Controlling wildlife reproduction

Hendrik Jan Bertschinger

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 [71]. 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 [50]. 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 [63]. 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 are 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 review reports about investigations on the possibilities of using contraception as a means of regulating the reproductive rate in elephants and a number of large African carnivore species [3]. 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 discusses 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 [14]. 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 [68]. 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 [71], 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, Windhoek, Namibia). 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. The first cheetahs were caught in 2000 and by December 2006, 137 had been removed from farmlands [62]. 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 [24] [64]. 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 [74]. 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, Pretoria, South Africa). 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 [31]. 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 [20] [60]. 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 [29].

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 advice 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 [72], this approach would not be expected to influence testosterone-related behaviour.

Anti-androgens [66] and GnRH agonists [8] 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 [8]. In this respect, GnRH agonists such as deslorelin are not able to down-regulate male reproductive function in cattle [11], horse [73] or donkey stallions (Bertschinger, unpublished, 2000).

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 F2α 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 adenohypophysis. 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 [33].

Recently a new player in the field of GnRH control has been discovered [6]. 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 (mammals with a placenta) [7]. The zona pellucida (ZP) surrounding the oocyte consists of a matrix of three (ZP1, ZP2 and ZP3) and, in some species, four major glycoproteins. Where there are three, ZP2 and ZP3 form a network of fibres that are cross-linked by ZP1 to build up a capsule surrounding the oocyte, which first appears in multi-laminar primary oocytes [76]. ZP3 is thought to provide receptor sites for sperm-binding since genetic deletion results in loss of ZP function accompanied by infertility in mice [7]. While ZP3 binds sperm, a process that is required to initiate the acrosome reaction, ZP2 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 (Figure 1 A), the acrosome reaction is induced during which the outer acrosomal membrane fuses with the plasma membrane to release zonalytic enzymes (Figure 1 B). This also exposes binding sites on the inner sperm acrosomal membrane which interacts with ZP2 and allows the motile sperm to penetrate obliquely through the ZP towards the oolemma.

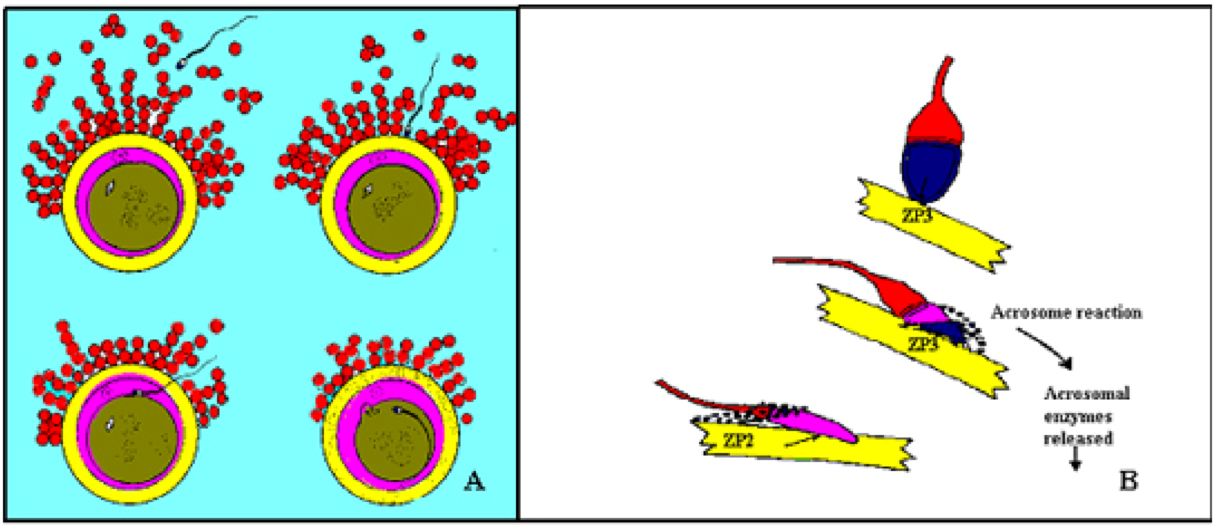

Figure 1. The process of fertilisation (A) and sperm-zona binding resulting in the acrosome reaction (B).

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 [44].

Short history of contraception

According to [16], for thousands of years the only form of contraception practiced on domestic animals was surgical castration. Male castration dates back to 7000 to 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 [16]. 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 [45], 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; Figure 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 [16]. 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. The first use of prostaglandin F2α to abort pregnancy in bitches was reported in 1973 [53].

