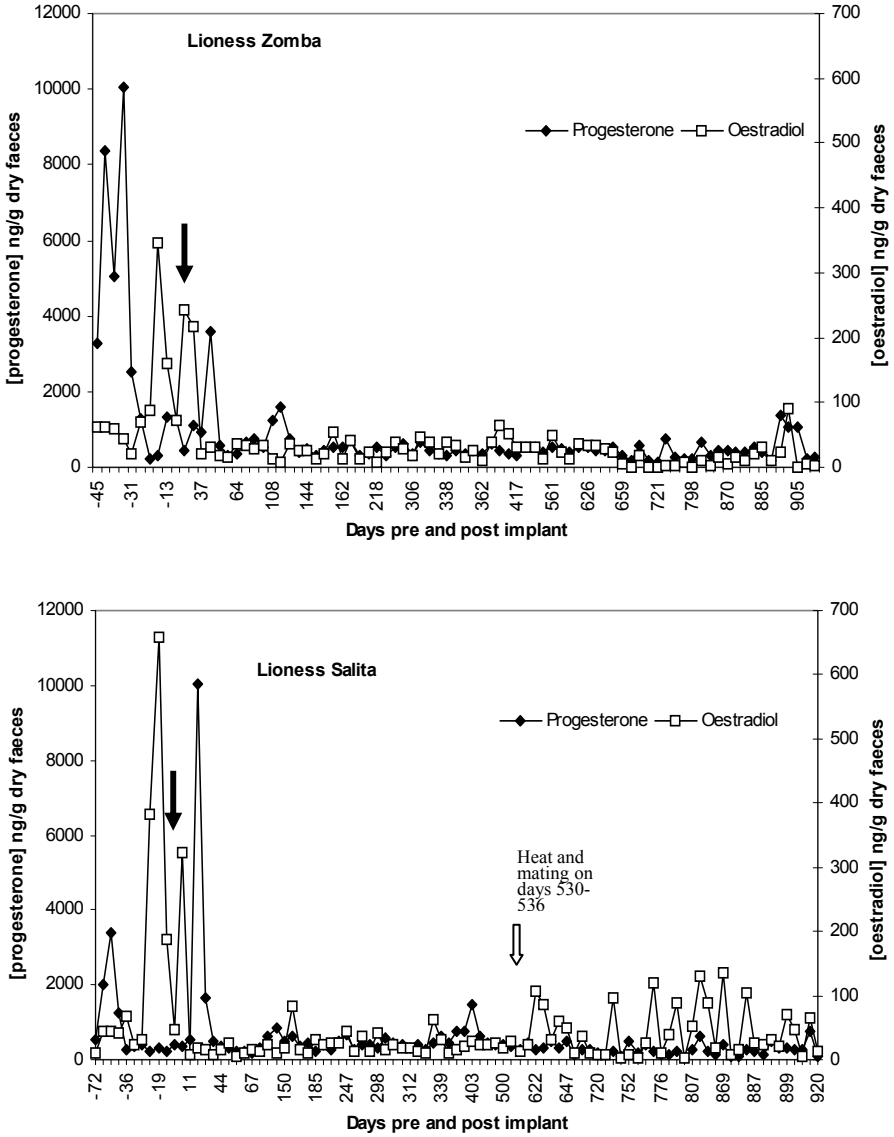

Figure 1: Faecal oestradiol and progesterone metabolite profiles of two lionesses in Sao Paulo Zoo before and after treatment with 9.4 mg deslorelin on Day 0 (solid black arrows).

Neither of the two lionesses that were treated during pregnancy produced cubs. This may have been due to the effects of the implants but may also have been due to poor mothering ability and eating of the cubs at birth, which is common in wild

captive felids. Previous experience in African wild dogs, however, showed that deslorelin implants do not cause abortion. Bitches gave birth to live puppies and were able to raise them unassisted (Bertschinger *et al.* 2001, 2002). Wright *et al*. (2001) found that use of deslorelin in two pregnant bitches (>5 pups and 2 pups, determined by means ultrasound examination) resulted in failure of each pregnancy around day 40 of gestation. Aborted puppies were found for one bitch, none found for the other.

As an additional tool to reproductive management of lions and tigers, we introduced the use of dinoprost as an abortifacient. Other than mild salivation, dinoprost produced no other side effects. The fact that females can be darted for follow-up treatment once recovered and mobile makes it a useful tool. It should be mentioned, however, that each one of these females was also implanted with deslorelin at the time of pregnancy diagnosis. Owing to the possible abortion induced by deslorelin in the two females described above, we cannot be absolutely sure that abortion was solely due to the prostaglandin. Previously, however, we have shown that dinoprost induces luteolysis in dioestrus non-pregnant cheetahs (H. J. Bertschinger, unpubl. data).

Following treatment, mating was seen in three lionesses (67 and 97 days, 12 months and 18 months respectively) during what was considered to be the post treatment anoestrus period. None of these females produced cubs that could be attributed to these observations. The female that was mated on Days 67 and 97 (3 x 4.7 mg) was immobilised and examined after each incident and found to have baseline SPC. The wild female mated after 12 months (9.4 mg) lay on her side instead of in the lordosis position during mating. The third lioness mated after 18 months (9.4 mg) also demonstrated a normal mating position. False mating or forced mating is known to occur in African lions and may be an expression of dominance by the male. Females will probably rather submit to the whims of the male than suffer the potentially dangerous consequences.

The data gained from the lions in Brazil is invaluable as it confirms the findings in the South African lions. Both lionesses show distinct oestradiol and progesterone profiles of cycling females prior to treatment with deslorelin. Following the implants, oestradiol peaks were seen in both females and a small and transient rise in progesterone metabolites were also observed. This is consistent with our observations in wild lions where females appear to be attractive to males without allowing them to mate within the first week post implant. Certainly, although we advise female and male separation in captive lions during the first three weeks, we have never seen pregnancies as a result of this attractive period. According to the faecal ovarian steroids, Lioness Zomba remained quiescent until close to the end of the observation period around Day 900 (30 months) when South African lionesses show reversal. The other lioness, Salita started showing signs of recovery earlier as can be seen in Fig. 1. Small oestradiol peaks started to occur at around Day 550 (17.7 months) and

continued to occur until the end of the observation period. She showed heat with mating at around the same time but neither this or the oestradiol peaks were associated with significant increases in progesterone. Perhaps it is these small oestradiol peaks that cause behavioural changes in our South African lionesses.

In conclusion the use of deslorelin implants allows managers of game parks and zoos to control reproduction in prides selectively and thus slow down the rate of population growth to whatever their requirements may be (adaptive management). From our results it would seem that either 3 x 4.7 mg or 9.4 mg implants can effectively used as contraception for lionesses and female tigers for a period of ~30 months or longer. Where contraception is to be maintained, we recommend the implementation of implants at 24-month intervals. Prolonged use of deslorelin implants for up to 8 years have produced no visible or measurable side effects.

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References

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

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

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

Brown, J.L., Wildt, D.E. (1997). Assessing reproductive status in wild felids by non-invasive faecal steroid monitoring. *International Zoo Yearbook* **35**, 173–191.

Brown, J.L., Wasser, S.K., Howard, J., Wells, S., Lang, K., Collins, L., Raphael, B., Schwartz, R., Evans, M., Hoyt, T., Wildt, D.E., Graham, L.H. (1993). Development and utility of fecal progesterone analysis to assess reproductive status in felids. In 'Proceedings of the Congresss of American Association of Zoo Veterinarians, St. Louis, 10-15 October 1993'. (Ed. R. E. Junge.) pp. 273–276. (Omni Press, Lawrence, KS.)

Brown, J.L., Wasser, S.K., Wildt, D.E., Graham, L.H. (1994). Comparative aspects of steroid hormone metabolism and ovarian activity in felids, measured noninvasively in feces. *Biology of Reproduction* **5**, 776–786.

Brown, J.L., Wildt, D.E., Wielebnowsky, N., Goodrowe, K.L., Graham, L.H., Howard, J.G. (1996). Reproductive activity in captive female cheetahs (*Acinonyx jubatus*) assessed by faecal steroids. *Journal of Reproduction and Fertility* **106**, 337–346.

Junaidi, A., Williamson, P.E., Cummins, J.M., Martin, G.B., Blackberry, M.A., Trigg, T.E. (2003). Use of a new drug delivery formulation of the gonadotrophin-releasing hormone analogue Deslorelin for reversible long-term contraception in male dogs. *Reproduction, Fertility and Development* **15,** 317-322. Doi: 10.1071/RD03039

Munson, L. In: Health risks of contraceptives in wildlife. (2001). In 'Proceedings of the 5th International Symposium on Fertility Control in Wildlife Skukuza, Kruger National Park, 19-22 August 2001' (Eds H. J. Bertschinger and J.F. Kirkpatrick.) pp12-13.

Munson, L., Bauman, J.E., Asa, C.S., Jöchle, W., Trigg, T.E. (2001). Efficacy of the GnRH analogue deslorelin for suppression of oestrous cycles in cats. *Journal of Reproduction and Fertility* **57** (Suppl.)**,** 269-273.

Munson, L., Gardener, I.A., Mason, R.J., Chassy, L.M., Seal, U.S. (2002). Endometrial hyperplasia and mineralization in zoo felids treated with melengestrol acetate contraceptives. *Veterinary Pathology* **39**, 419-427. doi: 10.1354/vp.39-4-419

Munson, L., Moresco, A., Calle, P.P. (2005). Adverse effects of contraceptives. In 'Wildlife contraception'. (Eds C. S. Asa and I. J. Porton.) pp 66-82. (The John Hopkins University Press: Baltimore, MD.)

Smithers R.H.N. (Ed.) (1983). *Panther leo*. In 'The mammals of the southern African region'. pp. 374-381. (University of Pretoria: Pretoria.)

Trigg, T.E., Wright, P.J., Armour, A.F., Williamson, P.E., Junaidi, A., Martin, G.B., Doyle, A.G., Walsh, J. (2001). Use of a GnRH analogue implant to produce reversible long-term suppression of reproductive function of male and female domestic dogs. *Journal of Reproduction and Fertility* **57(**Suppl.)**,** 255-261.

Viau, P.; Felippe, E. C. G.; Oliveira, C. A. (2005). Quantificação de esteróides fecais de fêmeas de onça-pintada (Panthera onca) mantidas em cativeiro: validação da técnica. *Brazilian Journal of Veterinary Research and Animal Science* 42, 262-270.

Wright, P.J., Verstegen, J.P., Onclin, K., Jöchle, W., Armour, A.F., Martin, G.B., Trigg, T.E. (2001). Suppression of the oestrous responses of bitches to the GnRH analogue deslorelin by progestin. *Journal of Reproduction and Fertility* **57**(Suppl.)**,** 263-268.

Chapter 4

Repeated use of the GnRH analogue deslorelin to down-regulate reproduction in male cheetahs (Acinonyx jubatus)

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Abstract

The GnRH analogue deslorelin, as a subcutaneous implant, was initially developed in Australia as an ovulation-inducing agent in mares. Its uses, for the suppression of reproduction in the domestic dog and cat and in other species, including humans, have been developed subsequently. Such implants have been used as a contraceptive modality in a variety of wild carnivores, both males and females. This paper describes the use of deslorelin implants as a contraceptive agent for cheetah males maintained in a semi-captive environment and housed in various camps together with females. Annually, male cheetahs were treated for 1 (n = 2), 2 (n = 7), 3 (n = 9), 4 (n = 3) or 5 (n = 1) consecutive years with an implant containing 4.7, 5.0 or 6.0 mg of deslorelin. On the first day of treatment and then on an annual basis, blood testosterone concentrations were analysed, testicular measurements were taken, appearance of penile spikes was determined, and semen was collected and evaluated. Pregnancy rates of mated or inseminated females were determined. A dose of 6 mg of deslorelin suppressed reproduction for at least 1 year, whereas with 4.7 and 5 mg of deslorelin, 3 of 17 males had a few non-motile spermatozoa in their ejaculates. All testosterone concentrations were basal at 1 year post-implant and no side effects were observed. We concluded that deslorelin implantation, at a dose of 6 mg, was a safe and reliable method of annual contraception in male cheetahs.

Keywords: Deslorelin; GnRH; Down-regulation; Contraception; Cheetah

1. Introduction

Population control by means of contraception has become an important tool in the management of wild carnivores in southern Africa. In most cases, especially with endangered species like the cheetah and African wild dog, a reversible method is required. The GnRH analogue, deslorelin, in a long-acting biocompatible subcutaneous implant (Peptech Animal Health, Sydney), was initially developed in Australia as an ovulation-inducing agent in mares. Its uses, for the control of reproduction in the domestic dog and cat and in other species, including humans, have been developed subsequently [1,2]. It has also been used as a contraceptive agent in a variety of wild carnivores, both males and females [3,4]. Contrary to the side effects reported in some female carnivores treated with gestagen implants [5], no adverse side effects have been observed with deslorelin. In most species, the continuous release of deslorelin from the implant down-regulates FSH and LH release, thereby controlling gonadal activity in both males and females. Previously, deslorelin (6 or 12 mg) was used once in each of six cheetah males. Animals examined 45 days after implant had undetectable blood testosterone concentrations, but their semen samples had high concentrations of spermatozoa. By 3 months, ejaculates from two males were azoospermic, the others becoming azoospermic later following treatment administration. All males remained azoospermic for at least 21 (12 mg) and 12 (6 mg) months after a single treatment [3,4]. The present paper describes the repeated use of deslorelin implants of various doses and release rates, on an annual basis, as a contraceptive agent in male cheetahs in a semi-captive environment.

2. Materials and methods

The cheetahs used in the present study were housed in enclosures (size, 10–1500 ha) in mixed sexes. Annually from 1999 to 2004, male cheetahs were treated for 1 (n = 2), 2 (n = 7), 3 (n = 9), 4 (n = 3) or 5(n = 1) consecutive years with an implant containing 4.7, 5.0 or 6.0 mg of deslorelin. GnRH analogue implant dose was 12 mg (1999), 6 mg (2000), 5 mg (2001) and 4.7 mg (2002–2004). Data collected on the first day of contraception and on an annual basis at 12–14-month intervals were: blood testosterone concentration, testicular measurements (from 2001), appearance of penile spikes, evaluation of semen collected by electro-stimulation [6], and pregnancy rates. Blood testosterone concentrations were determined by radioimmunoassay using a commercial kit (Coat-A-Count total testosterone kit; Diagnostic Products Corporation, Los Angeles, CA, USA) [4]. The presence of viable spermatozoa was used as an indicator of potential fertility [4]. Penile spikes were evaluated subjectively according to prominence on a scale of 1–3. Testicular measurements (length and width) were

carried out using vernier callipers. In the group treated twice, one male showing aberrant sexual behavior towards humans was given 9.4 mg deslorelin each year to correct behavioral abnormalities. Animal AJ138 (Table 1) died 2 days after it was immobilized for examination in 2004, 13 months after the last implant. Testes were fixed in 10% buffered formalin and prepared for histological examination.

A mixed model was used to determine the effects of cheetah, time and side (left or right testis) on the length and width of the testes. Cheetah was considered a random effect, whereas time and side of testis (left or right) were fixed. Times 0, 1, 2 and 3 were the times immediately before first, second and third contraceptive treatments, and 13 months after the third contraceptive treatment, respectively. Side of testis had no effect on either response variable and was removed from the model. A two-tailed Mann–Whitney test was used to compare the length of the testes of the younger animals versus the older animals, and a one- tailed Mann–Whitney test was used to compare the width of the testes over time or between young and old animals. For all statistical tests, $P < 0.05$ was considered significant.

3. Results

A dose of 6 mg suppressed reproduction for at least 1 year, whereas with 4.7 or 5 mg, 3 of 17 males had a few spermatozoa in the ejaculate, most of which were dead (Table 1) and 3 had normal ejaculates. Table 1 shows the results from 12 of the 14 cheetahs treated annually for 3 years in which a complete data set was available. Testosterone concentrations were reduced to basal concentrations at 1 year in eight animals, whereas four were at values still over the detection limit of the assay. The pattern in the remaining cheetahs treated from one to five times was comparable. Among the males with spermatozoa, three also had secondary signs of androgenic activity, as demonstrated by the prominence of penile spikes. During the second and third years of initial treatment, with the exception of sperm debris in one male, no additional ejaculates with spermatozoa present were seen. Testosterone concentrations were consistently basal and penile spikes were evaluated as either poorly or moderately developed in appearance from the second year of treatment.

Table 1.
Twelve cheetahs treated with 5 mg (2001) or 4.7 mg (2002–2004) deslorelin implants annually for 3 years

Observation	Cheetah ID and age during first examination in 2001											
	AJ3, 9 years	AJ132, 5 years	AJ133, 5 years	AJ138, 8.5 years	AJ187, 5 years	AJ225, 3.5 years	AJ226, 3.5 years	AJ227, 3.5 years	AJ255, 3.5 years	AJ256, 3 years	AJ259, 9 years	AJ261, 5 years
February 2001, Time 0												
R testis (mm)	25 x 19	27 x 19	27 x 17	26 x 19	25 x 18	27 x 19	30 x 20	25 x 20	27 x 19	23 x 16	29 x 19	27 x 19
L testis (mm)	27 x 19	27 x 19	27 x 18	26 x 18	29 x 18	31 x 18	29 x 21	27 x 20	26 x 18	24 x 17	24 x 20	28 x 17
Testosterone (nmol/L)	3.43	5.37	8.81	3.5	6.45	0	9.66	4.01	0.36	6.86	7.45	11.68
Penile spikes	3	3	3	3	3	3	3	3	3	3	3	3
Sperm	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Few [a]	Yes	Yes	Yes	Yes
Body weight (kg)	31	43	40	56	55	48	47	47	40	36	50	45
March 2002, Time 1												
R testis mm	24 x 16	25 x 18	24 x 17	24 x 19	25 x 17	24 x 18	26 x 15	22 x 14	24 x 19	17 x 11	22 x 17	24 x 18
L testis mm	24 x 16	26 x 18	25 x 17	24 x 19	24 x 18	23 x 17	24 x 14	22 x 14	23 x 19	17 x 12	21 x 17	23 x 17
Testosterone (nmol/L)	0	0.47	0	0.67	0	0	0.96	0	0	0	1.32	0
Penile spikes	2	3	2	2	2	2	2	1	2	1	2	2
Sperm	Few	Yes	None	None	None	Few [a]	Few [a]	None	Yes	None	None	Yes
Body weight (kg)	32	41	38	54	55	45.5	45	44	37	34	43	41
February 2003, Time 2												
R testis mm	21 x 16	21 x 12	23 x 12	20 x 13	20 x 12	21 x 12	18 x 13	19 x 16	20 x 13	18 x 11	16 x 14	18 x 12
L testis mm	22 x 16	20 x 12	21 x 13	20 x 14	22 x 12	20 x 12	20 x 14	19 x 14	19 x 12	17 x 12	17 x 14	17 x 12
Testosterone (nmol/L)	0.06	0	0	0	0	0	0	0	0	0	0	0
Penile spikes	2	1	1	2	1	1	1	1	2	2	1	1
Sperm	None	None	None	None	None	None	None	None	None	None	None	None
Body weight (kg)	31	40	38	54	57	47.5	46.5	46	38	35	44	45

Observation	Cheetah ID and age during first examination in 2001											
	AJ3, 9 years	AJ132, 5 years	AJ133, 5 years	AJ138, 8.5 years	AJ187, 5 years	AJ225, 3.5 years	AJ226, 3.5 years	AJ227, 3.5 years	AJ255, 3.5 years	AJ256, 3 years	AJ259, 9 years	AJ261, 5 years
March 2004, Time 3												
R testis mm	20 x 17	17 x 12	16 x 12	20 x 13	19 x 13	24 x 18	21 x 15	19 x 15	20 x 13	18 x 13	18 x 13	18 x 14
L testis mm	19 x 17	17 x 12	17 x 12	20 x 14	18 x 13	21 x 15	20 x 14	20 x 15	19 x 13	19 x 13	19 x 14	18 x 14
Testosterone (nmol/L)	0	0	0	0	0	0	0	0	0	0	0	3.27
Penile spikes	2	1	1	1	1	1	1	1	1	1	1	2
Sperm	Debris	None	None	None	None	None	None	None	None	None	None	None
Body weight (kg)	35	42	39	50.5	52	48	46	45	39.5	37	45	46

Penile spikes: 1 = poorly developed, 2 = moderately developed, 3 = prominent. Note: penile spikes cannot disappear once they have been formed, their size is regulated by androgens. Yes = presence of normal sperm cells.

[a] Dead sperm.

There were differences among cheetahs ($P < 0.001$) for testicular length and width. However, neither testicular length nor width before onset of contraception was correlated with age or body mass. Both the length and width of the testes differed among periods ($P < 0.001$). Length, as well as width, decreased from Time 0 to 1, with a further decrease to Time 2, but thereafter remained constant (Fig. 1). Cheetah differed in the testicular size response to repeated deslorelin treatments ($P < 0.001$); after the third treatment, the five younger animals (3–3.5 years at the beginning of the study) appeared to respond differently than the older animals (5–9 years at the beginning of the study). The length of the testes of the younger animals remained the same (number of testes = 6) or increased by 1–3 mm ($n = 4$) between

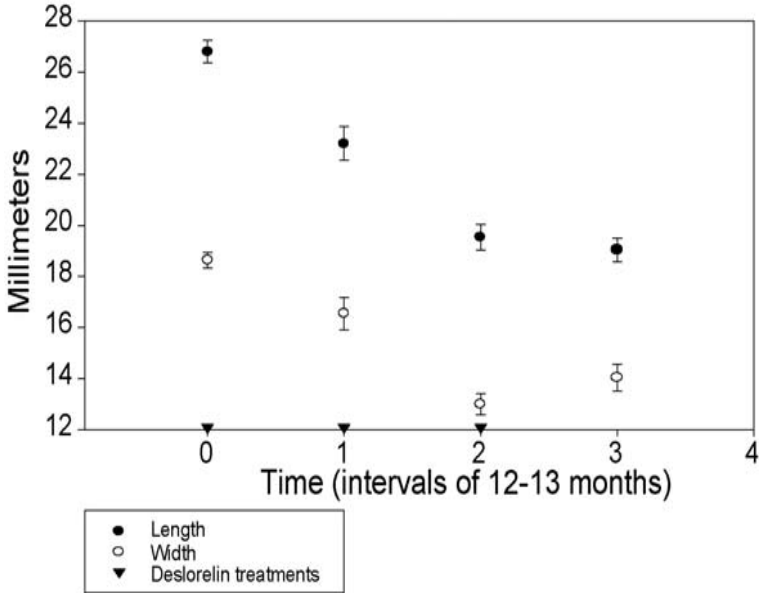

Fig. 1.Testicular dimensions of 13 cheetahs (*Acinonyx jubatus*) before and following three annual treatments of deslorelin (vertical bars indicate standard errors).

Times 2 and 3, whereas the testicular length in the older animals ($n = 7$) remained the same (number of testes = 4), decreased by 1–7 mm ($n = 8$) and increased by 2 mm in two testes ($P < 0.05$). The width of the testes remained constant or increased by 1–6 mm during Times 2 and 3. The testes of the five young animals tended to increase more in width than those of the seven older animals ($P = 0.07$).

The quality of the histological sections from animal AJ138 was suboptimal because

the animal was found several hours after he died. The testicular sections showed distinct suppression of spermatogenesis. The tubules contained mainly spermatogonia with occasional presence of spermatocytes and spermatids. No spermatozoa were visible in the tubules (Figs. 2 and 3).

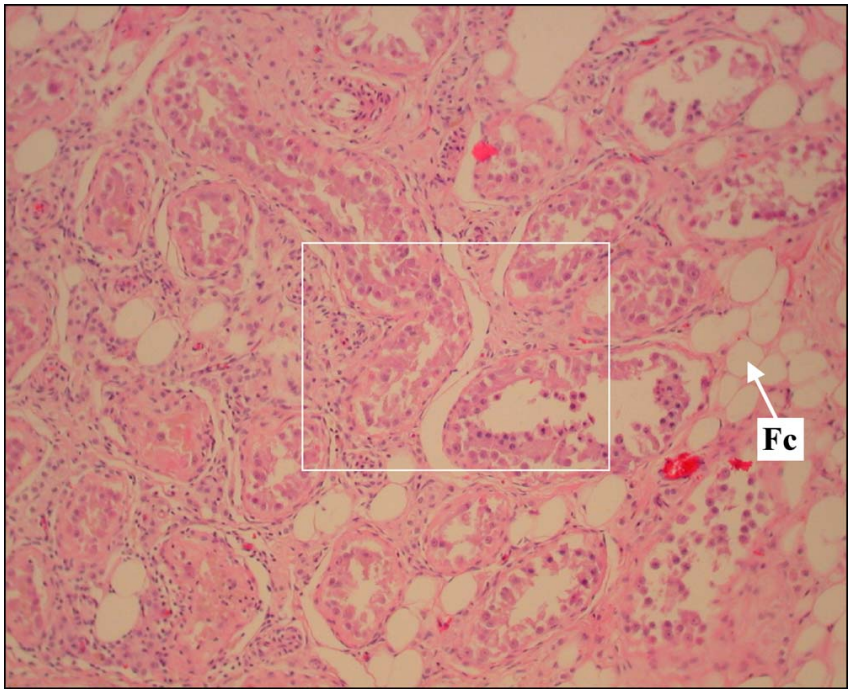

Fig. 2. Histological section (H&E; x100) of the testis of Cheetah AJ138 exposed to three annual deslorelin implants. The last implant was administered 12 months prior to death of the animal. The rectangular area is enlarged in Fig. 3. Fc = possible fat cells.

4. Discussion

The contraceptive efficacy of deslorelin in male cheetahs described previously [3,4] was confirmed in this extended study. Furthermore, between February 1999 and May 2004, no pregnancies were recorded. Deslorelin administered once a year at the doses described was effective at down-regulating testicular function in all males for a minimum period of 1 year. The down-regulation was reflected in basal or lowered blood testosterone concentrations and the absence of spermatozoa in most ejaculates 1 year after implant. The ejaculates observed in some males after 1 year of treatment were abnormal in sperm numbers and quality, suggesting that these males were infertile. It is not known if these abnormalities were the product of renewed

spermatogenesis or remnants in the epididymis after cessation of sperm production.

