ORIGINAL ARTICLE

Fertility suppression of some wildlife species in southern Africa—a review

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Contents

Generally speaking, southern Africa's wildlife populations in small-to-medium-sized protected game reserves (10,000–65,000 ha) reproduce at rapid rates which often lead overpopulation of certain species. Most commonly these are large predators such as lions, African wild dogs and cheetahs, and elephants. Overpopulation of large predators leads to depletion of prey species, breakouts into neighbouring communities and increased risks for disease transmission. An overabundance of elephants leads to habitat destruction which is to the detriment of not only other herbivores but also certain bird species. By far, the most acceptable and effective method of population control is contraception. Another problem, particularly in South Africa, is the large numbers of large predators that are held in zoos, wildlife sanctuaries or captive breeding facilities. Once again, there is a need for contraception to control the rate of reproduction. In this review, we discuss the methods that have been most commonly used for some wildlife species in southern Africa. The methods include hormonal control and immunocontraception. We also address the problem of androgen-related aggressive behaviours in elephant bulls and giraffe males and present solutions that have been used to ameliorate such behaviours.

1 | 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. 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 licence, but not veterinarians. It was only in the 18th and 19th centuries that veterinarians slowly entered the castration business. The possible use of intrauterine devices in animals may date back as far as 3,000 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).

It was only during 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, medroxyprogesterone acetate, became available in 1963. The development of "modern" contraceptive methods such as the GnRH super-agonists and immunological tools such as porcine zona pellucida (pZP) and GnRH vaccines did not begin until 20–30 years later.

Contraception to control the rate of reproduction is an interesting and potentially practical means of population control in wildlife, both captive and free-ranging populations. 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 ideal wildlife contraceptive should fulfil most of the following requirements: effective; allow remote delivery; be reversible, although this depends on requirements for individual species and conditions; have

TABLE 1 Extent to which potential wildlife contraceptive agents conform to the ideal properties (modified from Bertschinger et al., 2008a, 2008b)

Property	Surgical methods		Steroids		GnRH agonists		Immunocontraception	
	Gonadect	Vasec/FallT	Oral	Implants	Injectable	Implants	pZP	GnRH
Contraceptive efficacy								
Females	100%	100%	100%[a]	100%	≤100%	≤100%	70%–100%	70%–100%
Males	100%	100%[b]	Poor	Poor	≤100%[c]	≤100%[c]	No	70%–100%
Remote delivery	No	No	Only captive	No	Yes	No[d]	Yes	Yes
Reversible	No	No[e]	Yes	Yes	Yes	Yes	Yes	Yes[f]
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

[a]Only if given daily; [b]males can remain fertile for a number of weeks; [c]does not work in male ungulates; [d]remote delivery system being developed (Herbert & Vogelnest, 2007); [e]requires microsurgery—not feasible under field conditions and especially not in mega-herbivores: [f]long-acting formulations (GonaCon™) or longer-term use in males or pre-pubertal use in both sexes may be irreversible.
Gonadect, gonadectomy; Vasec, vasectomy; FallT, tying of fallopian tubes; NA, not applicable.

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 (Bertschinger et al., 2008b).

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

This article will concentrate on hormonal (also referred to as chemical) and immunological methods. In many wildlife species, surgical methods are impractical, irreversible and, in the case of gonadectomy, affect behaviour.

2 | ENDOCRINE CONTROL OF REPRODUCTION

Central endocrine control of reproduction is organized via the hypothalamo–pituitary–gonadal axis (Fig. 1). The hypothalamic liberin, GnRH, is central to this axis and is the primary controller of reproduction in both female and male mammals. GnRH stimulates the release of both FSH and LH. The gonadotrophic hormones control gametogenesis and gonadal steroid hormone production. Gonadal steroids are responsible for secondary sex characteristics, sexual behaviour and modulate GnRH, FSH and LH release. During the oestrous cycle, the deferential release of these gonadotrophic hormones is complex and depends on the pulsatile nature of GnRH release as well as feedback mechanisms from the ovary. The downstream target cells of FSH and

LH are the developing follicles (theca interna and granulosa cells). In the male, FSH specifically binds to Sertoli cell membrane receptors and is thus intricately involved in the regulation of spermatogenesis. Leydig cells on the other hand are targeted by LH which stimulates testosterone synthesis and secretion. Androgens also affect sperm production but more specifically the reduction division (spermatidogenesis) and spermiogenesis. The function of secondary reproductive organs such as the prostate gland is stimulated by testosterone metabolites, notably dihydrotestosterone (Hewit, 2001), but other hormones as well.