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

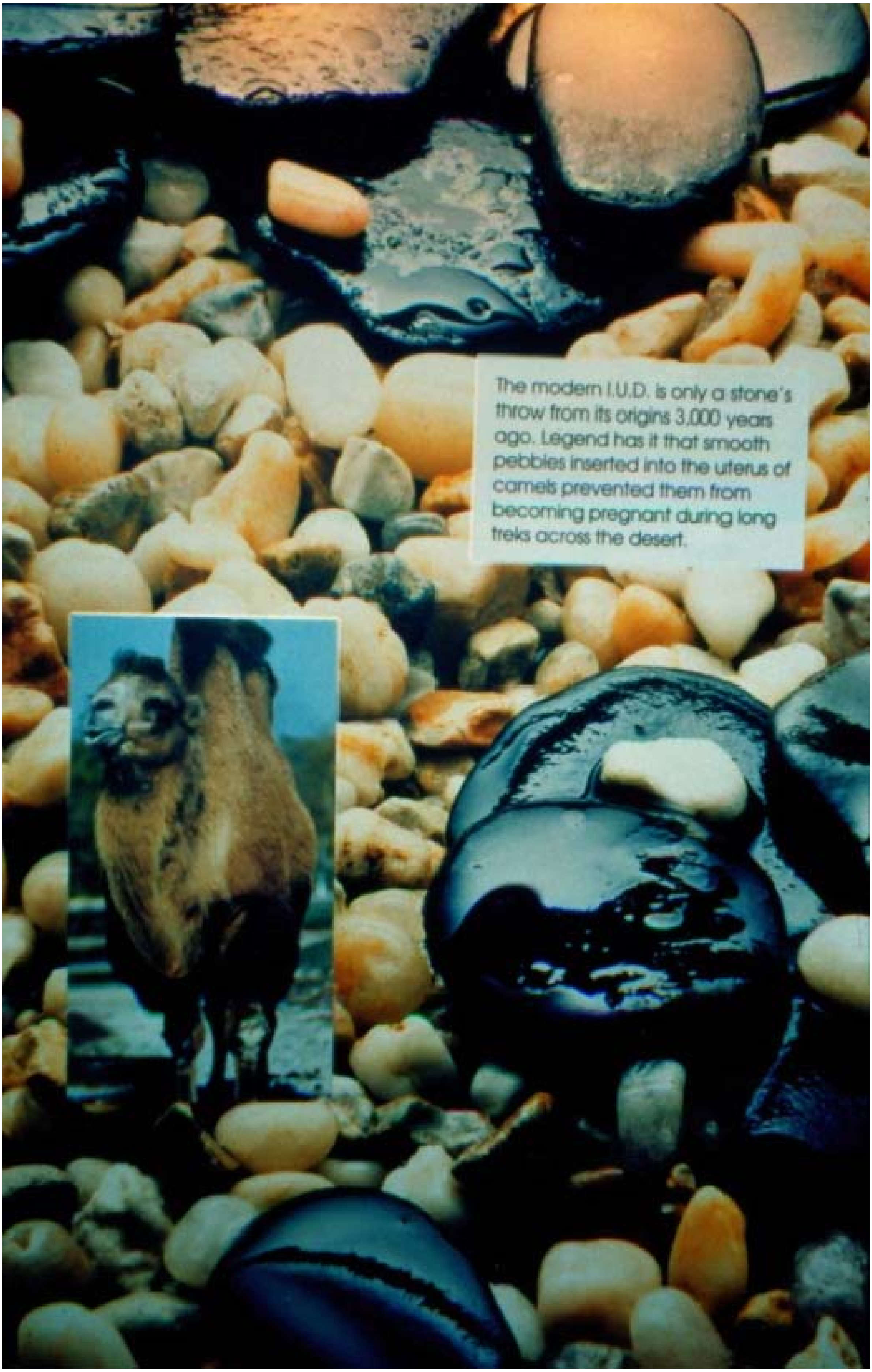
The modern I.U.D. is only a stone's throw from its origins 3,000 years ago. Legend has it that smooth pebbles inserted into the uterus of camels prevented them from becoming pregnant during long treks across the desert.