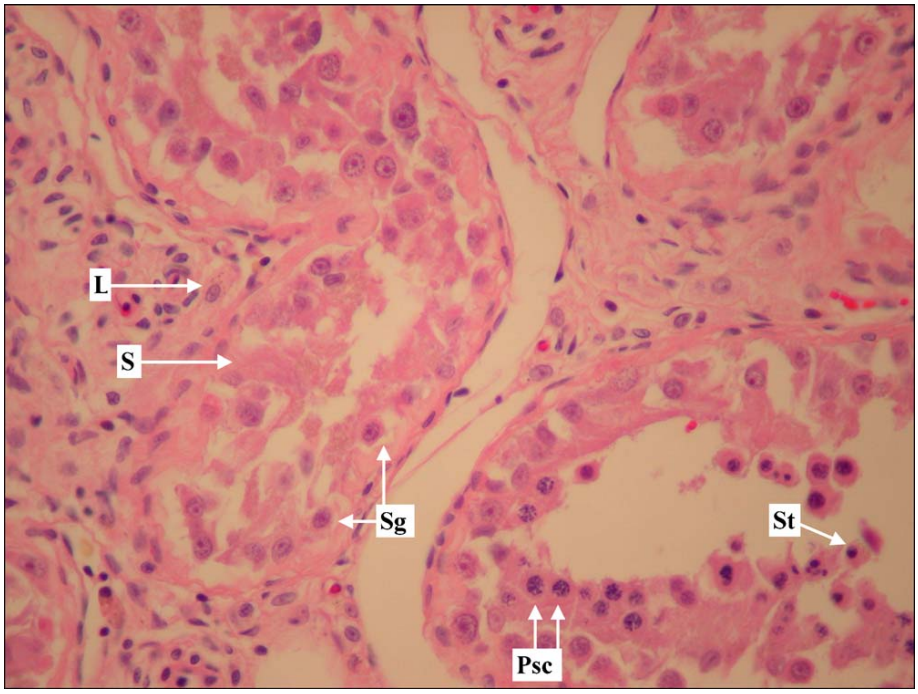

Fig. 3. Histological section (H&E; x400) of the testis of Cheetah AJ138. L = Leydig cell; Psc = primary spermatocytes; S = Sertoli cell; Sg = spermatogonia; St = spermatid.

Given that most spermatozoa were non-motile or sperm debris (including epithelial cells, sperm heads, sperm tails) we believe the latter to be true. Furthermore, if testicular size is an indicator of seminiferous tubule activity as in other species, the further reduction in size observed between the first and second year of treatment suggests a further decline in spermatogenesis. Perhaps spermatogenesis was either further down-regulated by the treatment or that the inactive cell stages of spermatogenesis take a long time to be totally inhibited. One year after the third implant, testicular size reached the minimum and no further changes were observed, except in the younger males where testicular size increased slightly. As shown in Table 1, testicular size increased without a corresponding increase in body weight. The testes histology realized from the tissues of the animal that accidentally died confirmed the previous observation of arrest of spermatogenesis.

Deslorelin effectively suppressed sexual behavior (no males were observed

attempting to mate) and inhibited penis spike development. The latter parameter was considered a good indicator of treatment efficacy by reflecting the suppressive effect on testosterone synthesis. No side effects, including significant changes in body weight, were seen in any of the males.

In conclusion, deslorelin was a safe and reliable method to inhibit, through pituitary GnRH receptor down-regulation, sexual function in male cheetahs. The optimal annual dose for male cheetahs was approximately 6 mg/animal.

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References

[1] Munson L, Bauman JE, Asa CS, Jöchle W, Trigg TE. Efficacy of the GnRH analogue deslorelin for suppression of oestrous cycles in cats. J Reprod Fertil 2001;Suppl. 57:269–73.

[2] Trigg TE, Wright PJ, Armour AF, Williamson PE, Junaidi A, Martin GB, et al. Use of a GnRH analogue implant to produce reversible long-term suppression of reproductive function of male and female domestic dogs. J Reprod Fertil 2001;Suppl. 57:255–61.

[3] Bertschinger HJ, Asa CS, Calle PP, Long JA, Bauman K, DeMatteo K, et al. Control of reproduction and sex related behaviour in exotic wild carnivores with the GnRH analogue deslorelin: preliminary observations. J Reprod Fertil 2001;Suppl. 57:275–83.

[4] Bertschinger HJ, Trigg TE, Jöchle W, Human A. Induction of contraception in some African wild carnivores by down regulation of LH and FSH secretion using the GnRH analogue deslorelin. Reproduction 2002;Suppl. 60:41–52.

[5] Munson L. Health risks of contraceptives in wildlife. In: Bertschinger HJ., Kirkpatrick JF., editors. Proceedings of the 5th international symposium on fertility control in wildlife. 2001. p. 12–3.

[6] Bertschinger HJ, Meltzer DGA. Reproduction in male cheetahs. Part 2: sperm morphology. In: Penzhorn BL., editor. Proceedings of a symposium on cheetahs as game ranch animals. 1998. p. 153–8.

Chapter 5a

Contraceptive potential of the porcine zona pellucida vaccine in the African elephant (*Loxodonta africana*)

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Abstract

Immunocontraception has been successful in controlling free-roaming equids; however, what is the potential for the immunocontraceptive control of the African elephant (Loxodonta africana)? The porcine zona pellucida (pZP) glycoproteins share antigenic domains with the African elephant zona pellucida (elZP) glycoproteins, and anti-zona pellucida serum antibodies have been successfully stimulated. To determine the cross-reactivity of the pZP and elZP, immunocytochemistry was evaluated by light and electron microscopy. Specifically, the binding of polyclonal antibodies against total heat-solubilized-porcine zona pellucida to fixed elephant ovary sections was evaluated. The elZP of primary, secondary and tertiary follicles was recognized by the rabbit-anti-pZP serum, but there was no apparent recognition of the primordial follicles. The ability of anti-pZP antibodies to recognize the elZP demonstrates that there is molecular homology between the pZP and elZP glycoproteins. This homology makes the African elephant a candidate for pZP immunocontraception. Three captive elephants were vaccinated with 400 μg pZP with a synthetic trehalose dicorynomycolate (S-TDCM) adjuvant. The elephants received 2 boosters of 600 μg pZP at 4 wk and 10 mo after the primary vaccination. The vaccinated female elephants developed significant (P<O.O5) titers to pZP over prevaccination levels. These levels persisted for 12 to14 mo after the third vaccination. This preliminary evidence shows that the female elephant can develop significant serum antibody levels to pZP. These levels of antibodies are comparable to those required in horses for successful immunocontraception. Thus, porcine zona pellucida immunocontraception might be used to control elephant populations.

Key words: immunocontraception, African elephant, zona pellucida

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Introduction

The density and mortality rates of populations of elephants in Africa vary greatly (18). In the Republic of South Africa, a combination of good park management and virtually no poaching has led to a minimum of 5% per annum elephant population growth (4). In 1990, the Convention on International Trade in Endangered Species (CITES) promulgated a ban on ivory trading, causing concerned members of the public to focus world attention on the plight of the African elephant. Public involvement and the inflow of financial support have led to decreased poaching in some African nations (17). The high elephant densities in Kruger National Park have caused concern over habitat destruction. In conjunction with the South African National Parks, an investigation was undertaken to determine if the porcine zona pellucida (pZP) glycoproteins have shared determinants with the elephant zona pellucida (elZP).

To date, pZP has been shown to be a highly versatile irmnunocontraceptive molecule. Injection of total heat solubilized pZP into horses resulted in contraception (11, 12, 24). The immunocontraceptive effect usually lasts for 12 mo and has been effective in reducing fertility of free-roaming horses (5, 6). The vaccine appears to be devoid of any major side effects (5). The pZP vaccine has been used for more than 8 yr in horses, with no adverse reactions; moreover, the vaccine does not affect the fetus of pregnant mares (5). Further, immunocytochemistry with rabbit anti-pZP polyclonal antibodies exhibited no cross-reaction with the brain, heart, lung, kidney, liver, bladder, stomach, small intestine, large intestine, skeletal muscle, skin, spleen, pancreas or lymph node of horses and dogs.[b] Western blots with anti-pZP polyclonal serum also revealed no detectable cross-reactivity with equine luteinizing hormone (LH) or equine chorionic gonadotropin (eCG).

The pZP has been shown to have cross-reactivity with the zona pellucida of many species, including humans (19), mice (21), rabbits (20, 25), horses (12, 15, 24), dogs (1, 13, 14), squirrels, monkeys (22) and 40 species of zoo animals (5, 8, 9).

The aim of this study was to determine if elZP has shared determinants with the pZP. On the basis of this evidence, the ability of a pZP immunocontraceptive to produce titers in adult female elephants was also explored.

[b]Fayrer-Hosken, personal communication 1999.

Materials and methods

Fixation of the Elephant Ovaries
Elephant ovarian tissues were cut into 3 to 5 mm^3 blocks with a sterile scalpel and placed into each of the following fixatives: Bouin's, neutral-buffered formalin and glutaraldehyde. The fixed samples was processed using the following methods. The tissues fixed in Bouin's and in neutral-buffered formalin were embedded in paraffin, whereas the tissues in glutaraldehyde were embedded in plastic.

Bouin's Fixation and Paraffin Embedding
Tissues were placed into 30 mL of Bouin's fluid (saturated aqueous picric acid, 75 mL; 37% formalin, 25 mL; glacial acetic acid, 5 mL) and fixed for 24 h at 4°C (2). Samples were washed under running water and were then placed for 1 h in 4 sequential 30 mL volumes of 50% ethanol (ETOH) at 4°C until the yellow stain disappeared. Following this, the samples were dehydrated, cleared, infiltrated and embedded in paraffin.

Neutral Buffered Formalin (NBF) Fixation and Paraffin Embedding
Tissues were placed into 30 mL of NBF (20%) and fixed for 24 h at room temperature (10, 23). Next, samples were dehydrated and embedded. All paraffin embedded samples for histology were then sectioned to 3 μm thickness on a Leitz microtome[c] and stained using Gill's hematoxylin and eosin (H&E; 3) using an automated processor.[d]

Glutaraldehyde Fixation and Plastic Embedding
Tissues were sectioned into 1 to 2 mm^3 cubes and fixed in 1% glutaraldehyde[e] in phosphate buffered saline (PBS), pH 7.4, at room temperature for 2 h. The samples were washed 5 times in chilled PBS and stored at 4°C overnight. After refrigeration, the samples were washed with 10 mL each of PBS and deionized water. They were then dehydrated through a series of ETOH solutions (30, 50, 75, and 95%) for 15 min each at 4°C and allowed to come to room temperature. Final dehydration steps included three, 30-min incubations in 100% ETOH at room temperature. Samples were infiltrated and embedded in JB-4®, a glycol methactylate-based plastic resin, [e] and 1.5 to 2.0-μm sections were obtained using a Sorvall JB-4 Porter-Blum microtome. The sections were placed on glass slides and, after drying, were stained with H&E using routine methods.

[c]Darmstadt, GDR.
[d]Hacker Instruments Inc, Fairfield, NJ.
[e]Polysciences Inc, Warrington , PA.

Preparation of the Anti-pZP Antibody

Male New Zealand White rabbits were vaccinated with 400 µg of highly purified pZP in Complete Freund's Adjuvant and then boosted twice 2 wk apart with 200 µg of highly purified pZP in Freund's Incomplete Adjuvant. Serum was harvested from the rabbits 2 wk after the last vaccination. To confirm the specificity of the antibodies for pZP, I-D and 2-D Western blot analyses were performed. In the Western blots, only pZP glycoproteins were stained, and all 3 families of pZP were recognized (pZP1, pZP3α, and pZP3β).

Elephant Oocyte Fixation and Embedment

Graafian follicle elephant oocytes was enrobed in 58% molten noble agar to facilitate handling, then promptly immersed in a fixative containing 1% paraformaldehyde, 1% glutaraldehyde and 0.1% picric acid in 0.1 M cacodylate-HCl buffer, pH 7.2. After several hours of fixation, the fixative was rinsed from the oocytes with several changes of buffer, followed by several rinses of deionized water to remove the buffer from the oocytes. A graded series of ethanol was used to dehydrate the oocytes to 95% ethanol. The oocytes were infiltrated gradually with 95% ethanol and LR White Medium Grade resin, a hydrophilic acrylic resin, [e] and then several long changes of 100% LR White resin. The oocytes were embedded in a gelatin capsule with fresh 100% LR White resin and were allowed to polymerize 24 h in a 58°C oven. The gelatin capsule was trimmed, and l-µm sections were obtained using a Reichert Ultracut S ultramicrotome[f]. Sections were placed on glass slides and allowed to dry with moderate heat before staining with 1% Toluidine Blue in 1% sodium borate.

Immunocytochemistry for Light Microscopy

All three fixation methods were evaluated for the tissue-fixation ability and the ability to preserve tissue antigenicity. The paraffin blocks were sectioned and the sections placed on slides. The slides were deparaffinized and blocked overnight in TRIS-buffered saline (TBS) containing 3% bovine serum albumin (BSA). Slides were washed and incubated with the primary antibody (rabbit-anti-pZP, 1:l00) in TBST (TBS with Tween 20) for 2 h. After incubation, the slides were washed with TBS. The slides were then incubated with the secondary antibody (protein A- 10 nm gold, 1: 100) in TBST for 1 h. After this step, the slides were washed in TBS, followed by triple distilled water. The staining of the slides was enhanced using standard silver enhancement, dried, and permanently mounted. The JB-4 blocks were sectioned and placed on slides. After the sections were allowed to dry, the slides were blocked in 3% BSA in TBST overnight in a wet chamber at 4ºC.

[e]Polysciences Inc, Warrington , PA.
[f]Leica Inc, Deerfield, IL.

After blocking for 24 h, the sections were washed in TBST and incubated in primary antibody, anti-pZP, at 1:500 in TBST for 2 h at room temperature in a wet chamber. After washing in TBST, the slides were then incubated in the secondary antibody (protein-A conjugated to 10 nm colloidal gold[g]) at 1:10 in TBST for 1 h at room temperature in a wet chamber. Next, the slides were jet washed in TBS and deionized water for 1 min each. The sections were enhanced using the silver-enhancement technique, and the reaction was stopped with deionized water after color development was observed. The sections were allowed to air dry, and coverslips were mounted with Flo-Texx®[h] prior to evaluation.

Immunocytochemistry for Transmission Electron Microscopy

Thin sections of the oocyte were obtained and mounted on Formvar carbon-coated nickel grids. The grids were blocked for 30 min in a TBS-BSA buffer solution (0.2 M Tris-buffered saline, pH 8.2 with 1% globulin-free BSA) with Tween 20 added (1 drop/mL buffer). After washing in buffer, the grids were incubated in the primary antibody (rabbit-anti-pZP) at 1: 16,000 in TBS-BSA overnight in a wet chamber at 4°C. The grids were washed in buffer before incubating in the secondary antibody, Protein-A conjugated to 10 nm colloidal gold, diluted 1:20 in TBS-BSA, 30 min at room temperature in a wet chamber. The grids were washed in buffer and then deionized water before continuing with a lo-min silver enhancement. After enhancement, the grids were washed in deionized water and poststained with 5% methanolic uranyl acetate and Reynolds lead citrate. The grids were observed with the JEM-1210 transmission electron microscope.[i]

Vaccination of Female Elephants

As a result of significant cooperation with zoos, 3 elephants were vaccinated with the pZP vaccine. A 30-yr-old African elephant (Elephant 1) at the Riverbanks Zoological Park and Botanical Garden, in Columbia, South Carolina; a 25-yr-old African elephant (Elephant 2) at the Greenville Zoo in Greenville, South Carolina; and a 20-yr-old Asian elephant (Elephant 3) at the Calgary Zoo, Calgary, BC, were vaccinated. Elephants 1, 2 and 3 were initially vaccinated with a 10% oil-in-water emulsion containing 400 μg of pZP and 5 mg of synthetic trehalose dicorynomycolate[j] (S-TDCM). Elephants 1 and 3 were initially boosted with 400 μg of pZP. Elephant 2, however, was boosted with 600 μg of pZP based on the initial serum antibody levels. For the subsequent boosters, all elephants received 600 μg of pZP in a 10% oil-in-water emulsion with 5 mg of S-TDCM adjuvant. Booster administrations were given after 4 wk and again 10 mo later.

[g]Ag-Enhancer, Sigma Chemicals, St Louis, MO.
[h]Lerner Laboratories, Pittsburgh, PA.
[i]JEOL USA Inc, Peabody, MA.
[j]Kindly provided by Dr. Terry Ulrich, Ribi Immunochem Research Inc, Hamilton, MT.

Enzyme Linked Immunosorbent Assay (ELISA) Detection of Anti-pZP IgG Levels in Elephants

For all assays, each data point was performed in triplicate, and each plate had a positive control (rabbit anti-pZP) and two negative controls (normal rabbit serum and no pZP in the well). Each well of the ELISA plates[k] was coated with 2 µg/well of pZP. The pZP was reconstituted from a lyophilized pZP stock to a concentration of 1 mg/mL in 0.02 M TRIS at pH 8.2. Fifty microliters of the stock solution (2 µg pZP) was added to the wells. The plates were incubated for 6 h at 4°C. The plates were then washed 3 times with TBS. To block the plates, 200 µL of TRIS buffered saline and TBST with 5% BSA[l] were added to each well. The covered plate was incubated at 4°C for 12 ho. The plates were removed from the refrigerator and allowed to warm to room temperature. The TBST was removed, and the wells were washed 3 times with TBS. To each well, 50 µL of the elephant serum at a dilution of 1:500 or 1: 1,000 were added and incubated for 4 ho. The elephant serum was poured off, and the plates were washed 3 times with TBS. The secondary antibody, rabbit anti-elephant IgG[m], was added at 50 uL/well (l:l,000 dilution in TBST) and incubated for 2 ho. Finally, 50 µL/well of the tertiary antibody (goat anti-rabbit with alkaline phosphatase) were added at a 1:2,000 dilution in TBST. The plates were then incubated for 2 h at room temperature. The plates were rinsed 3 times with TBS and dried. At this time, 200 µL of carbonate buffer were added to each well to adjust the pH to 9.8. After 5 min, the buffer was removed, and the plate was dried on paper towels. Finally, 50 µL/well of p-nitrophenyl phosphate in carbonate buffer at pH 9.8 were added. The color reaction was allowed to proceed for 30 min, after which it was stopped by adding 50 µL/well of 3 M NaOH. The plate was read at 405 and 492 nm.

Statistical Analysis

Mean serum antibody levels were compared statistically using an analysis of variance (ANOVA) and a Tukey's test to perform a pairwise comparison of the means. This was performed using computer software SAS[n] (version 6.21). Statistical significance was accepted at $P<0.05$.

[k]Immulon 96 well, Chantilly, VA.
[l]Fraction V, Sigma, St Louis, MO.
[m]Courtesy of Dr. Henk Bertschinger.

Results

Normal Histology

Primary (Figure 1A), secondary (Figure 2A), and tertiary follicles were easily identified in all fixed samples of ovarian tissue. Bouin's fixation provided the best preservation of the cellular detail. The primary follicles (Figure 1A) consisted of a single layer of granulosa cells surrounding the oocyte. The oocyte did not have a

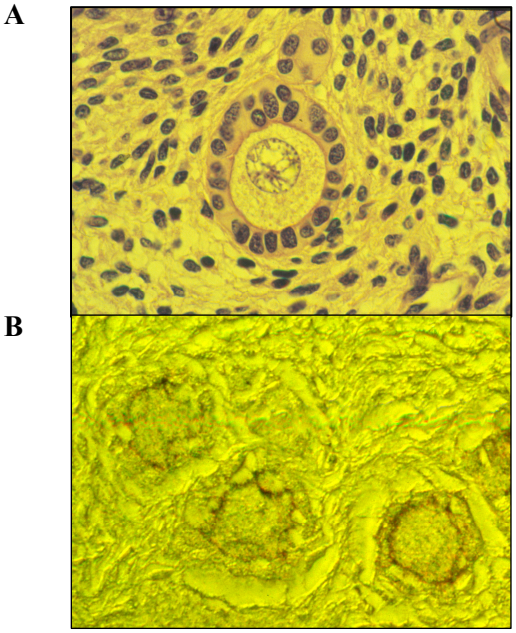

Figure l. A) Hematoxylin and eosin stained section of an elephant ovary at x 1000 magnification. The section shows a primary follicles that is located centrally surrounded by a single layer of granulosa cells. B) Immunogold staining using a polyclonal rabbit antibody against heat-solubilized porcine zona pellucida in a section of an elephant ovary at x 1000 magnification. This section shows 3 primary follicles surrounded by immunogold staining of the early elephant zona pellucida.

[n]SAS Institute, Cary, NC.

clearly visible zona pellucida at x 1,000 magnification. The secondary follicles (Figure 2A) had 2 to 3 layers of cuboidal granulosa cells, and the zona pellucida was clearly visible surrounding the oocyte. In the tertiary follicles, there were multiple layers of cuboidal to columnar granulosa cells, and the zona was clearly visible.

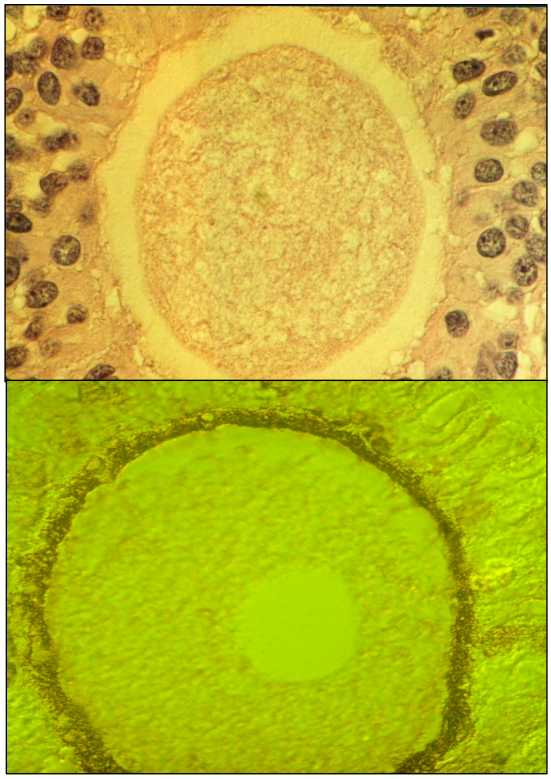

Figure 2. A) Hematoxylin and eosin stained section of an elephant ovary at x 1000 magnification. The section shows a secondary follicle surrounded by layers of granulosacells. The zona pellucida is clearly visible between the oocyte and granulosa cells. B) Immunogold staining using a polyclonal rabbit antibody against heat-solubilized porcine zona pellucida in a section of an elephant ovary at x 1000 magnification showing a secondary follicle. The follicle is surrounded by immunogold staining of the elephant zona pellucida.

Immunocytochemistry for Light Microscopy

In the primary follicles (Figure lB), immunogold deposits were concentrated at the oocyte- granulosa cell junctions. This formed a thin layer of gold staining at the cell junctions. Furthermore, here was diffuse staining of the primary oocyte cytoplasm, which exceeded that of the background and control sections. In the secondary (Figure 2B) and tertiary follicles, there was definitive staining of the zona pellucida; however, there was also some staining of intercorona radiata cell spaces.

Immunocytochemistry for transmission electron microscopy (TEM)
The zona of the control sections (x 8,000) had no gold staining (Figure 3A) of the zona pellucida, the ooplasm, or the perizonal area. In the sections treated (x 12,000) with anti-pZP (Figure 3B), the gold labeling can be clearly seen only over the zona pellucida. Some gold granules can be seen in the cytoplasm especially within dilated portions of the smooth endoplasmic reticulum.

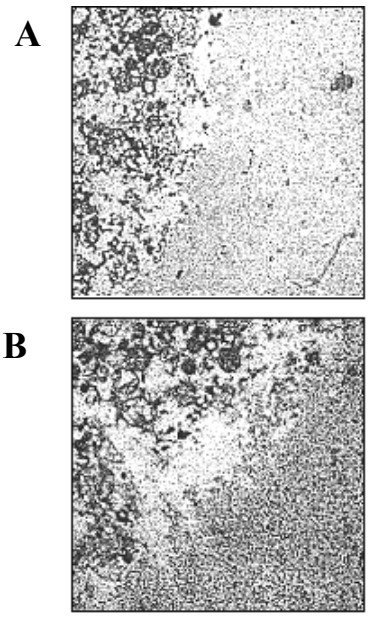

Figure 3. A) Immunogold staining using of a control elephant oocyte at x 8,000 magnification. Neither the ooplasm, the zona pellucida, or the space external to the zona pellucida show any immunogold staining. B) Immunogold staining using a polyclonal rabbit antibody against heat-solubilized porcine zona pellucida in an elephant oocyte at x 12,000 magnification. The elephant zona pellucida is clearly labeled with the immunogold staining. In addition, there is labeling of the ooplasm.

Serum Anti-pZP Levels
The serum levels of anti-pZP IgG rose with each successive vaccination (Figure 4). However, it was not until after the second booster that the levels remained elevated for at least 12 mo. The levels of anti-pZP IgG in Elephant 2 did not attain the levels of Elephant 1. Elephant 3, an Asian elephant, did not develop sustained levels until after the second booster.