Recently, new players in the control of reproduction have been discovered. Collectively known as kisspeptins and secreted by kisspeptin neurons in the hypothalamus, these hormones control the activity of
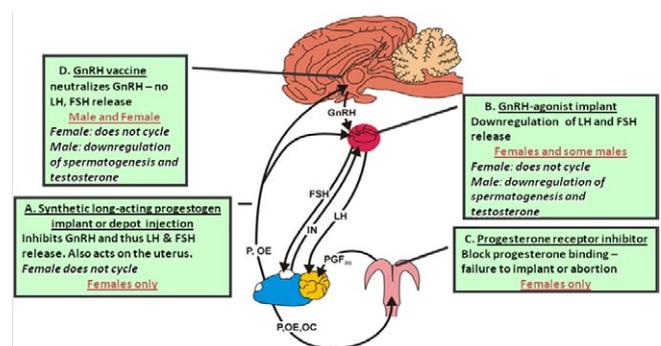
FIGURE 1 Endocrine control of female reproduction and mechanism of action of progestins (a), GnRH agonists (b), progesterone receptor inhibitors (c) and GnRH vaccines (d) (modified from Bertschinger et al., 2008b)

GnRH neurons. Furthermore, the kisspeptin neurons provide the links with the CNS, the environment, metabolic state and the reproductive system of an animal. Kisspeptins play a central role in puberty, and chronic administration of kisspeptin has been shown to induce precocious puberty in rats (Navarro et al. 2004). Kisspeptin administration in dogs (Albers-Wolthers et al., 2014) and ewes (Arreguin-Arevalo, Lents, Famerie, Nett, & Clay, 2007) resulted in LH peaks. Also, the photoperiod, which controls the release of melatonin from the pineal gland, is known to influence oestrous cycles of seasonal breeders. It is now established that melatonin exerts this effect via the kisspeptin neurons in the hypothalamus (Revel et al., 2007).

3 | HORMONAL METHODS OF FERTILITY SUPPRESSION

3.1 | Progestins or progestogens

Progestins have, for practical purposes, the same effect as progesterone on the hypothalamus and act by inhibiting the release of GnRH thus bringing about a downstream lack of FSH and LH release (Fig. 1A). As a result, a new oestrous cycle is inhibited (anoestrus). A range of oral progestins were developed for use in the dog and cat (e.g. ovarid, megestrol acetate; Jöchle, 2008) and the horse (altrenogest; Webel & Squires, 1982). Some of these preparations are used alone, or in conjunction with other agents (e.g. deslorelin implants; Wright et al., 2001), to control reproduction of a variety of zoo species. Daily intake is required to avoid a rebound release of GnRH. Thus, the use in free-ranging wildlife is out of the question. Long-acting silicone implants or depot injections containing progestins like melengestrol acetate have been extensively used to downregulate female reproduction in wild felids such as lions and tigers and some wild canid species. Although highly efficacious as contraceptive agents, their prolonged use resulted in a number of serious side effects such as pyometra, which were life-threatening (Munson, Moresco, & Calle, 2005). Provided no serious pathology had occurred, the effect of the progestins could be reversed by removing the implant — a process which required immobilization. Similar products are regularly used for the contraception of females in a range of primate species with far less side effects.

3.2 | GnRH super-agonists

During the late 1990s, a new hormonal method of contraception became available. The GnRH analogue deslorelin, a so-called GnRH super-agonist, was manufactured in a slow-release formulation that released deslorelin for periods of 6–12 months, or even longer, and was designed for use in dogs and cats (Munson, Bauman, Asa, Jöchle, & Trigg, 2001; Trigg et al., 2001). High continuous release of deslorelin from the subcutaneous biocompatible implant (Suprelorin, Virbac, France) acts by downregulating the release of FSH and LH from the anterior pituitary (Fig. 1B). This in turn leads to downregulation of gonadal activity and has the potential to be effective in both males and females. Following the successes achieved in male and female dogs and cats, preliminary trials were conducted in lionesses, male and