Figure 2. According to legend, the use of an intrauterine device (IUD) 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 (photograph taken at the 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 (Figure 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 (Figure 3B). Although highly successful as contraceptive agents, their prolonged use resulted in a number of side-effects, some of which were life-threatening [24] [64]. Provided no serious pathology had occurred, the effect of the progestins could be reversed by removing the implant – a process which required immobilisation.

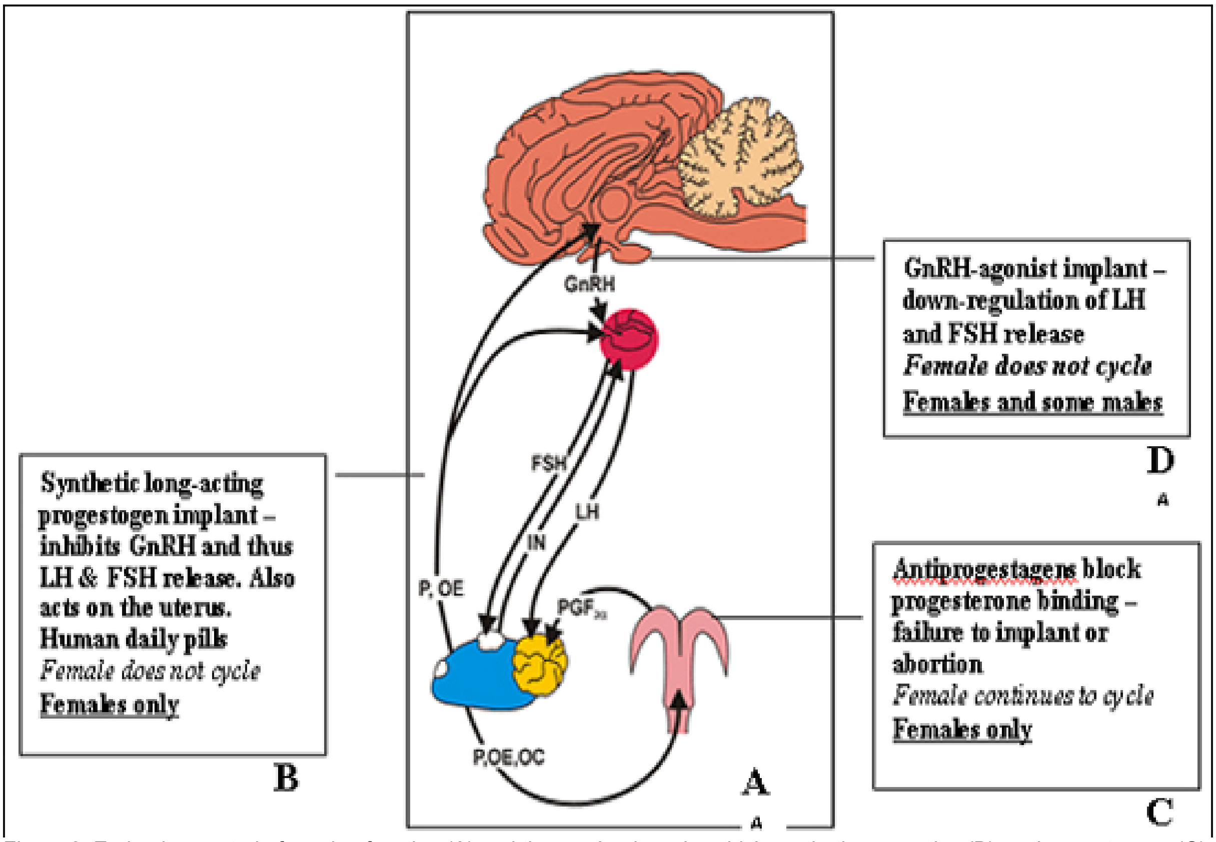

Figure 3. Endocrine control of ovarian function (A) and the mechanisms by which synthetic progestins (B), antiprogestagens (C) [48] and GnRH super agonists (D) exert their contraceptive effects.

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 [17]. 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 [67]. 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 [48], oestradiol treatment resulted in almost continuous oestrus that lasted at least 12 months [32] [36] [77]. No further trials were allowed with the drug.