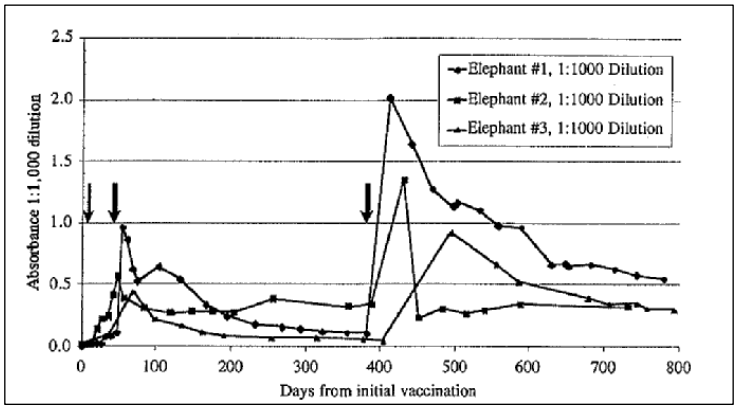

Figure 4. Serum levels of anti-pZP IgG in 3 captive elephants. The levels rose with each successivevaccination, but it rose the most and was sustained the longest after the second booster.

Discussion

The immunocytochemistry results reported here establish that there are shared epitopes between the elephant and pZP. This was demonstrated at the light microscopy level by immunogold staining of the zona pellucida of primary, secondary and tertiary oocytes, and then confirmed with TEM, which demonstrated that there was significant immunogold staining of the elZP. Moreover, the elZP might originate from the oocyte since immunogold staining of the dilated portions of the smooth endoplasmic reticulum was clearly visible, whereas it is not visible in any of surrounding cells. Hence, antibodies directed against the pZP would also recognize elZP. This data thus provides support for the hypothesis that the elephant vaccinated with pZP could be contracepted and it provides additional evidence that the pZP has significant homology with the zona pellucidae of many mammalian species.

For the vaccination trials, all elephants responded with significant ($P<0.05$) increases in blood levels of anti-pZP antibodies, specifically that of IgG. The magnitude of the antibody increases were equivalent to those of vaccinated immunocontracepted mares. There were, however, differences in serum IgG levels among elephants, and especially in the lower sustained levels in Elephants 2 and 3 prior to the second booster. However, in Elephant 2 these lower levels might have a nutritional origin, as her diet temporarily had low vitamin E levels. Vitamin E levels have been correlated with immune competence and specifically immunoglobulin levels. Once vitamin E supplementation was initiated, the antibody levels of Elephant 2 increased and were equivalent to those of Elephants 1 and 3. Elephant 3 was an Asian elephant, and this may also have contributed to the different response dynamics

in the phase prior to the second booster. Initial indications were that the titers after the first booster might be sustained for a sufficient time to serve as an acceptable immunocontraceptive agent. But only once the profile was completed did it become clear that a second booster would be needed for sustained levels. Hence the final quantity of serum IgG, after 2 boosters, appears to be sufficient for effective contraception in the elephant, but this needs to be proven in a free-roaming population.

The need for population management of African elephants will become a reality during the next century; however, it is also evident that culling will be increasingly unacceptable to the world community. Thus, alternative strategies must be provided to park and habitat managers in order to control elephant populations. These initial studies are pivotal in determining the potential role of the pZP immunocontraceptive vaccine.

The delivery of pZP vaccine and an adjuvant to free-roaming equids has already been shown to be a practical and economic reality (5). In addition, the pZP vaccine and adjuvant mixtures that have been reported in horses (5, 24) have had very few side effects such as lameness, swelling and abscesses. With the increasing availability of pZP for immunocontraception, there will be more widespread use of this technology for vaccinating animals. However, it is not clear if a remotely delivered pZP vaccine can control the number of free-roaming animals. The initial data from Assateague (5) Island indicate that a free-roaming population of animals can be controlled by remotely delivered pZP immunocontraception. The population of the Assateague horses is now kept at a constant by regular vaccination with pZP. Hence, the pZP technology is probably capable of affecting population control in the African elephant of South Africa.

What are the effects on herd dynamics and social behavior of immunocontraception on the free-roaming African elephant? This is an especially important question in this species (African elephant), whose social interactions may be among the most advanced in the animal kingdom. The potential impact of reduced numbers of calves on herds of the elephant are not known, but this is an important consideration to take into account, since the elephant is known to have highly evolved emotions and can comprehend the concept of death. Elephants will investigate dead relatives and have even been seen carrying the bones of dead relatives. Their bonds are so strong that they will attempt to assist sick, injured, and even dying elephants; the loss of group members results in changes in family cohesion and social bonding (17). Immunocontraception may also supplant the need for culling, thus nullifying its effects on the elephants. Reports by Moss (16) describe that entire family units are seldom culled, and the survivors of these herds are severely traumatized by the disruption of the matriarchal family units. Of the available population control options, it would seem that immunocontraception and reduced calf numbers would be the most

acceptable. The potential use of immunocontraception in controlling free-roaming African elephants is very encouraging, and it may provide an important tool for ecology and conservation of both the species and the habitat.

References

1. Bamezai AK, Mahi Brown CA, Talwar GP. Inhibition of penetration of canine zonae pellucidae by homologous spermatozoa in vitro using monoclonal antibodies raised against porcine zonae. J Reprod Immunol 1988; 13:85-95.
2. Deanesly R. Germ cell proliferations in the fetal horse ovary. Cell Tissue Res 1977; 185:361-71.
3. Hrapchak D.N. Selective staining with hematoxylin, applications and theory: a review. Am J Med Tech 1976; 42:371-379.
4. Joubert SCJ. Master plan for the management of the Kruger National Park: National Parks Board Conference, Skukuza, Republic of South Africa, 1986; 1-23.
5. Kirkpatrick JF. Management of Wild Horses by Fertility Control: The Assateague Experience, Denver CO: National Park Service Scientific Monograph, 1995; 32-35.
6. Kirkpatrick JF, Liu IKM, Turner JW, Jr., Naugle R, Keiper R. Long-term effects of porcine zonae pellucidae immunocontraception on ovarian function in feral horses (Equus caballus). J Reprod Fertil 1992; 94:437-444.
7. Kirkpatrick HF, Naugle R, Liu IKM, Bemoco M, Turner JW Jr. Effects of seven consecutive years of porcine zona pellucida contraception on ovarian function in feral mares. Biol Reprod Monograph 1985; 1:411-418.
8. Kirkpatrick JF, Colle PP, Kalk P, Liu IKM, Bernoco M, Turner JW, Jr. Immunocontraception of captive exotic species. II. Sika deer (Cervus niuuon). Axis deer (Axis axis) Himalayan tahr (Hemitraeus jemlahicus), Roosevelt elk (Cervus ellaphus roosevelt) muntjac deer (Muntiacus reeves), and sambar deer (Cervus unicolor). J Zoo Wildlife Med 1996; 27:482-495.
9. Kirkpatrick JF, Turner JW, Jr., Liu IKM, Fayrer-Hosken RA. Applications of pig zona pellucida immunocontraception to wildlife fertility control. In: Prospects of Zona Pellucida Glycoproteins for Contraception. J Reprod Fertil 1996; 50 (Suppl 1): 183-198.
10. Leveille MC, Roberts KD, Chevalier S, Chapdelaine A, Bleau G. Uptake of an oviductal antigen by the hamster zona pellucida. Biol Reprod 1987; 36:227-38.
11. Liu IKM, Bemoco M, Feldman M. Contraception in mares heteroimmunized with pig zonae pellucidae. J Reprod Fertil 1989; 85: 19-29.
12. Liu IKM, Shivers CA. Antibodies to the zona pellucida in mares. J Reprod Fertil 1982; 32 (Suppl): 309-313.
13. Mahi-Brown CA, Huang TT, Jr., Yanagimachi R. Infertility in bitches induced by active immunization with porcine zonae pellucidae. J Expt Zoo1 1982; 222: 89-95.
14. Mahi-Brown CA, Yanagimachi R, Hoffman HC, Huang TT, Jr. Fertility control in the bitch by active immunization with porcine zonae pellucidae: use of different adjuvants and patterns of estradiol and progesterone levels in estrous cycles. Biol Reprod 1985; 32: 761-772.
15. Miller CC, Fayrer Hosken RA, Timmons TM, Lee VH, Caudle AB, Dunbar BS. Characterization of equine zona pellucida glycoproteins by polyacrylamide gel electrophoresis and immunological techniques. J Reprod Fertil 1992; 96: 815-25.
16. Moss CJ. Some reproductive parameters in a population of African elephants, Loxodonta

africana. Proc 2nd Int Conf Human Anim Reprod 1992; 284-292.

17. Poole JH. Logistical and ethical considerations in the management of elephant populations through fertility control. Proc 2nd Int Conf Human Anim Reprod 1992; 278-283.
18. Prins HHT, Jeugd HPVD. Herbivore population crashes and woodland structure in East Africa. J Ecol 1993; 81: 305-314.
19. Sacco AG. Antigenic cross-reactivity between human and pig zona pellucida. Biol Reprod 1977; 16: 164-173.
20. Sacco AG, Palm VS. Heteroimmunization with isolated pig zonae pellucidae. J Reprod Fertil 1977; 51: 165-168.
21. Sacco AG, Subramanian MG, Yurewicz EC. Active immunization of mice with porcine zonae pellucidae: immune response and effect on fertility . J Expt Zool 1981; 218: 405-418.
22. Sacco AG, Subramanian MG, Yurewicz EC, DeMayo FJ, Dukelow WR. Heteroimmunization of squirrel monkeys (Saimiri sciureus) with a purified porcine zona antigen (PPZA): immune response and biologic activity of antiserum. Fertil Steril 1983; 39: 350-358.
23. Skinner SM, Mills T, Kirchick KJ, Dunbar BS. Immunization with zona pellucida proteins results in abnormal ovarian follicular differentiation and inhibition of gonadotropin-induced steroid secretion. Endocrinology 1984; 115: 2418-2432.
24. Willis LP, Heusner GL, Warren RJ, Kessler D, Fayrer-Hosken RA. Equine immunocontraception using porcine zona pellucida: A new method for the remote delivery and characterization of the immune response. J Eq Vet Sci 1994; 14:364-370.
25. Wood DM, Dunbar BS. Direct detection of two cross-reactive antigens between porcine and rabbit zonae pellucidae by radioimmunoassay and immunoelectrophoresis. J Exp Zoo1 1981; 217: 423-433.

Chapter 5b

Immunocontraception of African elephants

A humane method to control elephant populations without behavioural side effects.

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Concerted efforts to nurture elephant populations have resulted in elephant overpopulations in several areas, which in turn has led to damaging levels of browsing. As an alternative to culling entire family groups in order to control this damage, we have developed an immunocontraceptive vaccine from pig zona pellucida which safely and successfully controls free-roaming African elephants.

Immunocontraceptive vaccines cause the immune system to produce antibodies that prevent fertilization, without the side effects of hormonal contraceptives. The vaccine antigens are the proteins of the zona pellucida, the clear protein coat surrounding mammalian eggs. The surface structures of the elephant zona pellucida are very similar to those of the pig zona pellucida (pZP)[1-3].

Female zoo elephants vaccinated with pZP and an adjuvant all developed antibodies that persisted for 12-14 months[1,2], at a level equivalent to those found in horses given immunocontraception[4,5]. Based on this, we planned field trials in Kruger National Park, in conjunction with the South African National Parks.

Initial trials using 41 adult female elephants tested the efficacy of pZP as an immunocontraceptive. Elephants were located from a helicopter, and females to be anaesthetized (by aerial darting) were identified as non-pregnant by the presence of a calf smaller than 1 metre high. We then used ultrasound scans to confirm that females were not pregnant, and all non-pregnant animals were bled to obtain pre-vaccination serum samples. Twenty-one elephants were given an initial vaccination of pZP with adjuvant; 20 controls received a placebo.

All treated elephants wee fitted with radio-collars. The control females were fitted with numbered collars and paired in a family unit with a vaccinated elephant. The vaccinated elephants were located 6 weeks later and received a first booster, followed by another 6 months later. Both boosters were administered remotely with drop-out darts from a helicopter.

Twelve months after the initial vaccination, the elephants were recaptured and scanned for pregnancy. Of those treated with pZP, 19 were recaptured (two were not found because their radio-collars failed). Nine of the 19 were pregnant, ten were not. One of the pregnant elephants was in the last trimester of gestation (22 months) and gave birth to a healthy calf, showing that the vaccination of a pregnant elephant with pZP has no effect on gestation, the fetus or parturition. Eighteen of the 20 control elephants were located, 16 of which were pregnant. Therefore, significantly (χ^2, P=0.005) fewer vaccinated elephants (44%, 8/18) were pregnant than the control females (89%, 16/18).

Subsequently, we vaccinated ten elephants using a revised schedule. Females received an initial vaccination, followed by identical boosters delivered from a helicopter two and four weeks later. All elephants were fitted with radio-collars; five had global positioning satellite (GPS) collars (Lotek, Newmarket, Canada) which recorded their location hourly.

Of the ten elephants, two (20%) were pregnant after 10 months. This was significantly (χ^2, P=0.001) lower than the conception rate of the control elephants (89%, 16/18) and initial immunocontraception rates (44%, 8/18).

Female elephants with oestradiol implants have shown aberrant behaviour by separating off within the family unit (D.G., personal observation). GPS-collar location data indicated that there was no abnormal separation of the vaccinated females within a family unit over 8 months. This, combined with field observations of vaccinated females, suggests that the immunocontraceptive vaccine causes no behavioural abnormalities.

Finally, we tested the reversibility of immunocontraception, and its application for a second consecutive year. Of seven elephants from the group that had initially received immunocontraception, four were vaccinated with pZP and adjuvant, and three were not. Twelve months later, the seven elephants were captured and re-evaluated. Ultrasound scans showed that all three untreated females had conceived again, compared with none of the vaccinated elephants, although all were cycling. This indicates that the vaccine is reversible, and that it has no deleterious effect on the ovary and its cyclicity.

Elephants are intelligent and empathetic mammals, and culling is a last resort in controlling their numbers. Our immunocontraceptive study shows that free-roaming African elephants vaccinated with pZP are protected against conception. This pZP immunocontraception is safe and reversible and is thus a practical tool for controlling elephant populations

References

1. Fayrer-Hosken, RA, *et al Theriogenology* 47, 397 (1997)
2. Fayrer-Hosken, RA, *et al Theriogenology* 52, 835-846 (1999)
3. Fayrer-Hosken, RA, Bertschinger, HJ, Kirkpatrick, JF, Turner, JW & Liu, IKM. *Bulletin* 25, 18-21 (1997)
4. Willis, LP, Heusner, GL, Warren RJ, Kessler, D and Fayrer-Hosken, RA. *J.Eq.Vet.Sci*. 14: 364-370 (1994)
5. Kirkpatrick, JF, Turner, JW Jr, Liu, IK, Fayrer-Hosken, R & Rutberg, AT. *Reprod.Fertil.Dev*. 9, 105-110 (1997)

Chapter 6

Immunocontrol of reproductive rate of African elephant cows using porcine zona pellucida vaccine on seven private game reserves in South Africa

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Abstract

In southern Africa there is a need for elephant population control, especially in small to medium-sized, fenced reserves. The objectives of this study were to investigate the effects of porcine zona pellucida-immunocontraception on the reproductive rate as well as the safety during pregnancy of elephant cows in seven private game reserves in South Africa. A total of 108 individually identified cows were treated and monitored for 4 to 9 years, depending on when treatment commenced in each reserve. Primary vaccinations consisted of 400 or 600 μg porcine zona proteins with 0.5 ml Freund's modified complete adjuvant and boosters of 400 or 200 μg zona proteins with 0.5 ml Freund's incomplete adjuvant. Vaccine was delivered remotely: Year 1, primary plus two boosters 3-6 weeks apart; Year 2 onwards, annual boosters. Birth of calves was monitored continually and the result expressed as a percentage of cows treated on an annual basis. During Years 1 and 2, 38 (35.2%) and 24 (22.2%) calves were born, respectively. No more calves were born from Year 3 onwards. One cow conceived around the time of primary vaccination and a second between the primary vaccination and first booster. Two calves died soon after birth from unrelated causes. The remainder survived and were normal healthy calves. Sixty two cows (62.0%) have passed the 4-year and 24 (22.4%) the 6-year intercalving interval. The results show that it is possible to achieve a contraceptive efficacy of 100% in small to medium-sized free-ranging populations of African elephants.

Introduction

According to Kerley *et al.* (2008) the impact of African elephants (*Loxodonta Africana*) on ecosystems and biodiversity is difficult to assess. They improve conditions for other herbivores while negatively affecting a number of other animals. They decrease the diversity of plant species on the one hand while improving the landscape on the other. Be that as it may, the general consensus amongst reserve managers is that elephant populations, left uncontrolled in small to medium-sized reserves, will have a negative impact on habitat and thus biodiversity of the reserve concerned. Small reserves are defined as around 100 and medium-sized reserves around 500 km^2 (Mackey *et al.*, 2006). In South Africa many elephant populations were introduced into smaller fenced parks during the 1980s and 1990s. Previously maximum annual population growth rates were estimated at 4 to 7 % (Hanks and McIntosh, 1973; Calef, 1988) whereas recently they have been found to exceed 10 % (Mackey *et al.*, 2006). The rapid population increase known as irruptive growth (Mackey *et al.*, 2009) from density-independent population increase, may eventually lead to die-offs from starvation (Caughly, 1970).

The need to manage elephants, while controversial in the Kruger National Park (KNP), is well accepted in small to medium-sized fenced reserves (Mackey *et al.*, 2006). Traditionally culling has been regarded as the method of choice for controlling large populations (Slotow *et al.*, 2008). However, besides the opposition from many quarters, culling is hardly applicable to smaller populations. The practice is to cull entire breeding herds in order to avoid stress of family members left alive (Slotow *et al.*, 2008). In practice this probably seldom happens (Moss, 1992). In small populations this could mean removing all, half or a third of the breeding animals, depending on the size of the population. Despite being very costly, translocation is regarded as an ideal solution, however, in South Africa, habitat availability is limited (Delsink *et al.*, 2006).

Besides enlargement of parks the only other option to manage elephants is to decrease reproductive success by means of contraception. In selecting a contraceptive method for free-ranging mammals such as African elephants, the following requirements should be met. It must be efficient, reversible, safe, remotely deliverable, which largely determines the cost and have a minimal impact on the social behaviour of the target species (Kirkpatrick and Turner, 1991). Immunocontraception using porcine zona pellucida (pZP) vaccine satisfies all these requirements as has been shown in intensive studies in wild and domestic horses (Liu *et al*. 1989; Kirkpatrick and Turner, 2008) white tailed deer (Turner et al. 1992; McShea *et al.,* 1997; Rutberg and Naugle, 2008) and a number of other free-ranging and captive-held herbivores (Deigert *et al*. 2003; Frank *et al*. 2005; Kirkpatrick and Frank 2005; Kirkpatrick *et al.,* 2009). The putative mechanism for the success of pZP immunocontraception is the

production of antibodies that bind to ZP proteins of target animals' oocytes to prevent sperm binding (specifically to ZP3; Clarke and Dell, 2006), fertilisation and thus pregnancy. Fortunately zona proteins have been well conserved across mammal species and antibodies to pZP have been shown to recognise the African elephant ZP proteins (Fayrer-Hosken *et al.*, 1999).

Earlier immunocontraception trials on African elephants in the KNP showed that the porcine pZP vaccine is safe and effective as a contraceptive in African elephant cows and, in the short term, reversible (Fayrer-Hosken *et al.*, 1997; 1999; 2000). The final efficacy rate achieved was 80 % of vaccinated cows. This initial work was followed by an extensive study in the Greater Makalali Private Game Reserve (Makalali). The vaccine was shown to be 100% effective and, once all cows pregnant at inception of the program had calved, no more calves were born from the third year of the project (Delsink *et al.*, 2006; 2007).

This paper describes the effect of pZP vaccine on reproductive rate of free-ranging African elephant cows in one medium and six small reserves over periods of 4 to 6 years. Makalali is included in this study as previously reported data only covered the first 5 years after inception of the pZP contraception program (Delsink *et al.*, 2006; 2007). Another 5 cows were added to the program (2 in Year 2 and 3 in Year 3) that was not included in the previous papers. Also, dose rates of pZP antigen used during the first three years (2000-2002) were higher than used later on.

Materials and Methods

This is project, *Non-lethal control of African elephant (Loxodonta africana) Game reserves and respective elephant populations,* has been approved by the University of Pretoria's Animal Care and Use Committee, Project number: 36-5-251.

Game Reserves and elephants

The game reserves, their sizes, locations in South Africa and details of elephant populations are shown in Table 1.The elephants on each of the seven reserves were introduced by means of translocation and adult bulls were present on each reserve. Game Reserve, year of inception of the contraception program and number of cows of reproductive age (Laws, 1966; Lee *et al.,*1995) vaccinated during Year 1 were: Makalali, 2000, 18 cows (Deslsink *et al.,* 200); Mabula, 2002, 4 cows; Phinda, 2004, 19 cows; Shambala, 2004, 4 cows; Thornybush, 2004, 19 cows; Welgevonden, 2005, 35 cows and Kaingo, 2005, 4 cows. Additional cows were added during Years 2 (2 cows, Makalali) and 3 (3 cows Makalali), and in Years 4 (Mabula , 1 cow) and 5 (Makalali, 3 cows) were removed from the program so that they could be allowed to reverse. Either before or during the course of Year 1 each target animal was

individually identified (Delsink *et al.*, 2002). This allowed vaccination to take place on an individual cow basis. Prior to treatment, the populations typically had an inter-calving interval of 4.5-5 years and at inception of each program cows were at various unknown stages of reproduction. The Shambala population was captured and translocated to Entabeni Private Game Reserve at the beginning of Year 5 where no bulls of reproductive age were present.

Vaccine and vaccine delivery

The pZP antigen was produced by a modification of the methods described by Dunbar *et al.* (1980). The vaccine was manufactured at the Science and Conservation Centre, ZooMontana, Billings, Montana for the 2000/03 vaccinations. Thereafter, it was produced and supplied by the pZP Laboratory of the Department of Production Animal Studies, University of Pretoria. The During Year 1 each cow of reproductive age was given three pZP vaccinations: primary of 400 µg (600 µg at Makalali and Mabula) pZP in 1 ml phosphate buffered saline (PBS) with 0.5 ml Freund's complete modified adjuvant (Sigma Chemicals Co., St Louis, MO); two boosters of 200 µg (400 µg at Makalali and Mabula) pZP each in 1 ml PBS with 0.5 ml Freund's incomplete adjuvant (Sigma Chemicals Co., St Louis, MO). The intervals between vaccinations were 3-6 weeks. The four cows each in Shambala and Kaingo only received one booster during Year 1. This was followed by annual boosters with 200 µg (400 µg at Makalali and Mabula from 2000-2003; thereafter 200 µg) pZP in 1 ml PBS with 0.5 ml Freund's incomplete adjuvant. Shortly before use, the pZP antigen and adjuvant were mixed using two syringes joined by means of a connector. The fluid was pushed forwards and backwards between the syringes approximately 60 times creating a stable emulsion. Darts were then loaded with the emulsion. During the first three years at Makalali Dan-Inject® (DAN-INJECT ApS, Børkop, Denmark) darts with 60 mm needles were used (Delsink *et al.*, 2007). Thereafter and on the other reserves, Pneu-Dart® (Pneu-dart, Williamsport, USA) darts with 50 mm 13 gauge needles with gel collars were used. Elephants were either darted from the ground or a helicopter (Table 1). To facilitate the identification of cows within a group already darted during helicopter work, most cows were vaccinated with Pneu-Dart® mark and inject darts containing a pink dye (Wonder Mark®, Mafuta Products, Ventersdorp, RSA; Fig.1).

Monitoring of cows post vaccination

Cows on all game reserves were mostly seen one to three times a week but during wet periods spotting intervals were sometimes longer and as much as two weeks between sightings. Birth dates of new calves were taken as the date of first sighting. Mothers were identified with their calves from the close proximity and nursing of the claves (Delsink *et al*., 2002). Duration of gestation was taken as 22 months (Laws, 1966; Hodges *et al.,* 1994). Using this period, stage of gestation could be calculated in cows

pregnant at the time of inception of contraception or shortly thereafter. In order to simplify reporting, gestation was divided into trimesters as follows: 1st trimester, 0-8 months; 2nd trimester, 9-15 months and 3rd trimester, 16-22 months.

Data analysis

The total number of calves born per annum for Years 1 through to 6 were expressed as a percentage of the total number of cows treated each year. Expressing the annual reproductive rate as a calving percentage (calves born/annum/100 cows) was preferred to population growth rate because of the varying circumstances of each population. The χ^2 test was used to analyse annual differences in calving percentage. As the year of commencement of contraception differed between reserves (2000 to 2005) they were normalised so that the date of primary vaccination was the first day and 365 days later the last day of Year 1. The cows added to the trial during Years 2 and 3 at Makalali were also normalised to fit the data. Day 366 was then the start of Year 2 and so on. The staggered commencement also means that by Year 5 the total cow number decreased from 108 to 49 and in Year 6 to 23.

Results

Approximate calving data was available for five of the seven reserves prior to inception of contraception and varied from 16.7% to 25.0% in terms of annual calving percentage per cow of breeding age (Table 1). The mean calving percentages for Years 1 and 2 of the trial varied from 12.5% to 39.5% between reserves with an overall annual mean of 28.7% for 108 cows. This translates to 1.15 calves/cow per cycle of four years or an intercalving interval of less than 4 years and is more accurate than the estimates made prior to the start of contraception.

Following primary vaccination 38 calves were born during Year 1 and 24 during Year 2 providing calving percentages of 35.2% and 22.2%, respectively (Table 2). The difference between the years was significant ($\chi^2 = 4.81$; $p < 0.05$). No calves were born during years 3, 4, 5 and 6 ($p < 0.001$). With the exception of two, all calves (n=60) were conceived prior to the primary vaccination (Table 3). One calf was conceived around the time of primary vaccination and the other between the primary vaccination and the first booster. Of the 108 cows vaccinated during Year 1, 67 (62.0%) have passed the 4 year and 24 (22.4%) the 6 year intercalving interval.