female cheetahs, African wild dogs and leopards (Bertschinger, Trigg, Jöchle, & Human, 2002). With the exception of wild dog females, a contraceptive efficacy of 100% was achieved. It is now the most commonly used method of contraception for captive and free-ranging carnivores and can be used in many primates and a range of other species. Following the administration of the implant, an initial spike of FSH and LH is experienced before the pituitary gonadotrophs are downregulated. In females, this may induce a fertile oestrus in dogs (Trigg et al., 2001) and wild dogs (Bertschinger et al., 2001), but in large cats, such as cheetahs and lions, only a short rise in oestrogens and progesterone, which does not seem to be compatible with fertility, occurs (Bertschinger, De Barros Vaz Guimarães, Trigg, & Human, 2008a and Bertschinger et al., 2002, 2008b) . Nevertheless, many zoos will advocate suppression of oestrus with daily oral progestins starting 7 days prior to and continuing to 7 days after administering the implant (recommendations by the European Group on Zoo Animal Contraception). This regimen was first described for the domestic dog (Wright et al., 2001). In free-ranging cheetahs, lions and leopards, where this is not possible, we have never observed pregnancies following placement of the implants (Bertschinger et al., 2002, 2008a, 2008b). Reconception after single treatments with 1 × 4.7 mg and 2 × 4.7 mg implants occurs on average after 24 and 30 months in cheetahs (unpublished data) and lions (Bertschinger et al., 2008a, 2008b), respectively. Female lions, but not cheetahs, are prone to develop obesity after repeated treatments. We also demonstrated two reversals in one free-ranging female leopard following treatment with 1 × 4.7 mg. Reconception occurred after 24 and 26 months, respectively (unpublished data).

We have treated 66 male cheetahs with 1 × 4.7 mg deslorelin implants on an annual basis and some for up to 9 years without any side effects being recorded. Downregulation of fertility in males takes longer than in females with viable sperm being noted for up to 6 weeks post-treatment or longer (epididymal origin). During the first 2 years following annual treatment, a rapid decrease in testicular volume to 59.6% and 47.5% of original volume, respectively, is observed. This reflects a rapid decline in spermatogenesis. This was followed by a slower decline to reach between 29 and 24% of the original volume after 5–9 years of annual treatment (Fig. 2). We have demonstrated
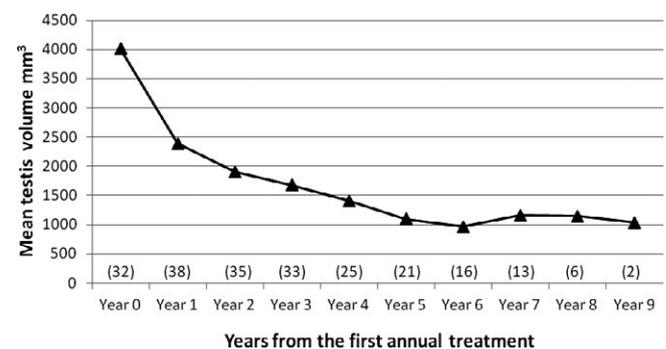
FIGURE 2 Effects of annual 1 × 4.7 mg Suprelorin implants on mean testicular volume from day of first treatment (Year 0) up to Year 9. Numbers in brackets indicate number of observations for each year

return to fertility in one male treated annually for 3 years. He was released into the wild with two females and was able to mate successfully 2 and 3 years after his last treatment. So far we have not been able to demonstrate reversal in males treated annually for 5 years or longer (unpublished data).

3.3 | Induction of abortion in some wild carnivores

Particularly when examining free-ranging carnivores for contraception, the pregnancy status of the animal will be unknown. If a female is found to be pregnant, we offer the owner the option of abortion, which can be combined with deslorelin treatment. We have used two methods in large carnivores: luteolytic prostaglandins and the progesterone receptor antagonist aglepristone (Fig. 1C).

3.3.1 | Luteolytic prostaglandins

Initially, we used dinoprost (Lutalyse, Zoetis, South Africa; Bertschinger et al., 2008a, 2008b) but now we have changed to cloprostenol (Estrumate, Intervet) which has much less effect on the smooth muscle of the GIT and is thus safer. Use one-third of the cattle dose (8 mg dinoprost and 170 μg cloprostenol) for a lion or tiger. It is important to administer three doses with the same dose on three consecutive or alternate days (Table 2). The follow-up treatments can be administered with a dropout dart. In African wild dogs, the side effects of dinoprost are similar to those observed in domestic dogs. We thus advise against the use of this drug in wild dogs.