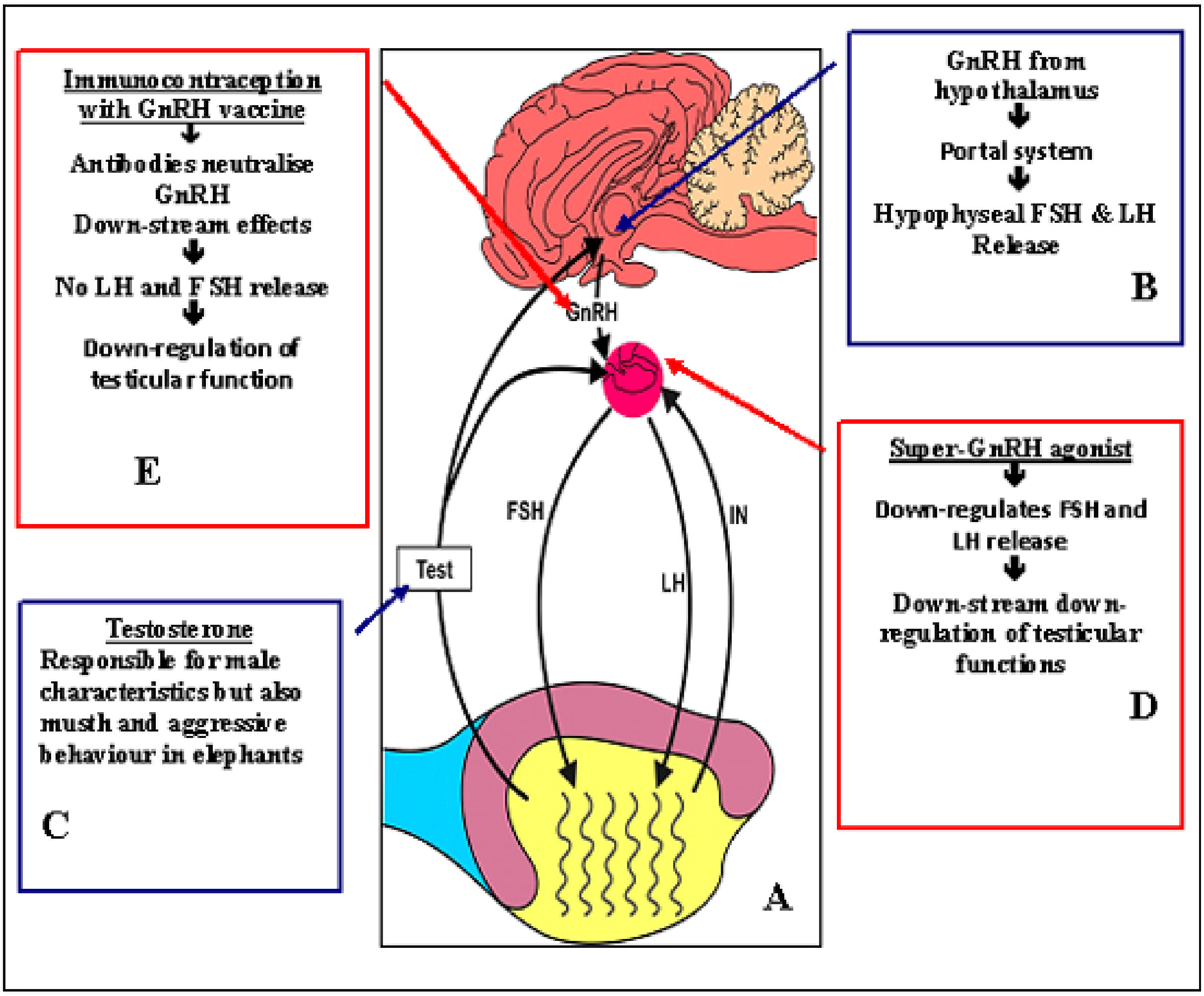

Figure 4. Endocrine control of testicular function (A-C), and the mechanisms by which GnRH super agonists (D) and GnRH vaccines (E) exert their contraceptive effects.

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 [27] [65], 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 (Figures 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 [1]. 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 ZP3 is thought to block sperm-zona binding and thereby prevent fertilisation from taking place (Figure 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 (Figure 4E). In theory, the vaccine should be effective as a contraceptive in both sexes.