Table 1: Elephant populations on the seven reserves where cows were treated with pZP vaccine

	Makalali	Mabula	Phinda	Shambala	Thornybush	Welgevonden	Kaingo
Size	24 500 ha	8 000 ha	22 800 ha	8 000 ha	11 548 ha	35 000 ha	8 461 ha
Population size (n) Year 1	47	11	92	10	35	117	9
Start of treatment	June 2000	May 2002	July 2004	July 2004	May 2005	Sept 2005	Oct 2005
Cows treated (n) Year 1	23[a]	4	19	4[b]	19	35	4[b]
Age range of cows (years) Year 1	12-50	13-16	10-35	19-25	6-31	9-44	10-40
Cows (n) calved before treatment	No data	3	18	No data	11	25	No data
Estimated mean calving% before treatment (number of years)[c]	21.7%[d]	25.0% (3)	21.0% (6)	No data	16.7% (6)	20.6% (6)	No data
Mean annual calving% during Years 1 and 2 of the study	32.6%	12.5%	39.5%	25.0%	15.8%	30.0%	25%

[a]18 cows were treated in 2000 (Delsink *et al.,* 2000); 2 added in 2001 and 3 in 2002
[b]Only vaccinated twice during Year 1 – primary vaccination was hand-injected
[c]Per number of cows judged to be of breeding age
[d]Adapted from Delsink *et al.* (2006)

Table 2: Number and percentage calves born to treated cows 1-6 years after the start of pZP vaccination

	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
Number of reserves	7	7	7	7	4	2
Cows treated	108	108	108	107[a]	49[b]	23
Calves born	38[c]	24[d]	0	0	0	0
Calving %	35.2%	22.2%	0%	0%	0%	0%

[a]One cow removed to allow reversal (Mabula)
[b]Another 3 cows removed to allow reversal (Makalali)
c and d significantly different ($\chi^2 = 4.81$; $p < 0.05$)

Table 3: Calves born after the primary vaccination showing the stage of gestation during primary vaccination and conception in relation to primary vaccination presuming a gestation period of 22 months (Hodges *et al.,* 1994).

Trimester of gestation at time of primary vaccination Number of calves			Conception in relation to primary vaccination Number of calves		
First trimester	Second trimester	Third trimester	Before	Around the primary	Between primary and 1st booster
22	19	21	60	1	1

From the calving dates it was apparent that 62 cows were pregnant at various stages of pregnancy. One calf died as a result of a physical injury soon after birth and another as a result of haemorrhage from the umbilicus at birth. The remaining calves were healthy and survived. Table 3 indicates the stage of pregnancy when the calves as foetuses were exposed to the primary vaccination. About one third (n=22) were in the first trimester and were thus exposed to possible effects of the vaccine as early as the embryonic stage and then at intervals for the remainder of pregnancy.

Discussion

The mean calving percentages of 28.7% during Years 1 and 2 of the trial was higher than those recorded in prior to inception of contraception in 5 of the 7 reserves. There are two possible reasons for these differences. Firstly, contrary to post-inception, birth dates of calves were not available in most reserves during the previous years and ages of calves were estimated according to shoulder height (Laws, 1966; Jachmann, 1988; Lee and Moss, 1995). Secondly, a number of cows in the trial only reached reproductive age around Year 1 of the trial and some were even younger. Although we compensated cow numbers to correct for this, figures quoted should only be regarded as estimates. The mean calving percentage for Years 1 and 2, on the other hand, are in agreement with recently published data for introduced populations (Mackey *et al.*, 2006) which quotes population growth rates of up to and even exceeding 10%. Our data for Years 1, 2 and the mean for the two years shows population growth rates of 12.7%, 7.1% and 9.4%, respectively. The fact that fewer calves were born during Year 2 than Year 1 is likely to be due to chance although the difference between the two years was statistically different.

As reported previously (Delsink *et al.,* 2006) no more calves were born from the third year onwards in this study. Although the number of cows decreased from after Year 4 as a result of the staggered dates of inception, no births were recorded by the end of Year 6. Expressed differently, 62% (n=67) of treated cows have passed the 4-year intercalving interval and 22.4% (n=24) the 6-year intercalving interval by the end of Year 6. For Makalali (excluding the cows taken off contraception) there were still no calves born to treated cows after 7 and 8 years.

The question that surely must be asked is, from when onwards in terms of the initial vaccinations are elephant cows infertile. Our data reflect that one cow conceived around the time of the primary vaccination when the antibody titre was either baseline or just starting to increase. A second cow conceived between the primary vaccination and first booster indicating that at least one booster is necessary to provide sufficient antibodies to block sperm-zona binding and thus a pregnancy from taking place. All remaining 60 cows that calved after inception of the program

conceived prior to the primary vaccination. Although, elephants in the reserves that were treated with the lower dose of pZP (400 µg, primary and 200 µg for boosters vs 600 µg, primary and 400 µg for boosters) have not been running as long as those in Makalali and Mabula, the protocol gave equivalent results from Year 3 onwards. Based on this we have routinely used the lower dosage regimen since the beginning of 2004. The doses required to achieve immunocontraception with pZP in the elephant are considerably smaller than is required for horses if one adjusts for body mass. Similarly the dose of GnRH used to immuno-regulate testosterone secretion in the pig (400 µg) is relatively much larger than is used for the same purpose in African elephant bulls (600 µg; DeNys *et al.*, 2010).

Curiously, the 95% efficacy of pZP immunocontraception achieved over a period of 17 years in wild horses (Kirkpatrick and Turner, 2008) was lower the 100% achieved in African elephant cows. The collective efficacy of pZP immunocontraception in 24 ungulate species 25 bears and 11 sea lions was 93.3% and ranged from 60% (nyala; *Taurotragus angasi*) to 100% in 16 other species such as Bison, Mountain goats, Wapiti, Fallow deer and moose (Frank *et al.,* 2005). Efficacies within the ungulate species varied from 60 to 83% in 6 species and 91.6-100% in the remaining 18 species. All animals reported in the above paper were held and treated in zoos. The one major advantage that possibly contributes to the success rate in elephants is the long interval of approximately 4 years between calves. This means that, with a gestation period of 22 months, the elephant cow takes approximately two years to conceive again. The precise physiology of the latter period is unknown but thought to be similar to lactation anoestrus seen in some domestic species like the sheep and the pig (Bertschinger *et al.*, 2008). Horses on the other hand can conceive within two weeks of giving birth during the so-called foal heat. Furthermore, at any one time, one can expect approximately 50% of cows to be pregnant (Bertschinger *et al.*, 2008). Thus in the elephant there is ample time during the presumed anoestrus and pregnancy periods to achieve good pZP antibody titres capable of preventing fertilisation and pregnancy later on. The very first two pZP-immunocontraception field trials in elephants recorded contraceptive success rates of only 56% and 80%, respectively (Fayrer-Hosken *et al.*, 2000). In both trials 400 µg and 200 µg pZP was used for the primary and booster vaccinations, respectively, but instead of Freund's adjuvants synthetic trehalose dicorynomycolatei (5 mg per vaccinations) was used as adjuvant. During the first trial (n=18; efficacy 56%) the boosters were administered 6 weeks and 6 months after the primary vaccination. In the second trial (n=10; efficacy 80%) two booster were administered at 2-weekly intervals. Another difference which may have been important is the selection of the target elephants. The only selection criterion in our trial was age and all cows estimated to be of reproductive age were vaccinated irrespective of age of calf at foot or being possibly pregnant. The selection of the cows in the Kruger National Park (Fayrer-Hosken *et al.*, 2000) was initially

based on the fact that they had a small calf (< 2 years old) at foot and later, after immobilisation and transrectal ultrasound examination, diagnosed as non-pregnant. The cows selected for treatment may have been in anoestrus or already have resumed ovarian cyclicity. Vaccination of cows that were about to or had already resumed ovarian cyclicity may have been too late to prevent a pregnancy. pZP antibody titres of these cows were never determined meaning that the precise reasons for differences between efficacies of the earlier trials and ours cannot be elucidated.

Just like as in the previous study in elephants (Delsink *et al.*, 2006), we clearly demonstrated the safety of pZP-immunocontraception during pregnancy. The loss of two out of 62 calves was accidental and unrelated to the use of the vaccine. Irrespective of the stage of pregnancy during vaccination, the 60 other calves were born healthy and viable and have survived until today. This means that no developmental abnormalities during pregnancy could be attributed to the use of the vaccine in elephants.

Conclusions

Immunocontraception using the pZP vaccine is highly effective as a method of birth control in African elephants. Calving in treated animals ceases two years after inception of the program. It is 100% safe for conceptuses at any stage of development. The delivery of the vaccine is remote and at no stage requires target animals to be caught or immobilized. The largest population treated so far is Welgevonden with 117 elephants of which 35 are cows of reproductive age. Despite the mountainous terrain of the reserve, a 100% efficacy was achieved meaning that the treatment of larger populations is feasible. According to population modelling of Mackey *et al.,* (2009) contraception of 75% of breeding-age females taking an annual mortality rate of 2-3% is sufficient to achieve an annual population growth of 0%. pZP-immunocontraception presents a proactive means of population control in elephants whereas culling is reactive, and once implemented, must continue indefinitely if it is to succeed. Reproductive rate in African elephants is density dependent (Laws, 1969; Laws *et al.,* 1975) and the response to culling will be an increase in this rate. To improve the practicality of immunocontraception and make the treatment of large populations possible, a slow or sequential release formulation is needed. This will mean only a single vaccination during the first year and maybe greater intervals later on.

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References

Bertschinger, Henk, Delsink, Audrey, van Altena, J.J., Kirkpatrick, Jay, Killian, Hanno, Ganswindt, André, Slotow, Rob, Castley, Guy. 2008. Chapter 6: Reproductive control of elephants. In: Elephant Management: A Scientific Assessment for South Africa. Eds RJ Scholes and KG Mennel: 257-328.

Calef, G.W. 1988. Maximum rate of increase in the African elephant. *African Journal of Ecology* 26: 323-327.

Caughly, G. 1970. Eruption of herbivore populations, with emphasis on Himalayan Tar in New Zealand. *Ecology* 51: 53-72.

Clarke, G.F., Dell, A. 2006. Molecular models for murine sperm-egg binding. *Journal of Biological Chemistry* 281: 13853-13856.

Deigert F.A., Duncan, A, Lyda, R.O., K. Frank, K., Kirkpatrick, J.F. 2003. Omminocontraception of captive exotic species. III. Fallow Deer (Cervus dama). *Zoo Biology* 22:261-268.

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

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

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

Denys, H.M., Bertschinger, H.J., Turkstra, J.A., Colenbrander, B., Palme, R., Human, A.M. 2010. Vaccination against GnRH may suppress aggressive behaviour and musth in African elephant (*Loxodonta africana*) bulls – a pilot study**.** *Journal of the South African Veterinary Association* 81, 8-15.

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

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

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

Frank KM, RO Lyda, JF Kirkpatrick. 2005. Immunocontgraception of captive exotic species. IV. Species differences in response to the porcine ziona pellucida vaccine and the timing of booster inoculations. Zoo Biol. 24:349-358.

Hanks, J., J.E.A. McIntosh. 1973. Population dynamics of the African elephant (*Loxodonta Africana*). *Journal of Zoology* 169: 29-38.

Jachmann H. 1988. Estimating age in African elephants: A revision of Law's molar evaluation technique. *African Journal of Ecology* 26, 51-56.

Kerley, G.I.H., P. Landman, L. Kruger, N. Owen-Smith. (2008). Chapter 3. Effects of elephants on ecosystems and biodiversity. In: Elephant management. A scientific assessment for South Africa. Eds RJ Scholes and KG Mennell, Wits University Press, 1Jan Smuts Avenue, Johannesburg: 146-205.

Kirkpatrick, JF, and J. W. Turner. 1991. Reversible fertility control in non-domestic animals. J. Zoo Wildl. Med. 22: 392-408.

Kirkpatrick JF, KM Frank. 2005. Fertility control in free-ranging wildlife. *In*: wildlife Contraception: Issues, Methods and Applications. Asa C, and Porton I (eds). Johns Hopkins University Press, Baltimore, MD. Pp. 17-25.

Kirkpatrick JF, A Turner. 2008. Achieving population goals in long-lived wildlife with contraception. *Wildlife Research* 35: 513-519.

Kirkpatrick JF, A Rowan, N Lamberski, R Wallace, K Frank, R Lyda. 2009. The practical side of immunocontraception: zona proteins and wildlife. *Journal of Reproductive Immunology* 83: 151-157.

Laws R**.** 1966. Age criteria for the African elephant *Loxodonta a. africana*. *East African Wildlife Journal.* 4, 1-37.

Laws RM. 1969. Aspects of reproduction in African elephants, *Loxodonta Africana*. *Journal of Reproduction and Fertility* Supplement 6: 193-217.

Laws RM, ISC Parker, RC.B Johnstone. 1975. Elephants and their habitats. Claredon Press, Oxford.

Lee P, Moss C**.** 1995. Statural growth in the African elephant (*Loxodonta africana*). *Journal of Zoology London*, 236, 29-41.

Liu IKM, M Bernoco, M Feldman. 1989. Contraception in mares heteroimmunized with pig zonae pellucidae. J. Reprod. Fert. 85:19-29.

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

Mackey, R.L., Page, B.R., Grobler, D., Slotow, R. 2009. Modelling the effectiveness of contraception for controlling introduced populations of elephants in South Africa. *African Journal of Ecology* 47:747-755.

McShea WJ, SL Monfort, S. Hakim, JF Kirkpatrick, IKM Liu, JW Turner, L. Chassy, L. Munson. 1997. Immunocontracetove efficiency and the impact of contraception on the reproductive behavior of white-tailed deer. J. Wildl.Manage. 61:560-569.

Moss CJ. 1992. Some reproductive parameters in a population of African elephants, *Loxodonta Africana. Proceedingd of the 2nd Conference on Human and Animal Reproduction.* 1992:284-292.

Rutberg AT, R Naugle. 2008. Population-level effects of immunocontraception in white-tailed deer (Odocoileus virginianus). Wildl. Res. 35:494-501.

Slotow, R., I. Whyte, M. Hofmeyr. 2008. Lethal management of elephants. In: Elephant management. A scientific assessment for South Africa. Eds RJ Scholes and KG Mennell, Wits University Press, 1Jan Smuts Avenue, Johannesburg: 370-405.

Turner JW, IKM Liu, JW Turner. 1992. Remotely-delivered immunocontraception of captive white-tailed deer. J. Wildl. Manage. 56:154-157.

Chapter 7

Vaccination against GnRH may suppress aggressive behaviour and musth in African elephant (*Loxodonta africana*) bulls – a pilot study

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Abstract

Aggressive behaviour and musth are constant problems in captive and sometimes in free-ranging African elephant bulls. Aggressive bulls are difficult and musth bulls almost impossible to manage without severely restricting their movement either by leg-chaining or using tranquillizers. This study investigated the relationship between faecal androgen metabolites (FAM) and faecal cortisol metabolites (FCM) concentrations and aggressive behaviour and tested a GnRH vaccine as a means of down-regulating aggressive behaviour and musth in 1 free-ranging and 5 captive elephant bulls. The bulls were non-aggressive (n = 3), aggressive (n = 2) or in musth (n = 1) at the onset of the study. The bulls were injected with a GnRH vaccine-adjuvant combination 3 or 4 times at 3- to 7-week intervals. Behaviour, FAM and FCM concentrations were measured during every week prior to vaccination until 4 months after the last vaccination. FAM concentrations were positively correlated with aggressive behaviour before the 1st vaccination. Androgen production, as reflected by FAM concentrations, was down-regulated in 3 out of the 6 immunised bulls. At least 2 bulls and possibly a 3rd showed behavioural improvement following GnRH vaccination and in all 3 temporal gland secretion ceased. No further aggressive behaviour was observed until the end of the study in any of the bulls. The results of this 1st GnRH immunization study suggest that it could be a useful method to control aggressive behaviour and musth in African elephant bulls.

Key words: aggressive behaviour, cortisol, down-regulation faeces, cortisol, GnRH vaccine, musth, testosterone.

Introduction

Musth, a condition exhibited periodically by adult male elephants[43], is associated with increased aggressive behaviour[11,41,43] and creates serious problems for the management of captive and free-ranging elephant bulls. Such bulls may even endanger the lives of both animals and humans. The characteristics of musth in Asian (*Elephas maximus*)[11,21,22] and African (*Loxodonta africana*)[20,41] elephant bulls are remarkably similar[43]. Apart from increased aggressive behaviour, the main signs of musth are heavy and continuous temporal gland secretion (TGS) and continuous urine dribbling[20,22,41]. Musth has been shown to be associated with increased androgen levels in blood[8,15,16,18,30,43,44], urine[5,42] and faeces[17,18]. Faecal androgen metabolites (FAM) and faecal cortisol metabolites (FCM), measured with an epiandrosterone and an 11-oxoaetiolcholanolone enzyme immunoassay (EIA) have been validated as a tool for non-invasive monitoring of endogenous secretion rates of testosterone and cortisol, respectively, in African elephant bulls[15,16,18].

Young free-ranging bulls often become problematic in smaller game reserves where there is a lack of natural hierarchical social structure and no or too few adult bulls to control them[47]. They generally enter musth at an earlier age and for longer periods than normal. Often the removal of the bull constitutes the only solution. The early appearance of long-lasting musth episodes also occurs in captive or domesticated bulls due to the decreased intensity of dominant relationships[43,45], good nutrition and a reduction in environmental stressors[8,43,44]. Bulls become less responsive to commands and difficult to control[12,22,25]. Generally they have to be restrained to such an extent that it becomes an animal welfare issue[25,30,43,50]. Often food and water supply are reduced[30,50] and tranquillizers may be employed to allow basic management procedures to continue[50]. In some instances, bulls have to be removed from working programs or even euthanased.

Consequently, there is an urgent need to develop methods to control musth and aggressive behaviour that could improve the well-being of the bulls and the safety of people and other animals. Reducing testosterone secretion could be a way to control musth. Surgical castration has been used[13,14,38] but is impractical, expensive and irreversible. The use of anti-androgens[36], GnRH agonists[6,10] and GnRH antagonists[6] has been investigated but with limited or no success. The use of gonadotrophin releasing hormone (GnRH) vaccines to down-regulate the hypothalamic-pituitary-gonadal axis could be a useful way to control musth and aggressive behaviour. Immunization with GnRH has been used successfully in many domestic as well as some non-domestic species[9,23,34,51] to control reproduction and androgen associated behaviours. It is reversible[23,28,29,34] and no adverse side effects have been recorded[23,26,27]. The aims of this study were to investigate a possible relationship between concentrations of faecal androgen and glucocorticoid metabolites and

aggressive behaviour and to test a GnRH vaccine as a means of controlling aggressive behaviour and musth in African elephant bulls.

Materials and Methods

Elephant bulls

Six elephant bulls were used in the study and individually named (see also Table 1).

Kinkel: intractable and hands-off management. Aggressive towards the dominant cow and had pushed her into the mote surrounding the elephant enclosure on a few occasions.

Thembo: wild and free-ranging on Tshukudu Game Reserve (Limpopo Province) but accustomed to the presence of people. Episodes of aggression towards people and other animals had been noted. The bull was captured on Day 66 (day of the 2nd booster) of the study because he was damaging fences and lodges adjacent to the reserve. He was translocated to Elephants for Africa for Ever, which trains elephants, at Moketsi in the Limpopo Province.

Toto, Chaka and Makuvhuzi: Imire Game Park (Zimbabwe): all 3 trained bulls were non-aggressive at the onset of the trial but had shown periods of aggression previously.

Grootvoet: ± 40 years old; wild and free-ranging on Shambala Private Game Reserve (Limpopo Province). His musth cycle had started 3 months earlier but due to TGS and urine dribbling was judged to be in full musth at the time of the 1st vaccination. He was considered to be the only sexually mature bull as he was older than 35 years of age[3].

Table 1: Vaccination protocols for the 5 captive and 1 free-ranging (Grootvoet) elephants.

	Bull (age in years) Location					
	Kinkel (22) Johannesburg Zoo	**Thembo (18)** Tshukudu Reserve	**Toto (18)** Imire Game Park	**Chaka (27)** Imire Game Park	**Makavhuzi (28)** Imire Game Park	**Grootvoet (40)** Shambala Reserve
Management status during study	Captive, intractable, hands-off	Free-ranging, relocated into captivity Day 66	Captive, trained and tractable	Captive, trained and tractable	Captive, trained and tractable	Free-ranging (wild), on game reserve, cows present
Behaviour during Stage 1	Aggressive	Aggressive	Non-aggressive	Non-aggressive	Non-aggressive	Aggressive, full musth
VACCINATIONS						
Primary						
Day	0	0	0	0	0	0
Method	Darted	Darted	Hand injection	Hand injection	Hand injection	Darted
Adjuvant	ISA 51	CoVaccine	CoVaccine	CoVaccine	CoVaccine	CoVaccine
1st Booster						
Day	21	21	49	49	49	21
Method	Darted	Darted	Hand injection	Hand injection	Hand injection	Darted
Adjuvant	ISA 51	CoVaccine	CoVaccine	CoVaccine	CoVaccine	CoVaccine
2nd Booster						
Day	43	66	196	70	70	50
Method	Darted	Hand injection[1]	Hand injection	Hand injection	Hand injection	Darted
Adjuvant	ISA 51	CoVaccine	CoVaccine	CoVaccine	CoVaccine	CoVaccine
3 rd Booster						
Day	64	-	-	-	-	-
Method	Darted	-	-	-	-	-
Adjuvant	CoVaccine	-	-	-	-	-

[1]While immobilised for relocation into captivity to Moketsi, tractable after three weeks

GnRH vaccine and adjuvants

The GnRH vaccine used in this study was previously described by Oonk *et al.*[39] and was provided by Pepscan Systems (Lelystad, The Netherlands). It is a modified GnRH-tandem-dimer-ovalbumin conjugate in which the GnRH molecules are modified by substituting L-glycine in the 6-position with D-lysine to enable conjugation to ovalbumin. The vaccine was developed for the immunocastration of male piglets[39]. Two different adjuvants were used: Montanide® ISA 51 (Seppic, Paris, France), consisting of manide oleate in mineral oil, and Covaccine™ (Covaccine B.V., Utrecht, The Netherlands), which is a proprietary product. Preparation of the vaccine with the Montanide® ISA 51 adjuvant was as follows: 1.5 m*l* ISA 51 was added to 1.5 m*l* vaccine containing 2 mg peptide conjugate in PBS buffer. The mixture was emulsified using two syringes and a connector. In the case of the Covaccine 1.5 m*l* the adjuvant was simply added to 1.5 m*l* vaccine and shaken briefly.

Vaccination protocol

All 6 bulls were immunised with the GnRH vaccine (Table 1). The bulls were vaccinated 3 times at intervals of 3-7 weeks. Despite clear instructions, Toto's 2nd booster was only administered 147 days after the 1st booster instead of the 21 days used for his stable mates. As a result of incomplete dart delivery of the ISA 51-GnRH emulsion during the 1st 3 vaccinations Kinkel was given a 3rd booster, this time using the Covaccine adjuvant. The vaccine was administered deep intramuscular into the semimembranosus-semitendinosus muscle mass by hand (25 mm 18-guage needle) or by means of a dart (5 m*l* Dan-Inject® dart fitted with 60 mm barbless needle; Dan-Inject ApS, Børkop, Denmark).

Collection and storage of faecal and serum samples

Faecal samples were collected on 4 to 5 consecutive days prior to the primary vaccination (Stage 1); 2 weeks after each vaccination (Stages 2, 3, 4 and 5; Stage 5, Kinkel only) and 2 (Stage 6; Thembo only) and 4 months (Stage 7) after the last vaccination. Sampling during Stages 4 and 7 was not possible for the Imire elephants and Grootvoet, respectively. An additional sample was collected from Grootvoet 3 months before the 1st vaccination. Immediately after defecation a 50 g aliquot was taken from the centre of a faecal ball, transferred to a labelled plastic zip-lock bag and transported on ice until freezing (-20 °C) 30 min to 4 hours later. In the case of Kinkel (Johannesburg Zoo), however, samples could only be collected in the morning once the bull had left his night room.

Blood samples were collected from 2 bulls while immobilised (Thembo and Grootvoet) and from the 3 tractable Imire bulls (Makavhuzi, Chaka and Toto) when feasible (Table 2). As Kinkel was neither tractable nor immobilised during the trial no serum samples could be collected from him. Once separated, the serum was stored at-20 °C until analysed.

Faecal steroid analyses

The extraction procedure used for both steroid groups has been described[33, 35]. Briefly, 0.5 g of thawed wet faeces was extracted with 80 % aqueous methanol. Following centrifugation 1 m*l* of the supernatant was transferred into a new vial and 5 m*l* diethylether plus 0.2 m*l* 5% $NaHCO_3$ added. This mixture was vortexed for 10 s, centrifuged (3000 x *g* for 15 min) and then frozen for 30 min at -70 °C. The diethylether supernatant was transferred into a new vial and dried down under a flow of nitrogen at 45 °C. The dried extracts were redisolved in 0.5 m*l* assay buffer.

The epiandrosterone EIA[40] used in this study was validated for African elephants[15]. The study demonstrated that concentrations of measured FAM are a reliable indicator of circulating blood testosterone concentrations in African elephant bulls. The 11-oxoaetiocholanolone EIA (measuring cortisol metabolites with a 3α-hydroxy-11-oxo-structure) was validated for African elephants and the measured FCM were demonstrated to be a reliable indicator of blood cortisol concentrations[16]. This EIA has been described in detail[35].

Serum testosterone

Total serum testosterone concentrations were analysed using a direct radioimmunoassay (RIA) kit (Coat-a-Count® Total Testosterone, Diagnostic Products Corporation, Los Angeles, CA). This assay has been validated in domestic animals[1,46] and used for wildlife species such as cheetah and African wild dogs[2].

Collection of behavioural data

Frequency of aggressive behaviours was assessed daily during Stages 1, 2, 3, 4, 5and 7 (Kinkel); Stages 1, 4 and 7 (Thembo) and during Stages 1, 2 and 7 (Imire bulls) using behavioural traits relating to aggression, musth and dominance as previously described[19,43,45]. In total 38 different behaviours related to aggression were used. Some of the more important ones were: head high, chin tucked in, shaking head, ear-flapping, waving, forward trunk swing, pushing, kicking, charging/advance towards, grabbing, throwing or destroying objects, tusking, rumbling, and urine dribbling. The monitoring frequency of the Imire bulls and Thembo were lower because of distance

constraints. Grootvoet was free-ranging and mostly solitary. Therefore behaviours towards other elephants could not be monitored. Observations allowed us to classify bulls as being in musth or non-musth, compare frequencies of aggressive behaviour before and after immunization, determine the dominance hierarchy between bulls and detect other possible behavioural and physical changes. A male in full musth exhibits temporal gland secretion and urine dribbling, or evidence of recent urine discharge[43].

Data analysis

For each animal, the samples and observations were grouped in Stage 1 (before primary vaccination), Stage 2 (after primary vaccination), Stage 3 (after first booster), Stage 4 (after second booster), Stage 5 (after third booster), Stage 6 (2 months after last booster) and Stage 7 (4 months after last booster). FAM were analysed by repeated measures of ANOVA. For each bull, differences in concentrations between different stages were analysed by means of one-way ANOVA with post hoc comparison using the Tukey-Kramer multiple comparison test. Mean FAM values for aggressive and non-aggressive non-musth bulls during Stage 1 were grouped and compared using the two-sample t-test. The non-parametric Spearman rank order correlation test was used to analyze the correlation between FAM and FCM for each individual bull. The α-level of significance was set at <0.05 for all the tests.

Results

Vaccination

None of the bulls showed injection-site reactions or signs of lameness at any stage after vaccination. Reaction of bulls to darting was brief and varied from mild surprise to brief confusion which ceased within 10 min. Hand-injection of the three tractable bulls was well tolerated. As mentioned under 'vaccination protocol', Toto's 2nd booster was overlooked and only administered 147 days (21 weeks) after the 1st booster instead of the 3-7 weeks required by the protocol. Vaccination using the Dan-Inject® darting system was found to be a practical means of administering the vaccine with the Covaccine adjuvant. The Montanide ISA 51 adjuvant was unsuitable for dart delivery because the emulsion was too viscous, resulting in incomplete injection of the vaccine. After this vaccine-adjuvant combination was once again unsuccessful during the 2nd booster vaccination of Kinkel, ISA 51 was abandoned as adjuvant. Hand injection was suitable to vaccinate trained bulls.

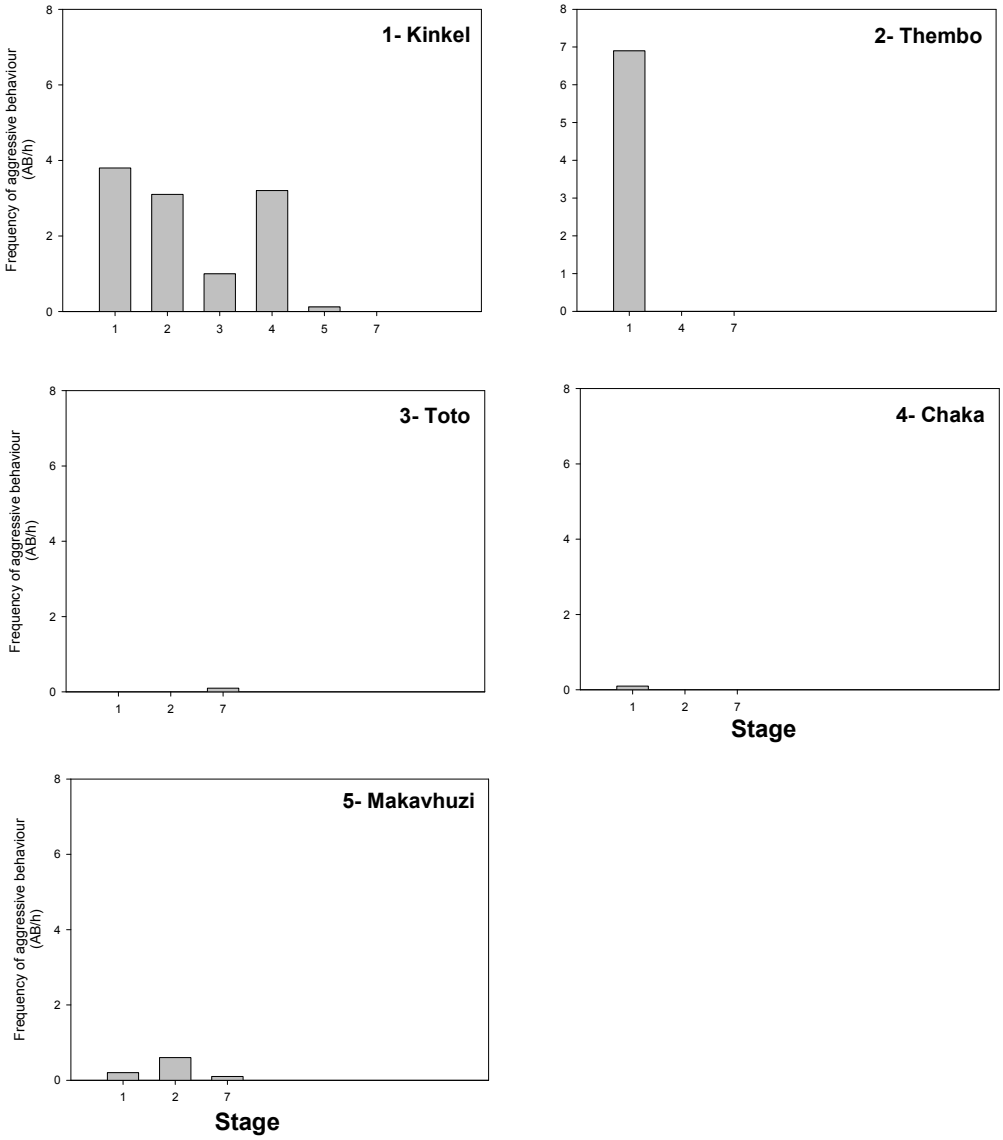

Fig. 1: Frequency of aggressive behaviour (number of AB per hour) as a function of the stage of immunization for Kinkel, Thembo, Toto, Chaka and Makavhuzi. (Stage 1 - before primary vaccination; Stage 2 - after primary vaccination; Stage 3 - after 1st booster; Stage 4 - after 2nd booster; Stage 5 - after 3rd booster; Stage 7 - 4 months after last vaccination).

Behaviour and GnRH vaccination

The frequencies of aggressive behaviour in relation to the stages of the study are shown in Fig. 1. The Grootvoet was the only bull in musth at the time and before the primary vaccination. He went out of musth 10 days after the 1st vaccination and his aggressive behaviour ceased completely. Kinkel and Thembo showed both aggressive behaviour and TGS without urine dribbling before the primary vaccination. In Kinkel, TGS ceased and aggressive behaviour was reduced after the 4th vaccination. The bull remained docile until the end of the observation
period. Thembo's aggressive behaviour and TGS ceased after the 2nd booster vaccination and no further aggressive behaviour or irritability was observed. Toto, Chaka and Makavhuzi did not show aggressive behaviour prior to vaccination. No changes in their behaviour were observed throughout the study. Chaka, however, came into full musth 10 months after the last vaccination, which was 6 months after the end of the trial.

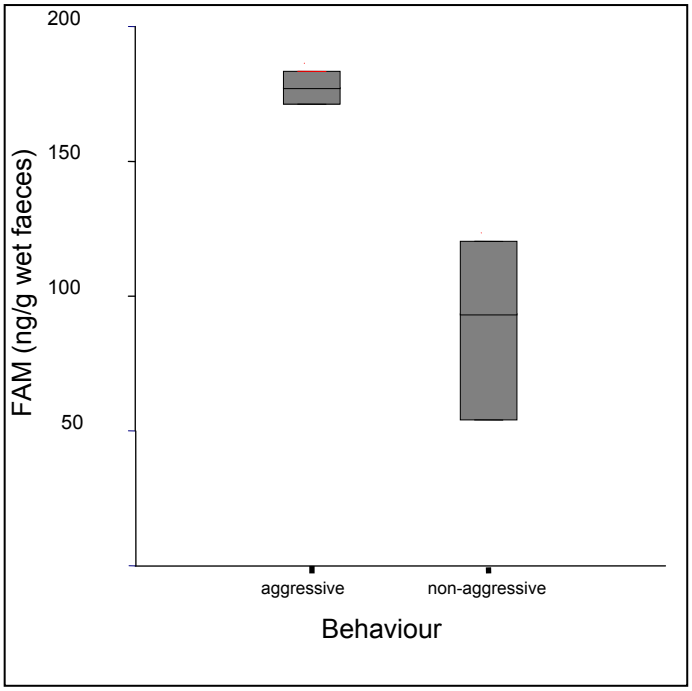

Fig.2: Grouped concentrations of faecal androgen metabolites (FAM) of aggressive (n = 2) and non-aggressive (n = 3) non-musth bulls during Stage 1 (before primary vaccination).

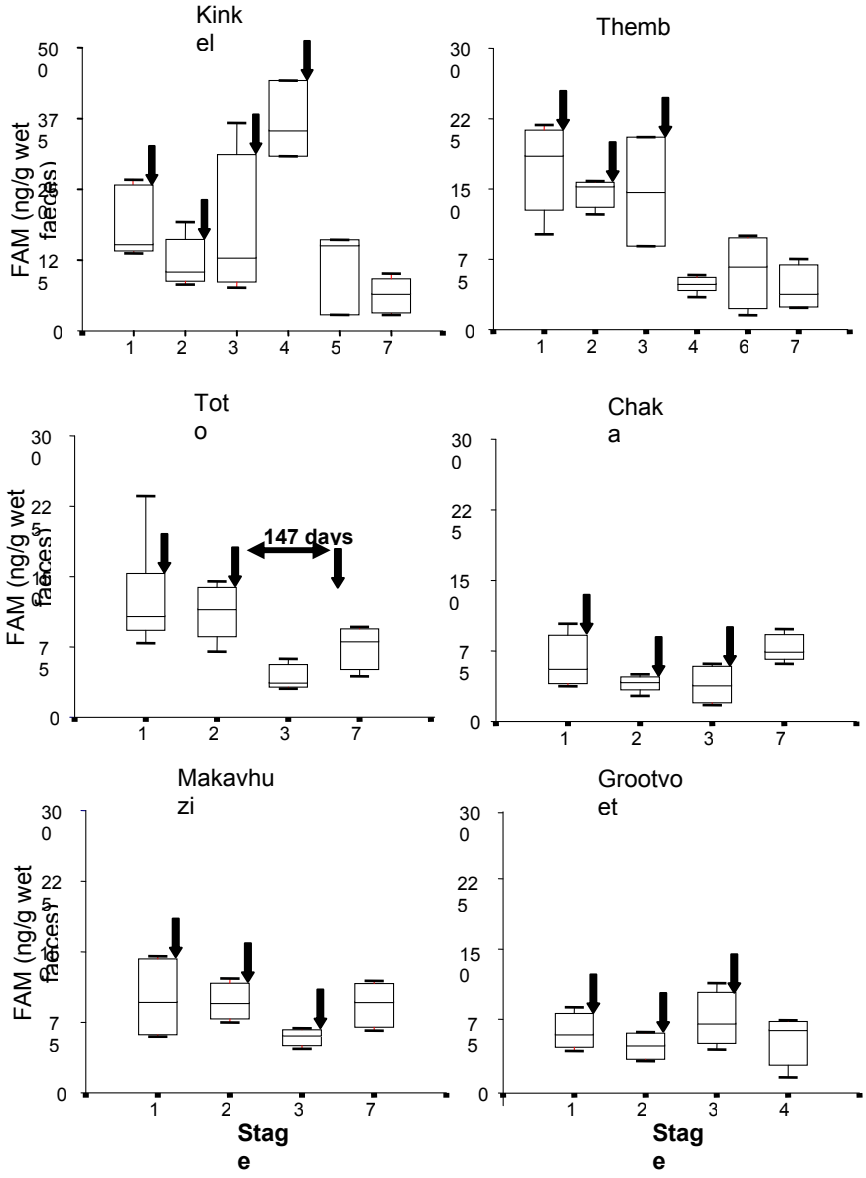

Fig. 3: Grouped concentrations of faecal androgen metabolites (FAM) for each of the 6 bulls. The boxes represent the median and the upper and lower quartile values of samples collected at different stages of vaccination. The whiskers show the range. Black arrows indicate vaccinations (Stage 1 - before primary vaccination; Stage 2 - after primary vaccination; Stage 3 - after 1st booster; Stage 4 - after 2nd booster; Stage 5 - after 3rd booster; Stage 6 – 2 months after the 2nd booster; Stage 7 - 4 months after last vaccination).

Faecal androgen metabolites (FAM)

The relationship between behaviour and FAM concentrations during Stage 1 is shown Fig. 2. The two aggressive bulls Kinkel and Thembo (179 ± 8 ng/g) had significantly ($P<0.05$) higher concentrations than the 3 non-aggressive bulls Makavhuzi, Chaka and Toto (97 ± 31 ng/g).

Fig. 3 shows the within bull effects of a GnRH vaccination on FAM concentrations. Significant differences between stages were observed in Kinkel ($P<0.05$), Thembo ($P<0.001$), Toto ($P<0.05$) and Chaka ($P<0.05$). Only Stages 1, 5 and 7 were taken into consideration for Kinkel due to the lack of complete administration when Montanide ISA 51 was used for the 1st 3 vaccinations. No significant differences were found in Makavhuzi and Grootvoet. The faecal sample collected from Grootvoet 3 months before the first vaccination (in full musth), however, had a FAM concentration (209 ng/g) approximately 3 times higher than the samples collected during Stages 1, 2, 3 and 4 (62 ± 24 ng/g).

Serum testosterone

The results of the serum testosterone are shown in Table 2. They were significantly correlated with FAM concentrations collected during the corresponding periods ($r = 0.83$; $P <0.005$).

Table 2: Serum testosterone concentrations of 5 elephant bulls.

	Bull **Serum testosterone concentration** (nmol/*l*)				
Time of sampling	**Thembo**	**Toto**	**Chaka**	**Makavhuzi**	**Grootvoet**
3 months before primary vaccine	-	-	-	-	152.4
On day of primary vaccination	-	2.7	0.7	2.2	-
12 days after primary vaccine	-	-	2.5	34.6	-
6 weeks after 1st booster	27.1	-	-	-	-
4 months after 2nd booster	-	-	0.4	2.5	-

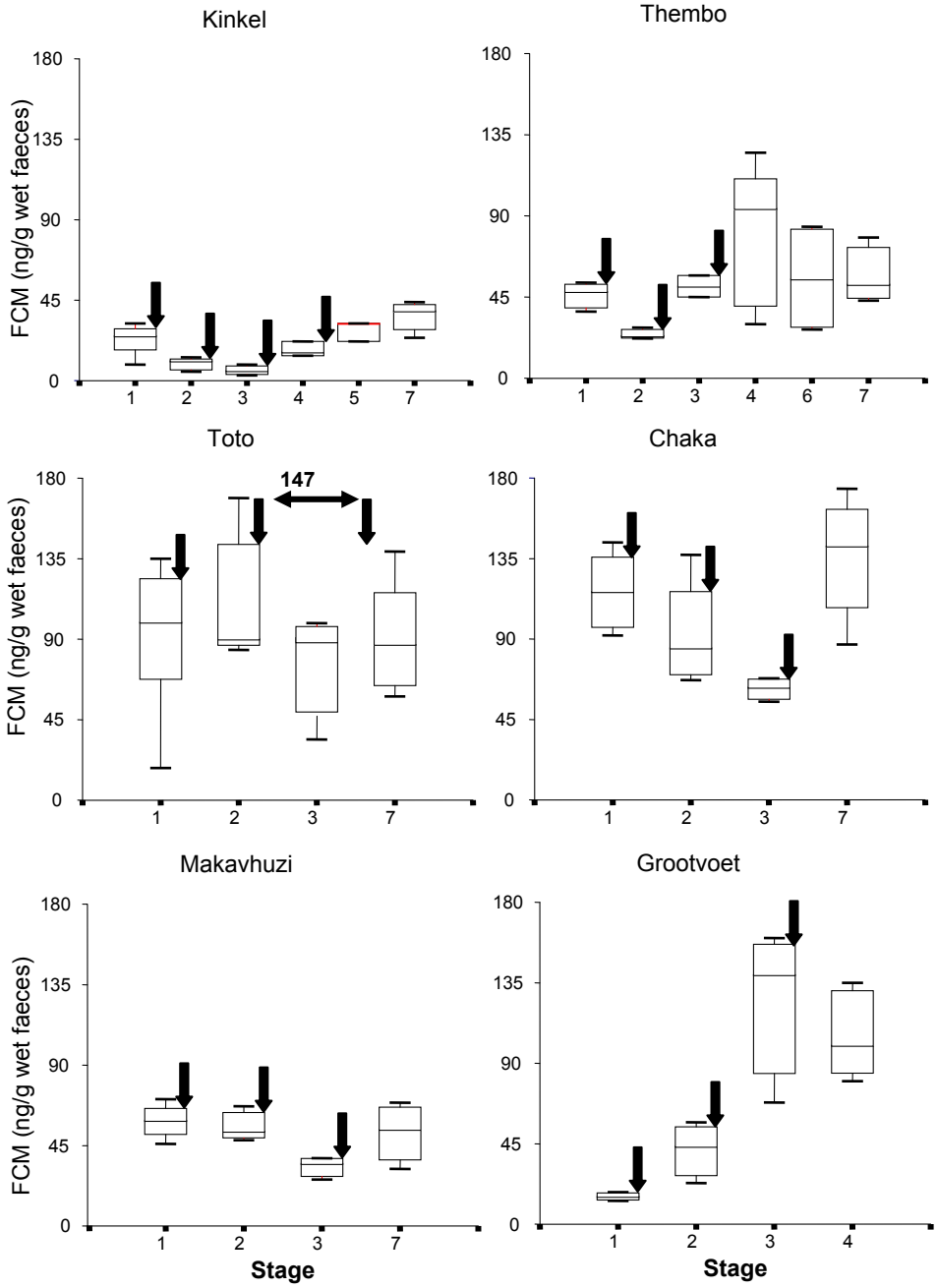

Fig. 4: Grouped concentrations of faecal cortisol metabolites (FCM) for each of the 6 bulls. For further details see legend of Fig. 3.

Faecal cortisol metabolites (FCM)

FCM concentrations in relation to the stages are shown in Fig 4. Again, only Stages 1, 5 and 7 were taken into consideration for Kinkel. Significant differences between stages were observed in Kinkel ($P = 0.05$), Thembo ($P = 0.05$), Chaka ($P < 0.01$), Makavhuzi ($P < 0.05$) and Grootvoet ($P < 0.001$). Wide variations were observed throughout in Toto and there were no significant differences between stages.

There were no significant correlations between FAM and FCM in Kinkel, Thembo, Toto and Grootvoet whereas positive correlations were found for Chaka ($r = 0.51$; $P < 0.05$) and Makavhuzi ($r = 0.48$; $P < 0.05$).

Discussion

This is the 1st study that reports the use of a GnRH vaccine to attempt down-regulation of musth or aggressive behaviour in African elephant bulls. Working with wildlife species like the African elephant often makes accessing a suitable sample size for research projects difficult, particularly when a new unproven drug is being tested. Another constraint is the accessibility of individual animals. It is not always possible to carry out treatments and sampling as required in a research protocol. The safety of vaccine-adjuvant administration was clearly demonstrated in the bulls of this trial.

When non-musth bulls were divided into aggressive (Thembo and Kinkel) and non-aggressive groups (Toto, Chaka and Makavhuzi) during Stage 1, behaviour was well correlated to FAM concentration. The mean FAM concentration of the non-aggressive bulls was significantly lower than the mean of the aggressive bulls. During this stage both aggressive bulls had permanent mucoid TGS without urine dribbling which is equivalent to the 1st and last stage of full musth (bulls entering or leaving full musth; Ganswindt, *et al.*[17,18]). Kinkel's behaviour only improved after the 3rd booster vaccination which was the 1st time vaccine delivery was complete. This was accompanied by cessation of TGS and a significant lowering of FAM concentrations during Stages 5 and 7. Thembo's behaviour, accompanied by a cessation of TGS, improved after the 1st booster. A significant decrease in FAM concentration was also seen. At the same time he was taken into captivity where he remained for the last 6 years. He is revaccinated (since 2006 with the commercially available GnRH vaccine Improvac, Pfizer Animal Health, Sandton, South Africa) every 6 to 8 months, is tractable and used for education purposes and elephant-back rides.

Of the 3 non-aggressive bulls, Toto also showed a significant decrease in FAM after the 1st booster (Stage 3). The other 2 bulls (Chaka and Makavhuzi) showed no significant changes, with concentrations having been low from the onset of the trial. They remained non-aggressive for the remainder of the observation period and for

another 6 months thereafter when Chaka came into musth. At the time, he and the 2 other bulls had not been revaccinated since the 2nd booster.

The results of Grootvoet are somewhat of an enigma. From a behavioural point of view, (presence of TGS and urine dribbling) he was in full musth during Stage 1. However, FAM concentrations during Stage 1 reflected those of non-musth, non-aggressive bulls. A faecal sample taken 3 months previously during full musth (same musth cycle) had a 3 times higher FAM concentration (209 ng/g), typically found in musth bulls. This was corroborated by a serum testosterone concentration of 152 nmol/*l* in a blood sample collected on the same day. Musth behaviours ceased 10 days after primary vaccination. In our opinion, given his lower FAM concentrations during Stage 1 and the fact that he had already been in full musth for at least 3 months, he was at the end of his musth cycle. The vaccine may or may not have assisted in ending musth. Certainly 10 days were sufficient to have allowed an initial rise in antibody titre.

It should also be mentioned that immobilisation of Thembo may have had a temporary influence on androgen secretion. At the end of Stage 3 when his FAM concentrations were still elevated and serum testosterone was above baseline concentrations (27 nmol/*l*) he was captured using etorphine and xylazine for relocation into captivity. Opioids and tranquillisers are known to temporarily decrease pulsatile release of GnRH[4,48]. Brown *et al.*[6] found reduced testosterone concentrations in a musth bull two days after capture but sensitivity to anaesthesia and the effects of capture seemed to be more specific in musth than non-musth bulls. On the other hand, repeated immobilisations of a captive Asian bull in musth did not suppress signs of musth, although androgens were not monitored in this bull[25]. Another factor that needs to be considered is the possible effect of stress in the case of Thembo. Chronic stress is known to decrease pulsatile release of GnRH and thus androgen secretion[24,31,37]. Prior to capture (Stages 1, 2 and 3) Thembo's faecal FCM concentrations were low (≈ 45 ng/g) with small variations. Following capture they were significantly higher during Stage 4 with a much larger variation (Fig. 4), but very similar to those of Toto and Chaka and Grootvoet following the 1st booster. Thembo was calm and tractable from the outset in captivity but the wider range during Stage 4 may reflect anxiety resulting from new experiences in captivity. The ranges narrowed progressively during Stages 6 and 7. A possible role of chronic stress can therefore not be excluded.

Previous attempts to control musth and aggressive behaviour, without resorting to castration, have yielded limited success and have only been tested on 1 or 2 bulls. Anti-androgens[36], GnRH super-agonists[6,10] or antagonists[6] were employed and the GnRH super-agonists produced more favourable results. Brown *et al.*[6] showed that the GnRH agonist Lupron Depot disrupts normal pituitary-gonadal function in free-ranging African elephant bulls. Initially testosterone was reduced to baseline concentrations. The testes, however, became hyper-responsive to gonadotrophic

stimulation. Similar results were obtained by one of the authors (HJB) with deslorelin implants in a donkey jack. The implants initially suppressed testosterone release but a rebound occurred after 10 days and testosterone concentrations actually increased in relation to pre-treatment concentrations. One study showed that administration of leuprolide acetate to a 52-year old Asian bull for several years decreased testosterone concentrations and musth could be prevented when the drug was administered during the 1st pre-musth manifestations[10]. Whether or not the GnRH super-agonists are reliable, the formulations used cannot be administered remotely by means of darting.

There appeared to no positive relationship between aggressive behaviour and faecal FCM concentrations. Kinkel, who showed a high frequency of aggressive behaviours during Stages 1-4 had consistently low concentrations of FCM. Thembo's concentrations were also low during Stage 1 when he exhibited aggressive behaviours. Mean concentrations and ranges increased after cessation of aggressive behaviour. In Grootvoet mean concentrations and ranges increased once musth ceased. Two of the non-aggressive bulls (Toto and Chaka) had high means and ranges of FCM concentrations almost throughout the study whereas FCM concentration in the 3rd non-aggressive bull was consistently lower throughout. It has reported that high androgen concentrations in African elephants seemed to suppress cortisol secretion[17,18]. The mechanism, however, could not be explained. A similar relationship between FAM and FCM concentrations could not be confirmed in our study.

Despite the limitations of this study, which include low sample size, some inconsistencies in the vaccination protocol and difficulties surrounding behavioural observations of two bulls, the results were encouraging. Most studies with GnRH vaccine in other species failed to produce an adequate response in 100 % of treated animals. It seems that a certain number of non-responders can be expected[23,32,49,51,52]. The reasons for individual variations are not completely understood. Age has clearly been shown to influence the results with older animals showing greater individual variations, less marked responses and shorter duration of effects[9,32,49,51,52]. In horses, 2 vaccinations with the modified GnRH-tandem-dimer vaccine-Covaccine adjuvant-combination were sufficient to suppress testosterone secretion in young sexually mature pony stallions but further boosters were generally needed in older stallions[49]. In blackbuck (*Antilope cervicapra*) and springbok (*Antidorcas marsupialis*), testosterone concentrations were reduced in young but not in adult rams[51]. The authors of a recent study[23] were able to suppress testosterone concentrations in 4 out of 5 stallions treated with a GnRH-protein conjugate (Equity™) for at least 6 months. The stallion that did not respond also showed the lowest antibody titres. The same stallion, however, showed a marked decrease in libido while 1 of the good responders with low testosterone concentrations demonstrated good libido. The behavioural response (libido suppression) was better than that reported by other authors[7,52]. These findings show that the control of sexual behaviour is complex and not only testosterone

dependent. It is also affected by factors such as age and previous sexual experience in the horse[49]. Rather than having non-responders in our trial the results in some of the bulls were inconclusive. Two aggressive non-musth young bulls responded whereas in the non-aggressive 3 young bulls a response was difficult to assess. One of these bulls did, however, show a reduction in androgen production. The sexually mature adult bull in the trial was inconclusive as he was probably going out of musth around the time of the primary vaccination.