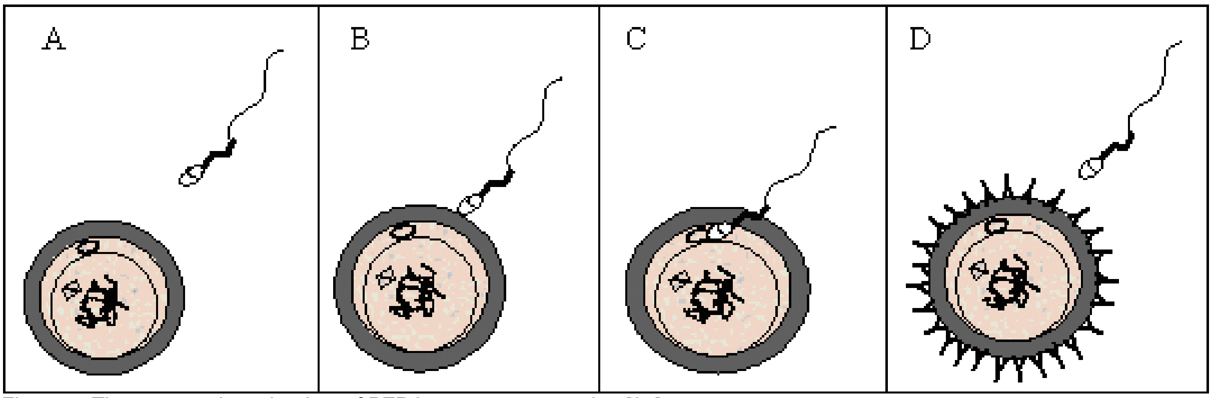

Figure 5. The proposed mechanism of PZP immunocontraception [34]
A - When the egg (oocyte) is ovulated into the Fallopian tube it is surrounded by a capsular layer known as the zona pellucida.
B - Before fertilisation can take place, the sperm must bind to one of thousands of receptor sites on one of 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. It was demonstrated that antibodies to a pZP vaccine could inhibit sperm-zona binding [26]. 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. The first to implement pZP vaccine successfully in domestic mares were Lui, c.s [21]. 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 [69], wapiti [70] and others [12]. The vaccine could be delivered remotely and was both safe and reversible.

GnRH immunocontraception was originally developed for the immunocastration of cattle [51]. 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 [25] [41] [43] [78]. GnRH vaccines have also been used to suppress fertility or reproductive behaviour in male feral pigs [55], stallions [15] [28] [37] [42], rams [52] and bison bulls [23]. GnRH vaccines have also been successfully used to down-regulate fertility and sex-related behaviour in domestic and wild mares [4] [47] [54].

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.

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

Table 1. Extent to which potential wildlife contraceptive agents conform to the ideal properties [2].
*Males can remain fertile for a number of weeks; **Only if given daily; ***Does not work in male ungulates; remote delivery system being developed ; *****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

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 [35]. 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 [46]. 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 [3]. 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 [3]. 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 [20] [60]. 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 [22].

It is difficult, if not impossible, for a single pharmaceutical product or treatment to meet all of the criteria for an ideal contraceptive [2] [3]. 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 [72]. 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, 2004). In the African wild dog, a further likely complication of repeated pseudopregnancies is pyometra [5]. 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 [2] 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 [27] and cats [65]. The same implants were therefore tested for their ability to control reproduction in wild carnivores, and yielded promising results [1]. A more extensive study in cheetahs (n = 31), African wild dogs (n = 21), lionesses (*Panthera leo*; n = 11) and leopards (n = 4) [3] 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): [27] [30] 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 [27]. 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 [30]. 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 [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 [3]. 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 [3]. 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 [38]. Since the African wild dog is highly seasonal with only one oestrus per year [5], 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 [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 [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, 2007). 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 [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 [1]. 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 [5]. 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 [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 [3]. 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 [3]. 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 [18] [21] [56] [57] [58] [59] [61]. 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 [3]. 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): [3]. 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 [9]. In addition, implementation of pZP contraception proved to be practical for free-ranging elephants [10], while no detrimental effects on social behaviour within or between the four herds treated or bulls on the reserve were observed [40].

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 [3]. 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 [2]. 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 [12]. 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 [12]. 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 [19]. 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 [7].

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 [25], down-regulate ovarian activity in mares [4], white-tailed deer [39], and zoo animals [75] and down-regulate reproduction in stallions [15] [73]. 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 [2].

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 [2]. GnRH vaccines developed for the immunocastration and down-regulation of androgen production responsible for boar-taint [11] [25], 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 [3]. 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 [13]. 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 [40], 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.

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Through Prof Ben Colenbrander we were able to access the first GnRH vaccine to ever be used for

the down-regulation of androgen-related behaviour in African elephant bulls. As a result, 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 using a GnRH vaccine produced by Pepscan in The Netherlands.. 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.

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.

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