Conclusions

The most significant finding of the current study was the effect of the GnRH vaccination on behaviour. Vaccination *per se* did not cause aggression or disturbance in any of the bulls and no other side effects were observed. At least 2 bulls and possibly a 3rd, showing aggressive behaviour prior to treatment, demonstrated substantial improvement. TGS also ceased. Aggressive behaviour did not recur within the 4-month observation period after the final booster. As expected, the non-aggressive bulls showed no changes in behaviour.

Acknowledgements

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References

1. Almond G W, Esbenshade K L, Smith C A, Richards G 1992 Effects of chronic gonadotrophin-releasing hormone agonist treatment on serum luteinizing hormone and testosterone concentrations in boars. American *Journal of Veterinary Research* 53: 22-25
2. Bertschinger H J, Trigg T E, Jöchle W, Human A 2002 Induction of contraception in some African wild carnivores by down-regulation of LH and FSH secretion using the GnRH analogue deslorelin. *Reproduction* Supplement 60: 41-52
3. Bertschinger Henk, Delsink Audrey, van Altena J J, Kirkpatrick Jay, Killian Hanno, Ganswindt Andre, Slotow Rob, Castley Guy 2008 Reproductive control of elephants. In Scholes R J, Mennel K G (eds) *Elephant Management: A Scientific Assessment for South Africa.* Wits University Press, Johannesburg: 257-328.
4. Blank M S, Fabbri A, Catt K J, Dufau M L 1986 Inhibition of luteinizing hormone release by morphine and endogenous opiates in cultured pituitary cells. *Endocrinology* 118: 2097-2101

5. Brannian J D, Griffin F, Terranova P F 1989 Urinary androstenedione and luteinizing hormone concentrations during musth in a mature African elephant. *Zoo Biology* 8: 165-170
6. Brown J L, Bush M, Wildt D E, Raath J R, de Vos V, Howard J G 1993 Effects of GnRH analogues on pituitary-testicular function in free-ranging African elephants (*Loxodonta africana*). *Journal of Reproduction and Fertility* 99: 627-634
7. Clement F, Vidament M, Daels P, Van der Meer F, Larry J L, Colenbrander B, Turkstra J 2005 Immunocastration in stallions: effect on spermatogenesis and behaviour. *Animal Reproduction Science* 89: 230-233
8. Cooper K A, Harder J D, Fredrick D L, Lodge G A, Peachey H C, Spellmire T J, Winstel D P, Clawson D H 1990 Serum testosterone and musth in captive male African and Asian elephants. *Zoo Biology* 9: 297-306
9. Curtis P D, Pooler R L, Richmond M E, Miller L A, Mattfeld G F, Quimby F W 2002 Comparative effects of GnRH and porcine zona pellucida (PZP) immunocontraceptive vaccines for controlling reproduction in white-tailed deer (*Odocoileus virginianus*). *Reproduction* Supplement 60: 131-141
10. de Oliveira C A, West G D, Houck R, Leblanc M 2004 Control of musth in an Asian elephant bull (*Elephas maximus*) using leuprolide acetate. *Zoo and Wildlife Medicine* 35: 70-76
11. Dickerman R D, Zachariah N Y, Fouraker M, McConathy W J 1997 Neuroendocrine-associated behavioral patterns in the male Asian elephant (*Elephas maximus*). *Physiology and Behavior* 61: 771-773
12. Eisenberg J F, McKay G M, Jainudeen M R 1971 Reproductive behavior of the Asiatic elephant (*Elephas maximus maximus*). *Behaviour* 38: 193-225
13. Foerner J J, Houck R I, Copeland J F, Schmidt M J, Byron H T, Olsen J H 1994 Surgical castration of the elephant (*Elephas maximus* and *Loxodonta africana*). *Journal of Zoo and Wildlife Medicine* 25: 355-359
14. Fowler M E, Hart R 1973 Castration of an Asian elephant, using etorphine anesthesia. *Journal of the American Veterinary Medical Association* 163: 539-543
15. Ganswindt A, Heistermann M, Borragan S, Hodges J K 2002 Assessment of testicular endocrine function in captive African elephants by measurement of urinary and faecal androgens. *Zoo Biology* 21: 27-36
16. Ganswindt A, Palme R, Heistermann M, Borragan S, Hodges J K 2003 Non-invasive assessment of adrenocortical function in the male African elephant (*Loxodonta africana*) and its relation to musth. *General and Comparative Endocrinology* 134: 156-166
17. Ganswindt A, Heistermann M, Hodges J K 2005a Physical, physiological and behavioural correlates of musth in captive African elephants (*Loxodonta africana*). *Physiological and Biochemical Zoology* 78: 505-514
18. Ganswindt A, Rasmussen H B, Heistermann M, Hodges J K 2005b The sexually active states of free-ranging male African elephants (*Loxodonta africana*): Defining musth and non-musth using endocrinology, physical signals, and behaviour. *Hormones and Behavior* 47 (1): 83-91
19. Garai M E 1997 The development of social behaviour in translocated juvenile African elephants Loxodonta africana (Blumenbach). PhD thesis, Universiteit van Pretoria
20. Hall-Martin A J, Van Der Walt L A 1984 Plasma testosterone levels in relation to musth in the male African elephant. *Koedoe* 27: 147-149
21. Jainudeen M R, Katongole C B, Short R V 1972a Plasma testosterone levels in relation to musth and sexual activity in the male Asiatic elephant, *Elephas maximus*. *Journal of Reproduction and Fertility* 29: 99-103
22. Jainudeen M R, Mc-Kay G M, Eisenberg J F 1972b Observations on musth in the domesticated Asiatic elephant (*Elephas maximus*). *Mammalia* 36: 247-261
23. Janett F, Stump R, Burger D, Thun R 2009 Suppression of testicular function and sexual behaviour by vaccination with GnRH (Equity^{Tm}) in the adult stallion. *Animal Reproduction Science* 115: 88-102

24. Katsiia G V, Todua T N, Gorlushkin V M, Chirkov, A M, Goncharov N P 1989 Effect of immobilization stress on the gonadotropic function of the hypophysis in male hamadryas baboons (*Papio hamadryas*). *Biulleten' Eksperimental'Noi Biologii i Meditsiny* 107: 231-234
25. Kock N, Kock M 1984 Management of two Indian elephants (*Elephas maximus indicus*) in a Middle Eastern zoo. *American Association of Zoo Veterinarians Annual Proceedings* 1984 75-81
26. Kumar N, Savage T, DeJesus W, Tsong Y Y, Didolkar A, Sundaram K 2000 Chronic toxicity and reversibility of antifertility effect of immunization against gonadotropin-releasing hormone in male rats and rabbits. *Toxicological Sciences* 53: 92-99
27. Ladd A 1993 Progress in the development of anti-LHRH vaccine. *American Journal of Reproductive Immunology* 29: 189-194
28. Ladd A, Tsong Y Y, Walfield A M, Thau R 1994 Development of an antifertility vaccine for pets based on active immunization against luteinizing hormone-releasing hormone. *Biology of Reproduction* 51: 1076-1083
29. Lincoln G A, Fraser H M, Fletcher T J 1982 Antler growth in male red deer (*Cervus elaphus*) after active immunization against LH-RH. *Journal of Reproduction and Fertility* 66: 703-708
30. Lincoln G A, Ratnasooriya W D 1996 Testosterone secretion, musth behaviour and social dominance in captive male Asian elephants living near the equator. *Journal of Reproduction and Fertility* 108: 107-113
31. López-Calderón A, Ariznavarreta C, González-Quijano M I, Tresguerres, J A, Calderón, M D 1991 Stress induced changes in testis function. *Journal of Steroid Biochemistry and Molecular Biology* 40: 473-479
32. Malmgren L, Andresen O, Dalin A M 2001 Effect of GnRH immunisation on hormonal levels, sexual behaviour, semen quality and testicular morphology in mature stallions. *Equine Veterinary Journal* 33: 75-83
33. Merl S, Scherzer S, Palme R, Möstl E 2000 Pain causes increased concentrations of glucocorticoid metabolites in horse feces. *Journal of Equine Veterinary Sciences* 20: 586-590
34. Miller L A, Johns B E, Killian G J 2000 Immunocontraception of white-tailed deer with GnRH vaccine. *American Journal of Reproductive Immunology* 44: 266-274
35. Möstl E, Maggs J L, Schrotter G, Besenfelder U, Palme R 2002 Measurement of cortisol metabolites in faeces of ruminants. *Veterinary Research Communications* 26: 127-139
36. Niemuller C A, Brown J L, Hodges J K 1998 Reproduction in elephants. In Knobil E, Neill J D (eds) *Encyclopedia of reproduction*. New York Academic Press, New York: 1018-1029
37. Norman RL 1993 Effects of corticotrophin-releasing hormone, testosterone and cortisol secretion in intact male rhesus macaques. *Biology of Reproduction* 40: 148-153
38. Olsen J H, Byron H T 1993 Castration of the elephant. In Fowler M E (eds) *Zoo and wild animal medicine: current therapy.* Saunders Company, Philadelphia: 441-444
39. Oonk H B, Turkstra J A, Schaaper W M, Erkens J H, Schuitemaker-de Weerd M H, van Nes A, Verheijden J H, Meloen R H 1998 New GnRH-like peptide construct to optimize efficient immunocastration of male pigs by immunoneutralization of GnRH. *Vaccine* 16:1074-1082
40. Palme R, Möstl E 1994 Biotin-streptavidin enzyme immunoassay for determination of oestrogens and androgens in boar faeces. In S. Görög (ed) *Advances of Steroid Analysis '93'* Akadémiai Kiadó, Budapest: 111-117
41. Poole J H, Moss C J 1981 Musth in the African elephant, *Loxodonta africana*. *Nature* 292: 830-831
42. Poole J H, Kasman L H, Ramsay E C, Lasley B L 1984 Musth and urinary testosterone concentrations in the African elephant (*Loxodonta africana*). *Journal of Reproduction and Fertility* 70: 255-260
43. Poole J H 1987a Rutting behavior in African elephants: the phenomenon of musth. *Behaviour* 102: 283-316
44. Poole J H 1987b Raging bulls. *Animal Kingdom* 90: 18-25
45. Poole J H, Granli P K Visual and Tactile Signals of African savannah elephants. Online at: http://www.elephantvoices.org/ (accessed January 2009).

46. Reimers T J, Lamb S V, Bartlett S A, Matamoros R A, Cowan R G, Engle J S 1991 Effects of hemolysis and storage on quantification of hormones in blood samples from dogs, cattle, and horses. *American Journal of Veterinary Research* 52: 1075-1080
47. Slotow R, van Dyk G, Poole J, Page B, Klocke A 2000 Older bull elephants control young males. *Nature* 408: 425-426
48. Stojilkovicacute S, Dufau M L, Catt K J 1987 Receptors and secretory actions of sigma/phencyclidine agonists in anterior pituitary cells. *Endocrinology* 121: 2044-2054
49. Stout T A E, Colenbrander B 2004 Suppressing reproductive activity in horses using GnRH vaccines, antagonists or agonists. *Animal Reproduction Science* 82-83: 633-643
50. Thakuria D B, Barthakur T 1996 Management of musth in a male African elephant by chemical sedatives in the Assam State Zoo, Guwahati. *Indian Veterinary Journal* 73: 339-340
51. Turkstra J A, Schaftenaar W, Klaver P, Meloen R H 2001 Immunization against GnRH to control fertility and sexual behaviour in zoo-animals. *Proceedings of the 40th International Symposium on Diseases of Zoo and Wildlife Animals*, *Rotterdam, The Netherlands,* 23-26May 2001: 313.
52. Turkstra J A, Van der Meer F, Knaap J, Rottier P, Teerts K, Colenbrander, Meloen R 2005 Effects of GnRH immunization in sexually mature pony stallions. *Animal Reproduction Science* 3-4: 247-259

Chapter 8

Summarising discussion

Habitat availability and the need for wildlife population control

Future preservation of biodiversity is clearly in the hands of the world's human population. However, much of what is required is beyond the direct control of conservationists. Matters such as pollution control to curb the growing effects of greenhouse gases on all forms of life on our planet, are mainly in the hands of politicians and economists. The latter refers to policy on carbon trading and energy production, both of which are highly flawed in terms of curbing global greenhouse gas emission. Unfortunately, South Africa is one of the main sinners in this respect and contributes 1.8% of the world's greenhouse gasses. The energy sector in South Africa is responsible for 87%, 96% and 94% of the country's CO_2, sulphur dioxide and nitrous oxide emission, respectively, because 90% of the country's energy is produced from coal (Bond *et al*., 2009). Adding to the seriousness of the situation is the controversial but approved loan of US$ 3.75 billion by the World Bank to build a new coal-fired power plant (Medupi Power Station) in the Limpopo Province (Suzanne Goldenberg, 2010). Besides adding 25 million tons of CO_2 to the atmosphere, it will seriously impact the ecosensitive wildlife areas in South Africa, Zimbabwe and Botswana. One of these is the World Heritage Site, Mapungupwe, the centre of the largest kingdom in the subcontinent of Africa.

The above illustrates how economics and politics override concerns about green-house gas emissions and global warming. Individuals can only make small contributions to slow down green-house emissions, and many of these measures are confined to the people who can afford to make the necessary changes. The poorer the population, the less likely they are to change. Economics and individual wealth are also the most important factors affecting human population growth. Birth rates in developed countries such as Spain and The Netherlands have reached very low levels during the past 5 to 10 years, and this is clearly linked to individual wealth. African countries are typical examples of the complete opposite; in many of these countries, people are getting poorer and birth rates have either increased or remained at high levels. Nevertheless, the infant survival rate has improved, and the combined result is an ever-expanding human population. For wild animals, particularly medium to large mammals, this is bad news because it means an ever-shrinking habitat. As a result, wildlife populations become fragmented into smaller fenced or unfenced areas and their survival is threatened by decreased food resources, trapping, poaching, culling motivated on the grounds of human-wildlife conflict or management needs, inbreeding depression and disease transmission between domestic and wildlife species. The survival of wildlife throughout the world is dependent on the availability of habitat that is sufficient in area and quality to sustain a variety of species. Zoos and wildlife sanctuaries can never substitute these requirements.

In order to preserve and improve currently available habitats we not only need to slow down the losses attributable to expanding human populations, but also to adequately manage designated wildlife areas. This includes managing many of the species found within these areas. This applies particularly to smaller fenced areas where an overabundance of certain species may impact on the habitat on the one hand, and/or on other species within the system on the other. Smaller restricted areas do not lend themselves to the establishment of an equilibrium situation between species. With regard to medium and large-sized mammals failing to reach equilibrium within a habitat area, there are a number of examples in Africa. These are the large predators and medium, large and mega herbivores (Chapter 1). Too many large carnivores in an area lead to prey species depletion and, if this is not addressed, to breakouts into neighbouring communities with livestock losses or even human fatalities. Too many herbivores can lead to habitat change (sometimes positive) and even eradication of sensitive plant species. The latter may impact on the diversity of animal life, an imbalance that may take decades to restore.

In addition to the problem in range countries, overabundance of many species exists in zoos and wildlife sanctuaries. Such species have a limited market (i.e. high risk of market saturation), and continued breeding leads to overcrowding, with related welfare issues, and an increased likelihood of inbreeding. Consequently there is a need to manage populations of both free-ranging (wild) and captive mammals.

There are various options for population control. These include lethal management (culling and hunting), translocation, enlargement of protected areas and fertility control (Chapter 1). It should be mentioned that while culling and translocation are effective, because they immediately reduce population density, they also tend to increase the rate of reproduction which means that, once you start the practice, you have to continue indefinitely. The effect of density on the rate of reproduction in elephants has been particularly well studied (Laws, 1969; Laws *et al.,* 1975). Fertility control, while it does not reduce the population immediately, will prevent that population from growing further. Moreover, depending of the level of fertility control adopted, the population can be allowed to increase slowly, stabilise or decrease as the mortality rate surpasses the birth rate. Using modelling for African elephants, the level of contraception required to stabilise a population has been estimated at 75% of adult females (Mackey *et al.,* 2009).

It is difficult, if not impossible, for a single pharmaceutical product or treatment to meet all of the criteria for an ideal contraceptive (Chapter 1; Bertschinger *et al*., 2008). The available options include surgical, hormonal and immune-mediated methods. Surgical neutering, besides being expensive and irreversible, affects behaviour and often leads to obesity, especially in females. Surgical approaches have therefore not been popular in wildlife. The one exception is vasectomy, which is quite frequently used in predators, such as lions and tigers, and has even been attempted in elephant

bulls (Stetter *et al.,* 2007). Vasectomies are easy to perform in large predators like lions, and has the advantage over castration of preserving sex-related behaviour; however, repeated heats in females as a result of the induced male infertility often leads to fighting between the sexes (Bertschinger; unpublished observations). In the African wild dog, a further likely complication of repeated pseudopregnancies is pyometra (Boutelle and Bertschinger, 2010). While laparoscopic vasectomies have been performed in elephant bulls in South Africa, the method is very expensive, results in the death of some bulls due to long anaesthetic times (Bertschinger *et al.,* 2008) while, in older bulls in good body condition, the vasa defferentia are very difficult to locate (J. Marais; personal communication). All surgical contraceptive techniques are, for practical purposes, irreversible.

Control of reproduction in large predator females using deslorelin implants

Previously, the GnRH super-agonist deslorelin, in the form of a long-term biocompatible subcutaneous implant (now marketed as Suprelorin [4.7 mg] and Suprelorin 12 [9.4 mg]: Peptech Animal Health, Sydney, Australia), was shown to be a highly effective agent for controlling reproduction in dogs (Trigg *et al.*, 2001) and cats (Munson *et al.*, 2001). The same implants were therefore tested for their ability to control reproduction in wild carnivores, and yielded promising results (Bertschinger *et al.*, 2001). A more extensive study in cheetahs (n = 31), African wild dogs (n = 21), lionesses (*Panthera leo*; n = 11) and leopards (n = 4) (Chapter 2) was then performed to further validate the suitability of the implants for listed species and to establish sensible dosages.

Based on the dose used in domestic dogs (6 mg: Trigg *et al.*, 2001; Wright *et al.,* 2001) the dose selected for cheetahs, wild dogs and leopards was a single 6 mg implant (1998-2002). This translated to an effective dose of 0.2 to 0.12 mg/kg for cheetahs and leopards (30-50 kg body mass) and 0.27 to 0.22 mg/kg for wild dogs (22-27 kg) compared to the > 0.25/kg used in domestic dogs (Trigg *et al.*, 2001). In lionesses, the doses initially used were either 12 mg (~0.08 mg/kg) or 15 mg (0.1 mg/kg).

The use of deslorelin implants is known to cause an initial rise in LH and FSH release followed by complete down-regulation of gonadotrophin release. The effect of the initial stimulatory phase in domestic dogs has been reported to include induction of pro-oestrus in 100% (9 of 9) of bitches treated; interestingly, two of the treated four bitches mated in this study became pregnant but subsequently lost their pregnancies at about 40 days of gestation (Wright *et al.,* 2001). In the same study, oestrus induction could be inhibited with 2 mg megestrol acetate kg^{-1} body weight for 21 or 14 days

starting either 14 or 7 days prior to deslorelin implant introduction. In cheetahs and lionesses, a brief period of sexual attractiveness was seen in some animals during the first 5 to 14 days following deslorelin treatment. In contrast to the domestic dog, however, this period did not appear to be fertile; in fact, mating was never observed. One of 11 cheetahs examined 3 months post-treatment had a marginally raised blood progesterone concentration, which would be compatible with late dioestrus. No lionesses were examined post-treatment, however, such that possible ovulation could not be determined. In a later study (Chapter 3), in which we monitored faecal oestrogen and progestin concentrations we were able to demonstrate post-treatment rises in oestrogen (Day 4 and Day 7) and progestin (Days 37-43 and Days 16-17) concentrations. These findings corroborated the post-treatment findings of field trials with cheetahs and lionesses. Despite males being attracted to early post-treatment females in this study, the treated females did not permit mating by the vasectomised males with which they were housed (one male with each female). The experience with African wild dog females was different (Chapter 2). Of 12 females treated with 6 mg implants, four ovulated post-treatment. And although heat was observed in only one of these females, she became pregnant and produced 7 pups. Another female regarded as a treatment failure became pregnant 3 months after treatment, when the implant was probably still releasing deslorelin. Thus, in contrast to the domestic dog, wild dog pregnancies were not aborted as a result of deslorelin treatment.

The duration of anoestrus following treatment with 6 mg deslorelin implants in cheetahs was at least 16 months (Chapter 2). Based on this, a treatment interval of 12 months is recommended for cheetah females treated with either 6 mg or the newer 4.7 mg (Suprelorin) implants. Similarly, in leopards, the period of contraception lasts at least 12 months, and a treatment interval of a year is recommended for prolonged contraception. In successfully treated African wild dogs (n = 10), by contrast, contraception lasted 5-14 months given that 6 females whelped between 7 and 16 months after treatment, while gestation lasts about two months in wild dogs (Creel & Creel, 2002). Since the African wild dog is highly seasonal with only one oestrus per year (Boutelle and Bertschinger, 2010), the combination of seasonal anoestrus combined with carefully-timed application of a 6 mg deslorelin implant should be able to postpone oestrus for up to 27 months .

Studies on the efficacy, dose and dose interval for deslorelin as a contraceptive continued with much larger numbers of female lions, and four female tigers (Chapter 3). While most of the females were free-ranging (n = 40), 23 plus the 4 tigers were treated in captivity. The dosages used were: 3 x 4.7 mg Suprelorin implants (43 treatments), 1 x 4.7 mg plus 1 x 9.4 mg (23 treatments), 2 x 4.7 mg (10 treatments) and 1 x 9.4 mg (50 treatments). Animals were treated once (n = 36), twice at intervals of 14-60 months (n = 12), three times at intervals of 11-33 months (n = 11), four times at intervals of 17-49 months (n = 2) and 5 times at intervals of 11-30 months (n = 6).

Two further lionesses each housed separately with a vasectomised male, were treated with a single 9.4 mg implant and monitored for faecal progestin and oestrogen profiles over 920 days (~30 months). All treatments were successful in suppressing oestrus for at least 23 months. The mean interval from treatment to reconception (complete reversal) was 30.1 months for the 3 x 4.7 mg treatment. Conception in the females that recovered took place during the second or third heat post-treatment. Reversal after 9.4 mg or 4.7 mg plus 9.4 mg was not established because the females had either been retreated or had not started to cycle at the end of the study period. It appears, however that, the 9.4 mg implants may last longer since four females had not started to cycle 30-36 months post treatment.

It is intriguing and very convenient that lionesses (~0.06 mg kg^{-1} for the 9.4 mg implants) appear to be more sensitive to the effects of deslorelin than domestic dogs (~0.25 mg kg^{-1} and ~0.47 mg kg^{-1} for the 4.7 mg and 9.4 mg implants, respectively). Currently, we advocate the use of 9.4 mg implants for lionesses. If contraception is to be maintained, we suggest retreatment after 18 months and thereafter every 24 months. To date we have had no failures using this approach. Moreover, there have been no serious side-effects that could be attributed to the use of deslorelin, even in lionesses treated for more than 10 years. We have however reported signs of heat at intervals of 2 to 18 months following treatment, and long before reversal has taken place. One such lioness that allowed mating 67 and 97 days after treatment was immobilised after each incident to establish if she had ovulated and was pregnant. In each case she had baseline blood progesterone concentrations. We observed similar behaviour in one of two lionesses treated with deslorelin. During the period when mating was observed, she had small oestrogen peaks without subsequent increases in progestins (Chapter 3).

Lionesses (or female tigers) that are immobilised for deslorelin treatment are routinely examined for pregnancy before implant introduction. Transrectal ultrasound is performed and blood collected for progesterone assay. In this way, pregnancies that are too early to detect by ultrasound can be suspected on the grounds of raised blood progesterone concentrations (>10 nmol/L). If pregnant, abortion is an option, although the decision is left to the owner. In contrast to domestic dogs it appears that deslorelin does not induce abortion in female lions. Two lionesses treated during pregnancy with deslorelin implants alone carried to term (Bertschinger, unpublished observations). We found treatment with the PGF2α analogue, dinoprost (Lutalyse; Pfizer Animal Health, Sandton, South Africa), to be a safe and effective method for terminating pregnancy in two tigers and three lionesses during ~3 weeks to 80 days of pregnancy (Chapter 3). The treatment consisted of three injections of 7.5mg dinoprost on consecutive or alternate days. Mild salivation was the only observed side-effect, and the hormone could be administered remotely via drop-out darts. All treated animals also received deslorelin implants on the day of examination.

In summary, deslorelin implants represent an ideal means of inducing contraception in lionesses and female tigers and cheetahs. The method is safe and reversible, and individual animals can be targeted to achieve the objectives of reserves or zoos. By contrast, the medium to long-term use of progestin implants, although effective, induces a number of side-effects, some of which are life-threatening. For this reason, the use of progestin implants in large predator females should be considered unethical.

Control of reproduction and sex-related behaviour in large predator males with deslorelin implants

Initial trials in male carnivores were performed on one African wild dog and two cheetahs (Bertschinger *et al.,* 2001). Each male was treated with a single 6 mg implant. The one wild dog was housed with 3 untreated females and treated at the beginning of the wild dog breeding season (Boutelle and Bertschinger, 2010). All three females in his group came into heat; the first, 3 weeks after the male was treated, and the other two more than 4 weeks after the male was treated. The first female was mated and gave birth to a litter 2 months later. The other two did not become pregnant. From one until 15 months after treatment, blood testosterone concentrations in the male wild dog were baseline. The two cheetah males were examined 3, 9 and 21 months after a single treatment with a 6 mg deslorelin implant. No or only a few dead sperm were found in the ejaculates, and blood testosterone concentrations remained baseline throughout. These excellent results were repeated in 4 new cheetah and 5 wild-dog males the following year (Chapter 3). This time, the wild dogs were treated in November (two months before the breeding season) thereby allowing ample time for down-regulation before the start of the breeding season. In treated wild dogs, testicular size was reduced from 45-50 x 23-27 to 28-38 x 16-20 mm, clearly indicating a down-regulation of spermatogenesis. The down-regulation was sufficient to avoid pregnancies during the breeding season, but 12 months after treatment one dog already showed reversal. This demonstrated the need to treat males on an annual basis for reliable contraception. Surprisingly, the new Suprelorin implants (4.7 mg and 9.4 mg), proved to be ineffective for contraception of male wild dogs, with some males not responding at all, others only partially and very few showing complete down-regulation. In male wild dogs, therefore, further research is required to develop a reliable protocol for contraception by GnRH agonist treatment.

More data is available on medium to long-term down-regulation of fertility in cheetah males (Chapter 4). Male cheetahs have been treated for 1 (n = 2), 2 (n = 7), 3 (n =9), 4 (n =3) or 5 (n = 1) consecutive years with an implant containing 4.7, 5.0 or 6.0 mg of deslorelin. The treatment with 4.7, 5.0 or 6.0 mg of deslorelin was

successful at preventing pregnancies in females placed in the same camps as implanted males. One and a half months after the treatment (6 mg) of two males, sperm were still present in the ejaculate even though blood testosterone concentrations were baseline. At 3 months after treatment, there were no sperm in the ejaculates of two other males (6 mg). This suggests that males remain fertile for at least 6 weeks after treatment and, therefore, should be separated from untreated females for about 2 months. The alternative would be to treat both sexes for the first year of a contraceptive programme but males only from the second year onwards. In 12 cheetah males treated annually for three years, mean testicular length and breadth decreased significantly each year for the first two years, and thereafter only minimally (Chapter 4). In terms of testicular volume, the reduction was to 61% and 40% of original volume, after one and two years, respectively, reflecting a rapid reduction in spermatogenesis. Annual treatment of males with 6 or 4.7 mg implants was found to be reliable for continued suppression. For proof of maintained efficacy, the absence of penile spikes was invaluable because their development is androgen dependent. Re-growth indicates failure of down-regulation.
In summary, deslorelin implant administration is a feasible method for reversible male contraception in large predators. It works extremely well in cheetahs, although for African wild dogs further research is required to establish effective dosages and treatment intervals with the new Suprelorin implants. Male lions were never targeted for treatment because of the probable negative effects on territorial behaviour and male characteristics.

Population control in African elephants; the South African experience

For herbivores that live in herds, like the African elephant, it is unrealistic to immobilise each animal for delivery of a contraceptive treatment. In such cases, remote delivery is essential. For this reason, the most practical approach to fertility control in elephants appeared to be immunocontraception using the porcine zona pellucida vaccine (pZP), a method that had been well researched in wild horses in the US and which had also been used successfully in a number of other wild herbivores (Kirkpatrick, 1995; Kirkpatrick *et al.*, 1985, 1992, 1996a, 1996b; Liu *et al.*, 1982, 1989). Because nothing was known about the effects of pZP immunocontraception on elephants, it was decided to first provide proof that antibodies to these proteins would recognise elephant zona pellucida (eZP); this was done by treating thin sections of elephant ovaries with anti-pZP antibodies raised in a rabbit and using protein-A conjugated to colloidal gold as a second (labelling) antibody. Examination of primary follicles using a light microscope showed immuno-gold deposits at the oocyte-

granulosa cell junction, while definite staining of the zona pellucida surrounding secondary and tertiary follicles was visible with light and electron microscopy (Chapter 5). Next, two African and one Asian elephant cows were vaccinated with pZP vaccine (400 or 600 μg) to assess the antibody response. All three cows developed acceptable titres following booster vaccinations. The combination of the proof of homology between pZP and eZP, and a humoral response in elephants vaccinated against pZP established pZP as a potentially useful contraceptive vaccine in African elephants.

During 1996-1999, the first two field trials using pZP immunocontraception were carried out on, respectively, 18 and 10 elephant cows in the Kruger National Park (KNP: Chapter 5). The results of the first trial were somewhat disappointing in terms of a contraceptive efficacy of only 56%. By decreasing the interval between the first and second boosters, however, the efficacy was increased to 80% during the second trial. In 2000, the elephant contraception trial was shifted to a private park (Makalali Private Game Reserve) which held a total of 72 elephants including 18 cows that could be identified individually for vaccination and monitoring thereby making the process more manageable and cheaper than in the KNP. The vaccination protocol was basically the same as for the 2nd trial in the KNP, although Freund's modified complete and Freund's incomplete adjuvants were used respectively for the primary and booster vaccinations, instead of trehalose dicorynomycolate. All cows were treated three times at 3-4 week intervals during Year 1, followed by an annual booster. Cows that were pregnant at the time of initial vaccination gave birth to normal calves within 2 years. During Year 3 and for the following 3 years of the program, no more calves were born thereby demonstrating 100% contraceptive efficacy (Delsink *et al.*, 2006). In addition, implementation of pZP contraception proved to be practical for free-ranging elephants (Delsink *et al.,* 2007a), while no detrimental effects on social behaviour within or between the four herds treated or bulls on the reserve were observed (Delsink *et al.,* 2007b).

During the following 7 years, another 12 game reserves were added to the pZP immunocontraception program. However, because it takes at least 4 years to prove efficacy from inception of a program (4-5 year inter-calving interval), only 7 (including Makalali) of the programs have been running long enough to evaluate. For the reserves that can be evaluated, the total number of cows treated is 108 and, as in Makalali, no calves have been born from Year 3 after inception of pZP vaccination (Chapter 6). The 62 (57.4%) cows pregnant at different stages of gestation (1st, 2nd and 3rd trimesters) during initial treatment, all gave birth to normal healthy calves. The only side effect noted in a small percentage of cows was temporary swelling presumed to be due to abscesses at the vaccination sites (Bertschinger *et al.,* 2008). Two small populations with 4 cows each were only treated twice during the first year but still

recorded 100% contraception by year 3, indicating that the 2nd booster during Year 1 may be unnecessary; larger numbers are required to prove this suspicion.

The 100% efficacy achieved using pZP immunocontraception compares well with the results in other species. The collective efficacy of pZP immunocontraception in 24 ungulate species, 25 bears and 11 sea lions was 93.3%, and ranged from 60% (nyala; *Taurotragus angasi*) to 100% in 16 other species such as Bison, Mountain goats, Wapiti, Fallow deer and moose (Frank *et al.,* 2005). Efficacies within the ungulate species varied from 60 to 83% in 6 species, but reached 91.6-100% in the remaining 18 species. All animals reported in these studies were however held and treated in zoos (Frank *et al.,* 2005). In wild horses, for which the largest data set is available, extending back as far as the mid-eighties, the overall result was 95% efficacy (Kirkpatrick and Turner, 2008). These were all free-ranging horses and the conditions of delivery were thus similar to the elephants in our studies. The slightly better response achieved in elephants can possibly be attributed to the long inter-calving interval of this mega-herbivore which allows ample time between calves to achieve antibody titres that are capable of preventing fertilisation. The mare, depending on environmental conditions, by contrast, breeds soon after foaling (foal heat) during late spring or summer.

pZP immunocontraception has thus been shown to be reliable, and the implementation practical, in small to medium-sized elephant populations. Probably the single most important reason why the method cannot currently be employed on large populations, like that in the KNP, is the cost of delivery which ranges from R 700-1000 per vaccination. The first year which requires three vaccinations costs about R 2500 per cow. The vaccine itself contributes only R 880 to the cost, meaning that the remainder is from professional fees and, in particular, hiring a helicopter. The cost could be markedly reduced if the number of vaccinations could be reduced to one during the first year, followed by biennial (i.e. every 2nd year) boosters. To achieve this goal, there is a need to develop and test slow-release formulations like polymer gels or microcapsules. Currently, vaccine production is also labour-intensive, and the product potentially risky in terms of inter-species disease transfer. Even though the risk is small, it would be better to develop a synthetic or recombinant vaccine using viral vectors that cannot replicate and are thus safe to use (c.f. canary pox vector expressing glycoproteins from canine distemper; Merial Literature Update, 2004). To date, attempts to synthesise vaccine proteins using bacterial cultures have produced poor results, probably because the glycoproteins produced in bacteria show N-linked protein glycosylation instead of eukaryotic O-linked protein glycosylation. In this respect, appropriate glycosylation of the polypeptide backbone appears to be necessary to ensure the correct quaternary structure of ZP proteins required for fertilization (Clark and Dell, 2006).

It would also be wise to investigate other potential targets for contraceptive vaccines such as GnRH. GnRH vaccines have the potential to work in both male and female animals, and have been used to successfully immunocastrate pigs (Oonk *et al.,* 1998), down-regulate ovarian activity in mares (Botha *et al.,* 2008), white-tailed deer (Curtis *et al.,* 2002), and zoo animals (Turkstra *et al.,* 2001) and down-regulate reproduction in stallions (Stout and Colenbrander, 2004; Janett *et al.,* 2009). A possible advantage of GnRH immunocontraception is that, if successful, it would induce anoestrus, a state that elephant cows probably spend most of their (non-pregnant) lives in (Bertschinger *et al.,* 2008).

Down-regulation of aggressive behaviour and musth in African elephant bulls

African elephant bulls are frequently kept under unnatural conditions, even in small to medium fenced reserves. These include proximity to humans and other mammals, lack of herd structure for pre-pubertal bulls and absence of dominant bulls to influence young post-pubertal bulls. Lack of social interaction with matriarchs and dominant bulls, means that young bulls are not disciplined or suppressed; as a result, they may come into musth much earlier than normal. Aggression related to androgen production then becomes a huge problem, especially if bulls do come into musth since testosterone concentrations then reach levels that are 10 to 30 times higher than outside this period of enhanced sexual behaviour (Bertschinger *et al.,* 2008). GnRH vaccines developed for the immunocastration and down-regulation of androgen production responsible for boar-taint (D'Occhio, 1993; Oonk *et al.*, 1998), seemed to be a suitable candidate to address testosterone-driven behavioural problems in elephant bulls. A pilot trial was carried out in 5 captive (3 non-aggressive and 2 aggressive) and one free-ranging bull in musth (Chapter 7). At the beginning of the trial, aggressive behaviour was positively correlated with faecal androgen metabolites (FAM). The bulls were then vaccinated against GnRH three or four times at 3 to 7-week intervals. Both aggressive bulls responded with improved behaviour and decreased FAM following treatment. Musth ceased in the adult free-ranging bull after treatment, although it is possible that he spontaneously exited musth. The captive bulls remained non-aggressive until the end of the study, 4 months after the primary vaccination. Treatment has continued in one of the captive bulls approximately every 6 months, and he has been tractable since 2003 to the present, despite being 26 years old in 2010. These results have been sufficiently encouraging to suggest that GnRH vaccines could be useful for down-regulating aggressive behaviour and musth, not only in African, but also in Asian elephants where androgen-driven behaviour frequently results in the loss of human lives. Once again, a slow-release formulation

would be useful to avoid the frequent boosters currently required. However, the fact that many captive Asian bulls are also used for breeding, means that the effect of the vaccine on the reproductive organs and reproductive function, including spermatogenesis, needs to be investigated. In free-ranging African elephant bulls on the other hand, down-regulation of spermatogenesis as a means of contraception would be a potential advantage.

Concluding remarks

Fertility control represents a proactive approach to population management for various mammalian wildlife species. In large predators, deslorelin implants have proven to be useful contraceptives in species such as lions, tigers and cheetahs. It does however, appear that certain species, like the wild dog, are more difficult to down-regulate than others. Whether this relates to differences in peripheral deslorelin concentrations achieved or to actual concentrations at the effector site is unknown. Determining peripheral deslorelin concentrations in species like the lion, cheetah and wild dog may provide some answers. LH response to GnRH stimulation at various intervals after deslorelin treatment may also be informative (Herbert *et al.,* 2004). Additionally, the ability to deliver the implants remotely would make this method of contraception much more appealing to reserve managers.

Immunocontraception with pZP of elephant cows has been shown to be 100% effective in small to medium populations. Future research should concentrate on development of synthetic or recombinant vaccines that would be safer and less labour-intensive to manufacture. A slow-release vaccine would reduce implementation costs and enable use on larger populations. Although studies are ongoing (Delsink *et al.*, 2007b), there is also a need to expand behavioural studies on treated populations. Down-regulation of androgen-related behaviour in elephant bulls also requires more intensive studies on animals (African and Asian) of various ages to determine whether the treatment is capable of suppressing the annual musth cycles and to establish the effects of GnRH vaccination on male fertility.

References

1. Bertschinger, H.J., C.S. Asa, P.P. Calle, J.A. Long, K. Bauman, K. Dematte, W. Jöchle, T.E. Trigg. 2001. Control of reproduction and sex related behaviour in exotic carnivores with the GnRH analogue deslorelin: preliminary observations. *Journal of Reproduction and Fertility*, Supplement 57: 275-283.
2. Bertschinger, Henk, Audrey Delsink, J.J. van Altena, Jay Kirkpatrick, Hanno Killian, Andre Ganswindt, Rob Slotow & Guy Castley. 2008. Reproductive control of elephants. In: Elephant management. A scientific assessment for South Africa. Eds RJ Scholes and KG Mennell, Wits University Press, 1Jan Smuts Avenue, Johannesburg: 357-328.

3. Bertschinger, H.J., C. Herbert, M.A. De Barros Vaz Guimarães, T.E. Trigg & A. Human. 2009. Managing the reproductive rate of some large carnivores using the GnRH super-agonist implant Suprelorin®. British Veterinary Zoological Society, York, 7-8 November 2009: 12-17
4. Bond, P., R. Dada & G. Erion (eds). 2009. Climate change, carbon trading and civil society. Negative returns on South African investments. University of Kwa-Zulu Natal Press, Scottsville, South Africa: pp 231.
5. Botha, A.E., M.L. Schulman, H.J. Bertschinger**,** A.J. Guthrie, C.H. Annandale & S.B. Hughes. 2008. The use of a GnRH vaccine to suppress mare ovarian activity in a large group of mares under field conditions. *Wildlife Research* 35: 548-554
6. Boutelle, S.M. & H.J. Bertschinger. 2010. Reproductive management in captive and wild canids: contraception challenges. *International Zoo Year Book* 44: 109-120.
7. Clarke, G.F. & A. Dell. 2006. Molecular models for murine sperm-egg binding. *Journal of Biological Chemistry* 281: 13853-13856.
8. Creel, S. & N.M. Creel. 2002. *The African wild Dog. Behavior, ecology, and conservation.* Princeton NJ: University Press.
9. Curtis P.D., R.L. Pooler, M.E. Richmond, L.A. Miller, G.F. Mattfeld & F.W. Quimby. 2002 Comparative effects of GnRH and porcine zona pellucida (PZP) immunocontraceptive vaccines for controlling reproduction in white-tailed deer (*Odocoileus virginianus*). *Reproduction* Supplement 60: 131-141
10. Delsink, A.K., J.J. Van Altena, D. Grobler, J. Kirkpatrick, H. Bertschinger & R. Slotow. 2006. Regulation of a small, discrete African elephant population through immunocontraception in the Makalali Conservancy, Limpopo, South Africa. *South African Journal of Science* 102, 403-405.
11. Delsink, A.K., J.J. van Altena, D. Grobler, H. Bertschinger, J.F. Kirkpatrick & R. Slotow 2007a. Implementing immunocontraception in free-ranging African elephants at Makalali Conservancy. *Journal of the South African Veterinary Association* 78(1), 25–30.
12. Delsink, A., J.F. Kirkpatrick, J.J. Van Altena, D. Grobler, H.J. Bertschinger & R. Slotow. 2007b. Lack of social and behavioural consequences of immunocontraception in African elephants. 6th International Conference on Fertility Control for Wildlife. York, UK, 3-5 September: 31.
13. D'Occhio, M.J. 1993. Immunological suppression of reproductive functions in male and female mammals. *Animal Reproduction Science* 33: 345-372.
14. Frank, K.M., R.O. Lyda & J.F. Kirkpatrick. 2005. Immunocontgraception of captive exotic species. IV. Species differences in response to the porcine ziona pellucida vaccine and the timing of booster inoculations. *Zoo Biology* 24: 349-358.
15. Goldenberg, Suzanne (guest writer), Guardian, 9 April 2010.
16. Herbert C.A., T.E. Trigg, M.B. Renfree, G. Shaw, D.C. Eckery & D.W. Cooper. 2004. Effects of a gonadotropin releasing hormone agonist implant on reproduction in a male marsupial, *Macropus eugenii. Biology of Reproduction* 70: 1836-1842.
17. Janett F., R. Stump, D. Burger & R. Thun. 2009. Suppression of testicular function and sexual behaviour by vaccination with GnRH (Equity™) in the adult stallion. *Animal Reproduction Science* 115: 88-102
18. Kirkpatrick, J.F. 1995. Management of Wild Horses by Fertility Control: The Assateague Experience, Denver CO: National Park Service Scientific Monograph: 32-35.
19. Kirkpatrick J.F., R. Naugle, I.K.M. Liu, M. Bemoco & J.W. Turner Jr. 1985. Effects of seven consecutive years of porcine zona pellucida contraception on ovarian function in feral mares. *Biology of Reproduction Monograph.* 1:411-418.
20. Kirkpatrick J.F., I.K.M. Liu, J.W. Turner Jr., R. Naugle & R. Keiper. 1992. Long-term effects of porcine zonae pellucidae immunocontraception on ovarian function in feral horses (Equus caballus). *Journal of Reproduction and Fertility*. 94:437-444.
21. Kirkpatrick J.F., P.P. Colle, P. Kalk, I.K.M. Liu, M. Bernoco & J.W. Turner Jr. 1996a. Immunocontraception of captive exotic species. II. Sika deer (Cervus niuuon). Axis deer (Axis axis) Himalayan tahr (Hemitraeus jemlahicus), Roosevelt elk (Cervus ellaphus roosevelt) muntjac deer

(Muntiacus reeves), and sambar deer (Cervus unicolor). *Journal of Zoo and Wildlife Medicine*. 27:482-495.
22. Kirkpatrick J.F., J.W. Turner Jr., I.K.M. Liu & R.A. Fayrer-Hosken. 1996b. Applications of pig zona pellucida immunocontraception to wildlife fertility control. In: Prospects of Zona Pellucida Glycoproteins for Contraception. *Journal of Reproduction and Fertility*. 50 (Suppl 1): 183-198.
23. Kirkpatrick, J.F. & A. Turner. 2008. Achieving population goals in long-lived wildlife with contraception. *Wildlife Research* 35: 513-519.
24. Laws, R.M. 1969. Aspects of reproduction in African elephants, *Loxodonta Africana*. *Journal of Reproduction and Fertility* Supplement 6: 193-217.
25. Laws, R.M., I.S.C. Parker & R.C.B Johnstone. 1975. Elephants and their habitats. Claredon Press, Oxford.
26. Liu I.K.M. & C.A. Shivers. 1982. Antibodies to the zona pellucida in mares. *Journal of Reproduction and Fertility*. 32 (Suppl): 309-313.
27. Liu I.K.M., M. Bemoco & M. Feldman. 1989. Contraception in mares heteroimmunized with pig zonae pellucidae. *Journal of Reproduction and Fertility*. 85: 19-29.
28. Mackey, R.L., B.R. Page, D. Grobler & Slotow, R. 2009. Modelling the effectiveness of contraception for controlling introduced populations of elephants in South Africa. *African Journal of Ecology* 47:747-755.
29. Merial Literature Update. 2004. Bulletin Number TSB-4-0019-FTB, Veterinary Services, Merial Limited, Duluth GA, US.
30. Miller, L.A., B.E. Johns & G.J. Killian. 2000. Immunocontraception of white-tailed deer with GnRH vaccine. *American Journal of Reproductive Immunology* 44: 266-274
31. Oonk, H.B., J.A. Turkstra, W.M. Schaaper, J.H. Erkens, M.H. Schuitemaker-de Weerd, A. van Nes, J.H. Verheijden & R.H. Meloen. 1998. New GnRH-like peptide construct to optimize efficient immunocastration of male pigs by immunoneutralization of GnRH. *Vaccine* 16:1074-1082
32. Stetter, M., D. Hendrickson, J.R. Zuba, M. Briggs, D. Grobler, L. Small, J.J. van Aletna. 2007. Laparoscopic vasectomy as a potential population control method in free ranging African elephants (*Loxodonta Africana*). *Proceedings of the American Association of Zoo Veterinarians*, Annual Meeting, Knoxville, TN, USA: 185-188.
33. Stout, T.A.E. & B. Colenbrander. 2004. Suppressing reproductive activity in horses using GnRH vaccines, antagonists or agonists. *Animal Reproduction Science* 82-83: 633-643.
34. Tempelhof, Ilse. Beeld, 12 May 2010.
35. Trigg, T.E., P.J. Wright., A.F. Armour, P.E. Williamson, A. Junaidi, G.B. Martin, A.G. Doyle & J. Walsh. 2001. Use of a GnRH analogue implant to produce reversible long-term suppression of reproductive function of male and female domestic dogs. *Journal of Reproduction and Fertility* Suppl. 57: 255-261.
36. Turkstra, J.A., W. Schaftenaar, P. Klaver & R.H. Meloen. 2001. Immunization against GnRH to control fertility and sexual behaviour in zoo-animals. *Proceedings of the 40th International Symposium on Diseases of Zoo and Wildlife Animals*, *Rotterdam, The Netherlands,* 23-26May 2001: 313.
37. Wright, P.J., J.P. Verstegen, K. Onclin, W. Jochle, A.F. Armour, G.B. Martin & T.E. Trigg. 2001. Suppression of the oestrous responses of bitches to the GnRH analogue deslorelin by progestin *Journal of Reproduction and Fertility Supplement* **57** 263-268.

Samenvatting

Voor behoud en verbetering van huidige natuurgebieden is het niet alleen nodig om de geleidelijke inkrimping van de natuurgebieden - veroorzaakt door bevolkingsgroei - tegen te gaan, maar ook de populaties van de wilde dieren in die gebieden adequaat te beheren. Dit betreft dan vooral relatief kleine, omheinde gebieden waar een overschot van een bepaalde diersoort een grote invloed heeft op de leefomgeving, vooral op andere diersoorten en planten. Hierdoor kan een duurzaam evenwicht tussen dieren en planten niet tot stand komen. Hiervan zijn in Afrika duidelijke voorbeelden bekend zoals voor enkele grote roofdierensoorten en voor middelgrote, grote en mega herbivoren (Hoofdstuk 1). Een te groot aantal grote vleeseters in een bepaald gebied zal de beschikbaarheid van prooidieren verminderen of deze dieren zelfs doen verdwijnen. Wanneer geen maatregelen worden genomen, zal het uitbreken van dergelijke roofdieren naar naburige leefgemeenschappen of dorpen leiden tot verlies van vee maar ook mogelijk menselijke slachtoffers ten gevolge hebben. Een overmaat aan herbivoren kan een negatieve invloed hebben op de leefomgeving en zelfs de oorzaak zijn van het verdwijnen van plantensoorten. Dit laatste kan wederom de diversiteit van diersoorten beïnvloeden en een onbalans veroorzaken die zelfs in het verloop van jaren nog niet herstelt. Behalve dat dergelijke problemen zich voor kunnen doen in de natuurlijke leefomgeving van deze diersoorten, kan een te sterke toename in het aantal dieren van een bepaalde soort een probleem vormen in dierentuinen en wildparken. Voor dergelijke dieren is vaak maar een beperkte afzetmarkt. Een voortdurende voortplanting heeft overbevolking tot gevolg met een daarmee gepaard gaande dierenwelzijnproblematiek en een vergrote kans op inteelt.

Er zijn verschillende mogelijkheden om populatiegrootte te controleren zoals het doden of verplaatsen van dieren, het beperken van het aantal nakomelingen of het uitbreiden van het gebied. Daarbij moet worden aangemerkt dat hoewel doden of verplaatsen een direct effect heeft, deze methoden ook leiden tot een stimulering van de voortplanting met als gevolg dat men door moet blijven gaan met de gekozen aanpak. Geboortebeperking heeft op korte termijn geen resultaat, maar zal voorkómen dat de populatie verder toeneemt. Bovendien kan de populatiegrootte worden gereguleerd door het aantal geboortes aan te passen aan het sterfte cijfer.

Het is onwaarschijnlijk, zo niet onmogelijk, dat één geneesmiddel of één behandelingsmethode als ideaal kan worden gezien voor contraceptie (Hoofdstuk 1). De beschikbare methodes zijn gebaseerd op chirurgische, hormonale of immunologische benaderingen. Castratie van mannelijke of vrouwelijke dieren is duur

maar beïnvloedt ook gedrag en leidt vaak tot vetzucht, vooral bij vrouwelijke dieren. Derhalve zijn operatieve methodes weinig toegepast bij dieren in het wild, met uitzondering van vasectomie (het onderbreken van de zaadleiders) bij roofdieren zoals leeuwen en tijgers. Het wordt ook incidenteel toegepast bij olifanten.

Als meest geschikte methode voor geboortebeperking bij grote roofdieren kwam de behandeling naar voren met de GnRH super-agonist deslorelin (Suprelorin [4.7 mg] en Suprelorin 12 [9.4 mg]: Peptech Animal Health, Sydney, Australie) in de vorm van een onderhuids implantaat. Deze methode bleek effectief bij de geboorteregulatie van honden en katten. De eerste testresultaten met de implantaten bij wilde vleeseters leken veelbelovend. Een uitgebreidere studie werd uitgevoerd bij het jachtluipaard (*Acinonyx jubatus*; n=31), de Afrikaanse wilde hond (*Lycaon pictus*; n=21), de leeuw (*Panthera leo*; n=11) en het luipaard (*Panthera pardus*; n=4) (Hoofdstuk 2). In deze studie werd geschiktheid van dit geneesmiddel getest evenals de te gebruiken dosis. Deslorelin veroorzaakt een initiële een stijging van LH en FSH gevolgd door een complete blokkering van verdere afgifte van deze hormonen door de hypofyse. Bij de hond (*Canis lupus familiaris*) veroorzaakte de initiële stijging een pro-oestrus (beginnende loopsheid) bij alle teven en wanneer deze dieren werden gedekt leidde dit tot dracht met een daaropvolgende abortus rond de 40ste dag van de graviditeit. Sommige jachtluipaarden en leeuwinnen waren in de eerste 5 tot 14 dagen na behandeling seksueel attractief voor partners. Deze dieren werden echter, in tegenstelling tot de teven, niet gedekt door een aanwezige partner en dus bleef dracht uit. In een vervolg onderzoek werd bij twee leeuwinnen (Hoofdstuk 3) na de behandeling (Dag 4 en Dag 7) een stijging van fecale oestrogenen waargenomen en verhoogde concentraties van prostagenen (Dag 37-43 en Dag 16-17). De resultaten waren bij de Afrikaanse wilde hond geheel anders (Hoofdstuk 2): vier van de twaalf dieren hadden ovulaties (eisprong) na het aanbrengen van een 6 mg implantaat. Eén van deze dieren werd loops, gedekt en wierp zeven pups. Een ander dier werd drie maanden na inbrengen van het implantaat gedekt en drachtig, dus in een periode wanneer nog afgifte vanuit het implantaat plaatsvond. Dus in tegenstelling tot de situatie bij de gedomesticeerde hond, treedt bij de Afrikaanse wilde hond geen abortus op na deslorelin behandeling. Bij de overige honden was de behandeling wel succesvol en werkte gedurende 5-14 maanden. Aangezien bij deze diersoort seksuele activiteit seizoensgebonden is met slechts één dekmoment per jaar, zou de combinatie van de seizoensanoestrus met een op het goede moment uitgevoerde behandeling (6 mg implantaat) het dekken (tijdens het jaarlijkse voortplantingsseizoen) moeten kunnen voorkomen.

Bij het jachtluipaard bleef na deslorelin behandeling (6 mg implantaat) bronstgedrag voor minstens 16 maanden achterwege (Hoofdstuk 2). Op basis van deze resultaten wordt een 12 maandelijkse behandeling aanbevolen voor vrouwelijke

jachtluipaarden met een 6 mg delorelin implantaat of met het nieuwere 4.7 mg (Suprelorin) implantaat.

De effectiviteit, dosering en behandelingsinterval van deslorelin als middel voor geboortebeperking werden nader onderzocht bij 40 wilde leeuwinnen, 23 leeuwinnen in dierentuinen en 4 tijgers in dierentuinen. Dosering van Suprolerin en behandelingsinterval varieerden (zie Hoofdstuk 3). Herstel van de ovariumactiviteit na stopzetting van de behandeling kon niet worden geëvalueerd omdat behandelingen vaak werden gecontinueerd. Thans wordt voor de leeuw het gebruik van het 9.4 mg implantaat aanbevolen, een tweede dosering na 18 maanden en vervolgdoseringen elke 24 maanden. Tot nu toe is dit effectief gebleken. Bijwerkingen van deze deslorelin behandeling zijn niet waargenomen, zelfs niet bij dieren die meer dan 10 jaar zijn gevolgd. Wel werd een enkele keer oestrus gedrag waargenomen tussen 2 tot 18 maanden na behandeling wat gepaard kon gaan met een geringe oestrogeen verhoging en lang voordat een herhalingsbehandeling werd toegepast.

Behandeling van mannelijke vleeseters met deslorelin werd uitgevoerd bij de Afrikaanse wilde hond en het jachtluipaard. Initieel onderzoek toonde aan dat bij deze diersoorten de zaadcelvorming en de testosteronproductie met dit middel konden worden onderdrukt. Bij Afrikaanse wilde honden (n=5) , behandeld twee maanden voor de start van periode van het voortplantingsseizoen, nam de testisomvang af en werden geen drachten veroorzaakt. Na 12 maanden trad bij één dier herstel op, derhalve wordt aanbevolen de dieren voor een effectieve geboortebeperking jaarlijks te behandelen. Helaas bleken de nieuwe implantaten (Suprelorin 4.7 mg en 9.4 mg) niet effectief of leidden slechts tot een gedeeltelijke onderdrukking van de zaadcelvorming. Verder onderzoek is derhalve noodzakelijk om tot een betrouwbaar, doeltreffend protocol te komen voor behandeling van mannelijke dieren bij Afrikaanse wilde honden met een GnRH agonist.

Behandeling van mannelijke jachtluipaarden met een GnRH agonist ter onderdrukking van hun bevruchtend vermogen bleek effectief op middellange en lange termijn. Tweeëntwintig dieren werden behandeld gedurende een periode variërend van 1 tot 5 jaar met implantaten van 4.7, 5 of 6 mg deslorelin. Vrouwelijke dieren die waren gehuisvest in aanwezigheid van een behandeld mannelijk dier werden niet drachtig. Bij 12 dieren die gedurende drie jaar waren behandeld was de testisomvang significant afgenomen. Een na-behandeling van één keer paar jaar was voldoende om het gewenste effect te continueren.

Voor herbivoren die in groepsverband leven is, zoals de Afrikaanse olifant (*Loxodonta africana*), is het niet realistisch om voor elke behandeling die moet leiden tot geboortebeperking de dieren te immobiliseren. De meest geschikte methode bleek immuuncontraceptie met gebruik van een porcine zona pellucida vaccin (pZP: eischaal

rond de varkenseicel), een methode die succesvol was gebleken, niet alleen bij wilde paarden in natuurparken in de Verenigde Staten van Amerika, maar ook bij andere in het wild levende herbivoren. In eerste instantie werd de toepasbaarheid bij de olifant getest door histologische coupes van een ovarium van een olifant bloot te stellen aan pZP antilichamen waarbij specifieke kleuring optrad. Daarnaast bleken olifanten na toediening van een pZP vaccin specifieke antilichamen te vormen (Hoofdstuk 5). Van 1996 tot 1999 werden vervolgens in twee veldexperimenten in het Kruger Park 18 en 20 olifanten met het vaccin behandeld (Hoofdstuk 5). De resultaten waren in het eerste experiment iets teleurstellend (contraceptie bij 56% van de dieren) maar na inkorten van de termijn tussen de eerste injectie en de vervolginjectie nam de effectiviteit toe to 80%.

In de daaropvolgende acht jaren werden 108 (olifant)koeien behandeld in 7 privé wildparken (Hoofdstuk 6). Het vaccinatie protocol was in principe gelijk aan dat van het tweede experiment in het Kruger Park, al had het adjuvans een andere samenstelling. Alle koeien werden individueel behandeld: drie injecties werden met een tussentijd van 3-4 weken van afstand toegediend (dartgun), gevolgd door jaarlijkse een herhalingsinjectie. Er werden geen kalveren meer geboren na het tweede jaar van behandeling. De 62 (57.4%) dieren die bij de start van het project drachtig waren (eerste, tweede en derde trimester) brachten alle een gezond kalf ter wereld. Slechts bij een beperkt aantal dieren werd een tijdelijke zwelling op de injectieplaats waargenomen. Bij acht dieren die bij aanvang slechts twee maal geïnjecteerd werden bleek de behandeling ook effectief, zodat een derde injectie in jaar 1 waarschijnlijk niet nodig is. Anticonceptie met pZP bij de Afrikaanse olifant blijkt dus effectief, veilig en toepasbaar in kleine tot middelgrote wildparken. De enige echte reden waarom toepassing in grote parken zoals het Kruger Park niet realistisch lijkt zijn de kosten ter grootte van 700-1000 Rand per injectie.

Mannelijke Afrikaanse olifanten (stieren) worden vaak op een onnatuurlijke manier gehouden, zelfs in de kleine tot middelgrote wildparken. Het betreft dan vooral de nabijheid van de mens en andere zoogdieren, gebrek aan de specifieke kuddestructuur met name voor prepuberale stieren en de afwezigheid van dominante volwassen stieren die het gedrag van de jonge volwassen stieren beïnvloeden. De afwezigheid van sociale interactie met de matriarch van de kudde en met dominante stieren heeft tot gevolg dat jonge stieren niet aan discipline onderhevig zijn of in hun gedrag worden beperkt; tengevolge hiervan komen zij al op jonge leeftijd in musth (bronst). Agressie gerelateerd aan testosteron wordt dan een groot probleem, zeker tijdens de musth als de betreffende concentraties 10-30 maal hoger zijn dan buiten de musth periode. De GnRH vaccins, ontwikkeld om de androgeen afhankelijke geslachtsgeur van beren (mannelijke varkens) te voorkomen, zouden mogelijk geschikt zijn om de testosteron-gerelateerde gedragsproblemen bij stieren tegen te gaan. In eerste instantie werden 5 gehouden olifanten (3 niet-agressief, 2 agressief) en

één in het wild levende olifant behandeld (Hoofdstuk 7). Bij de start van het onderzoek was agressief gedrag duidelijk gecorreleerd met de concentratie aan androgene hormonen in de feces (FA). De stieren werden 3 of 4 keer gevaccineerd met een tussentijd van 3-7 weken. De beide agressieve stieren verbeterden hun gedrag en FA daalde sterk. Bij de enige in het wild levende stier ging de musth over. De gehouden stieren kwamen gedurende de studie niet in musth. De behandeling werd bij een van de dieren gecontinueerd met een frequentie van één injectie per 6 maanden. Dit volwassen dier bleef gedurende de volledige periode handzaam en hanteerbaar. Deze bevindingen duiden erop dat GnRH vaccinatie een geschikte wijze is om androgeen-afhankelijk agressief gedrag en musth te voorkomen, niet alleen bij de Afrikaanse olifant maar ook bij de Aziatische olifant waar de androgeen-afhankelijke agressie jaarlijks mensenlevens kost.

Geboortebeperking is een proactieve handelwijze met betrekking tot het populatiemanagement van dieren in het wild. Bij grote roofdieren zoals leeuwen, tijgers en jachtluipaarden zijn deslorelin implantaten geschikt voor dit doel. Bij enkele diersoorten, zoals de Afrikaanse wolf werkt dit minder goed. Of dit komt door te geringe perifere concentraties van deslorelin of door te lage werkelijke concentraties bij de receptor is onbekend. Bepaling van dergelijke concentraties bij diersoorten als de leeuw, de tijger en de Afrikaanse wilde hond zouden hierin duidelijkheid kunnen scheppen. Een al of niet aanwezige LH respons na GnRH stimulatie op verschillende tijden na behandeling met deslorelin zou ook informatief zijn. De mogelijkheid om implantaten toe te dienen vanaf een zekere afstand (dartgun) zou de toepasbaarheid van deze behandeling vergroten en deze methode in de ogen van de wildparkbeheerders aantrekkelijk maken.

Immuuncontraceptie van olifantkoeien met behulp van pZP is 100% effectief gebleken bij gebruik in kleine tot middelgrote wildparken. Toekomstig onderzoek zal zich bij voorkeur richten op de ontwikkeling van synthetische of recombinant vaccins die veiliger zijn dan het huidige preparaat en ook makkelijker geproduceerd kunnen worden. Onderzoek naar een slow-release vaccin dat de toedieningkosten verlaagt en toepassing in grotere populaties mogelijk maakt, is thans gaande. Daarnaast wordt uitgebreider gedragsonderzoek verricht bij kuddes waarin dieren zijn behandeld. Ook is nader onderzoek noodzakelijk naar de invloed van onder andere leeftijd van met GnRH vaccin behandelde stieren op parameters als musth frequentie en intensiteit en bevruchtend vermogen.

Samenvattend kan worden gesteld dat reversibele beperking van de voortplanting en van het geslachtsgebonden gedrag van groot belang zijn voor het management van dierpopulaties in het wild,

Acknowledgments

My interest in wildlife was kindled at an early age. For this my parents Felix and Trudy Bertschinger are clearly to blame. Firstly, when I was four years old we moved to a farm (Farm Waid) in the Hekpoort district about 80 km northwest of Johannesburg. Initially I was petrified of most animals, especially the Afrikander oxen that came with the farm, and seemed so fierce, large and threatening. To boot, the toilet was a long-drop outside the house, and harboured all sorts of creepy-crawlies, some of which lived under the toilet seat. My elder brother Alex and I imagined being attacked by these fiendish things and, as a result, no leisure time was spent in that little house. Soon I forgot my fears of things creepy-crawly and larger and Alex and I spent our free time roaming every inch of that farm. We acquired the first edition of Robert's Birds and were very soon keen birders and also (rather shockingly from a conservation point of view) collected eggs and nests of our feathered friends. Once we were lucky enough to find a dead vulture and took the talons home to add to our collection. My mother soon 'nosed' in on our prized possession and the talons disappeared mysteriously when we were asleep one night. Although we had a number of wildlife experiences on the farm, most of which were birds, small antelopes, snakes and monitor lizards, my first real exposure to wildlife was in 1948 (age seven) when my mother, Alex and I and Mrs Wetchen and her two daughters took a trip to the Kruger National Park (KNP). In those days there was no need to book accommodation in the KNP, particularly in winter when the northern part of the park was open (in summer it was closed because of impassable roads and malaria). So we drove in at Phalaborwa gate and north towards Shingwedzi. We were late and had to drive over the speed limit to get there before the camp gate closed. About 10 km form Shingwedzi we surprised four cheetahs who, to avoid us, jumped over the bonnet of the car. What an introduction to this beautiful cat! Arriving at camp we learned that all the bungalows were occupied. The camp warden said that this was no problem as they would simply give us some beds under the Lala palms, which are still there today. In those days the camps were surrounded by a low fence and the entrance gate was simply a boom – not much of a barrier to most kinds of African wildlife. Lions could be heard roaring and hyenas calling throughout the night. That remains one of the most memorable nights of my life. The other memorable thing about that holiday was encounters with elephants. The Mopani bush along the Shingwedzi River simply crawls with elephants. The roads were narrow and winding and you were often surprised by or surprised big bulls who loved to use the roads. Alex and I weren't exactly brave but the two girls were so scared that they spent most of the time on the

floor of the car. I am convinced that elephant bulls have a weird sense of humour. They are very intelligent creatures and very quickly notice that 'cars' are afraid of them - so what better fun than to scare a few cars every day. Good material for Gary Larson. From then onwards, we visited the KNP as a family at least once but sometimes twice a year. Farm Waid eventually turned out to be a dairy farm – yet another factor that influenced my future. Early on I learnt to inject and dose cattle and help with the milking shifts. Once a month our veterinarian Dr Boswell would visit the valley and part of his tour was to drop in at our dairy. He stripped to the waste, put on his gum boots and carried out his pregnancy and post partum checks. When done, he would give his boots to a stable hand called Zulu (no doubt because he was a Zulu) to clean and would say 'Aikona faga manzi pagati' – 'don't put water in my boots'. Each time Zulu would have a good laugh and go off to wash the boots. Dr Boswell was probably the first cattle reproduction/herd health vet in South Africa and it was thanks to him more than anyone else that I studied veterinary science. I am of course deeply grateful to my parents for sending me to a wonderful boarding school (Kingswood College in Grahamstown) for my secondary education. They were some of the best years of my life made possible by a school that provided us with not only a sound education but also life skills which cannot be overemphasised. Some of the good friends I made at school, like Menno Meinesz, Andy Anderson, Chris Huddy and Ron Whytock, remain amongst my best friends today.

In 1960 I started my veterinary degree at the University of Pretoria (Tucks).I spent my first year on main campus doing the basic subjects Physics, Chemistry, Botany and Zoology. Having attended an English primary and secondary school the switch to Afrikaans during the first year at Tucks was quite a shock to the system. I coped by translating everything straight into English. There were only a handful of English speaking students at the time and naturally, one quickly found one another. I spied this very pretty girl amongst this group and that's how I met my future wife Renate. We have been sole mates ever since, married in 1965 and produced two lovely children Adrian and Natalie. In the meantime they have made us proud grandparents of six grandchildren whom we love and enjoy – more so in short stretches than too long. I dedicate this work to my family, but especially to my wife Renate for everything she has meant and done for me.

The Onderstepoort student days were very special. We started as a class of 30 and by the time we reached final year there were 24 of us left. We worked hard and played hard as well – this seems to be true for veterinary students all over the world. Having obtained the BVSc degree in 1965 we very soon left for Switzerland where I worked at the Faculty of Veterinary Science in Zurich for 9 years in the field of reproduction. Besides completing the DrMedVet degree at the University of Zurich I was lucky enough to work together with a good friend of mine, Ewald Isenbügel, who became the Zoo veterinarian and was a huge fan of Africa and wildlife in general. Once again

this and the few wildlife cases that we tackled together renewed my interest in wildlife. We returned to South Africa in 1975 and in 1976 I moved to the Veterinary Faculty at Onderstepoort. Once again fate was kind to me. Through two good friends of mine, Brough Coubrough and Woody Meltzer, I managed to become involved at the de Wildt Cheetah Research Centre (now called 'The Ann van Dyk Cheetah Centre') – I am proud to say that I am still associated with de Wildt which is owned by my very good friend Ann van Dyk. The experience and expertise that I gained through working at de Wildt and with Ann, Woody and Brough was the impetus I needed for my future work in wildlife reproduction. Nowhere else in the world would it have been possible gain so much experience in working with cheetahs and African wild dogs. While other researchers were collecting semen from one, two or three cheetahs we worked on 10 to 20 a year. I am deeply indebted to Ann for allowing me to work with these beautiful animals and to Brough and especially Woody for teaching me much of what I know about large African carnivores. The first treatment (1998) of African wild dogs with the contraceptive implant deslorelin took place at de Wildt and, with few exceptions; all the dogs reported in our publications were treated there.

By the early nineties I had given very little thought to wildlife contraception, having concentrated on promoting fertility of species like cheetah and African buffalo rather than suppressing it. My interest in wildlife contraception was mainly sparked by the debate around culling and culling methods of elephants in the Kruger National Park. At the time I had absolutely no idea of wildlife contraception other than the use of progestin implants in carnivores like lions and tigers. Reading the literature, the only method that seemed to be in any way feasible to apply was immunocontraception. Fortune, in the form of Jay Kirkpatrick, once again smiled on me. Here was a man who had been using the porcine zona pellucida (pZP) vaccine to control the fertility of wild horses for more than a decade. And so the first studies on the immunocontraception of elephants in South Africa commenced in 1995. Without Jay I would never have become involved in this field. I certainly had neither the knowledge nor experience to tackle such a project. He was also instrumental in helping us to set up our own pZP vaccine production lab by motivating the funding of the lab by the HSUS and allowing Annemarie Human to be trained is his lab in Billings, Montana. Without the financial support of the HSUS/HSI and the moral support of Andrew Rowan and now Teresa Telecky, elephant contraception in South Africa would surely have fizzled out after the initial study in the KNP. The study that followed in Makalali, where initially I was not involved, was also central to the development of elephant contraception. Audrey Delsink ('The Matriarch' or TM) is acknowledged for the great study she carried out there in collaboration with Jay, JJ van Altena, Douw Grobler and the staff of Makalali. JJ played a huge role in developing the practical aspects of vaccine delivery to elephants in Makalali, Phinda, Thaba Tholo, Shambala and Kaingo. After Makalali, which we regard as our flagship

model for elephant immunocontraception, several new game reserves have made use of immunocontraception. It is important to acknowledge that each of these game reserves have covered all costs of application and monitoring themselves. I would thus like to thank the following parks and people for their contributions towards the success of elephant immunocontraception: Mabula (Jock McMillan, Danie de Bruyn and Drs Pierre Bester and Hendrik Hansen), Phinda (Kevin Pretorius, Jaco Mattheus and Tarryne Dickerson), Thornybush (Mike Pieterse, Melodie Ahlers and Dr Pete Rogers), Thaba Tholo (Ruben Els), Kaingo (Nick Callichy), Shambala, Welgevonden (Andrew Parker and Dave Powrie), Tembe Elephant Park (Wayne Matthews, Nick de Goede and Dr Dave Cooper) and Karongwe (Kobus Haveman and Dr Peter Rogers).

At about the same time I started searching for a better alternative than progestin implants to control fertility of large African carnivores. Wolfgang Jöchle, whom I had met during my time in Zurich, helped to access a new slow-release GnRH agonist called deslorelin. This is also when my association with Tim Trigg (Peptech Animal Health) started and with the help of Tim and Wolfgang we started treating the first African wild dogs (de Wildt), Lions (Mabula Private Game Reserve and National Zoo in Pretoria) and cheetahs (Africat Foundation, Namibia). The initial results were highly successful and soon lead to more animals and species being treated. Once again there are a number of game reserves and sanctuaries that should be mentioned. These are: Mabula (Danie de Bruyn and Patrick), National Zoo in Pretoria, Africat Foundation (Carla Conradie, Dave Houghton) Johannesburg Zoo Phinda (Kevin Pretorius, Jaco Mattheus), Thornybush (Mike Pieterse and Jock Orford), Entabeni (Jan Lessing), Welgevonden (Andrew Parker), Lion Park (Kevin) and Rhino and Lion Park (Ed Hearn) to name some of them. There were a number of veterinary colleagues involved in the capture of many of these animals. Some are: Drs Peter Rogers, Mark Jago, Pierre Bester, Hendrik Hansen, Clare Speedy, Leon Venter, Ian Espie, Michelle Barrows, Katja Köppel, Chap Masterson and Dave Cooper.

Another aspect of wildlife reproduction that became apparent while working with the deslorelin implants was the control of sex-related behaviour in wildlife. It could be achieved using deslorelin in primates and carnivores but this drug did not appear to work in males of some species like domestic cattle and donkeys. It seemed unlikely therefore that it should work in elephant bulls. I had read quite a bit about GnRH vaccines by then and Ben Colenbrander managed to access (and purchase) a vaccine from Pepscan in The Netherlands. So, in 2003 an MSc student of mine, Helen DeNys, started what turned out to be an exciting pilot trial to down-regulate aggressive behaviour and musth in African elephant bulls. I wish to thank Helen, Ben and Johan Turkstra for their valuable contributions. Very soon the project expanded; mainly involving captive but also some free-ranging bulls. Rory Hensman played a huge role in encouraging the use of GnRH vaccines in his elephants placed at Elephants for Africa Forever all over South Africa. Other collaborators have been

Jabulani Elephant Camp, Johannesburg Zoo, Phinda, Bowmansville Zoo, Shambala, Karongwe and many more. More than 35 elephant bulls have been treated, mostly successfully, with GnRH vaccines.

So why, so late in life, would I choose to attempt a PhD degree. It's something that I have always wanted but have never had the opportunity to do. I mentioned this during one of my visits to Prof Ben Colenbrander (promoter) and he never let up since then. Thanks to Ben I have finally completed the task but only thanks to his persuasive efforts. At times it felt like having a second wife. Thank you Ben, not only for the opportunity, but also the help and encouragement you have provided throughout. Thanks also to my other promoter Prof Tom Stout, who has been less persuasive but extremely helpful all along. I never believed that I am a literary genius. At the same time I did not realise that my English grammar was that bad. I would also like to thank Anne-Marie Human who was my laboratory technologist for many years. She carried out most of the hormone assays for the carnivore and elephant bull studies, introduced pZP vaccine production to our lab and made many of the batches we used on elephants in South Africa. She was always friendly, extremely willing to help and a true friend.

I would also like to dedicate this thesis to my Dutch birth father, Kryn Hijbeek, who died after an unfortunate accident in Johannesburg a few months before I was born. I often wonder what you were like and what would have happened to our family if you had lived. Would we have stayed in South Africa, would I have become a vet, met Renate and hundreds of other questions? We will never know but half of me came from you and for that I am truly thankful.

Curriculum vitae

Henk Bertschinger was born on 16 June 1941 in Johannesburg. He matriculated in 1959 at Kingswood College in Grahamstown. He completed his veterinary degree (BVSc) at the University of Pretoria in 1965 and after working for a year in government service in Pretoria he spent the following nine years at the 'Institut für Zuchthygiene', Faculty of Veterinary Science, University of Zurich. Key clinical activities were bovine and porcine andrology and in 1975 he obtained the DrMedVet degree with the following research title 'The hereditary occurrence of diploid spermatozoa in the semen of Brown Swiss bulls'. In 1975 he returned to South Africa and after working for one year at the Onderstepoort Veterinary Research Institute he joined the Department of Physiology, Pharmacology and Toxicology, Faculty of Veterinary Science, University of Pretoria, as senior lecturer teaching physiology and later biochemistry. In 1983 he joined the Department of Reproduction as associate professor and in 1987 was promoted to full professor and head of department. He was then transferred to the Faculty Wildlife Unit in 2001 as acting director and remained there until he retired in 2006. Presently he is emeritus professor as well as extraordinary professor in the Department of Production Animal Studies. Having grown up on a farm in South Africa and being stimulated by visits to the Kruger National Park as a child he has always shown a keen interest in wildlife. Significant professional involvement, however, started in 1975 when he became involved in reproductive research and clinical work at the de Wildt Cheetah and Wildlife Centre. At first this involvement was confined to cheetahs but later it included wild dogs, also at de Wildt. Gradually the number of species expanded to include lions, leopards, buffalo, rhinos and elephants. Over the years the emphasis has also changed somewhat from promoting fertility to contraception of many of these species as well as control of aggressive behaviour. He was also a founding member of the European College of Animal Reproduction, has served on many Faculty, national and international committees and is currently the President of ICAR.