3.3.2 | Aglepristone (Alizine®, Virbac, South Africa)

Alizine® is registered for use in domestic dogs in South Africa, and the recommended dose to induce abortion is 1 ml/3 kg (30 mg aglepristone) repeated a day later. We used Alizine® as an extra-label drug to successfully induce abortion in African wild dogs and lions. Five 3- to 4-year-old captive wild dogs weighing 20–25 kg were each treated *once* with 8 ml Alizine® (approximately the same dose prescribed for the domestic dog). The Alizine® was injected intramuscularly in two

TABLE 2 Induction of abortion in lions and tigers with dinoprost (modified from Bertschinger et al., 2008a, 2008b)

Species	SPC (nmol/L)	Stage of gestation	Dinoprost treatment
Tiger	177.92	<3 weeks	7.5 mg on 3 alternate days; 2 weeks later
Lion	194.08	3 weeks	7.5 mg on 3 consecutive days from PD
Lion	107.29	3 weeks	7.5 mg on 3 consecutive days from PD
Tiger	63.70	70 days	7.5 mg on 3 alternate days from PD
Lion	212.80	80 days	7.5 mg on 3 consecutive days from PD

sites using a pole syringe while the dogs were trapped in a crush. Stage of pregnancy varied from 21 to 55 days. Abortion took place 5–7 days after treatment, and no complications were observed.

Fourteen 3- to 9-year-old lionesses weighing an estimated 150–210 kg and during the first to third trimester of gestation were successfully aborted with Alizine®. The immobilized lionesses were given a *single* total dose of 35–45 ml each (administered in several sites) which is ≈70% of the dose recommended for the domestic dog. Abortion ensued after 3–5 days, no foetal remains were seen and only one female was seen with a serosanguineous vaginal discharge on Day 3 post-treatment. All lionesses were immobilized 7–10 days after treatment for the purpose of contraception when they were all healthy.

4 | IMMUNOCONTRACEPTION

Immunocontraception relies on carefully selecting peptides that are involved in critical steps of reproduction. Including such a peptide or peptides as antigens in a vaccine provokes the production of antibodies that neutralize the endogenous molecule or block a particular process. Examples of vaccines commonly in use are GnRH and native porcine zona pellucida (pZP) vaccines.
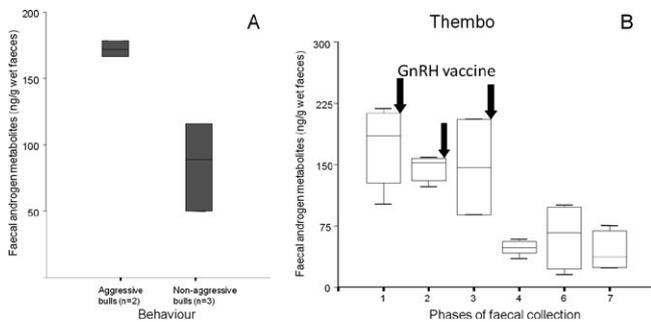
4.1 | GnRH vaccines

The method involves the use of GnRH vaccines that stimulate the production of anti-GnRH-specific antibodies. The antibodies neutralize endogenous GnRH and, in so doing, suppress downstream endocrine mechanisms that control reproductive functions of both females (Fig. 1D) and males. GnRH immunocontraception was originally developed for the immunocastration of cattle (D'Occhio, 1993), but one of the main reasons for further development of these vaccines was to use them as an alternative to surgical castration for the control of boar taint in pork (Dunshea et al., 2001). GnRH vaccines have also, amongst others, been used to suppress fertility or reproductive behaviour in male feral pigs (Killian, Miller, Rhyan, & Doten, 2006) and white tailed deer (Miller, Johns, & Killian, 2000). In mares, they have also been applied successfully to downregulate fertility (Botha et al., 2008) and sex-related behaviour (Elhay et al., 2007).

The association between aggressive behaviour, especially during musth, and testosterone concentrations is clearly established in African elephant bulls. Bulls with raised testosterone levels and particularly during musth, when peak levels are reached, are very difficult to manage in captivity and also in smaller game reserves in South Africa. The positive relationship between the frequencies of aggressive behaviours and faecal androgen metabolites (FAM) is shown in Fig. 3A. In 2003, we initiated a study to see whether testosterone and thus aggressive behaviour could be downregulated in six bulls with GnRH vaccine treatments. The bulls that were aggressive (*n* = 2) or in musth (*n* = 1) at the start of treatment responded very well. Fig. 3B shows the effect of three treatments with a GnRH vaccine (Pepscan Systems, Lelystad, the Netherlands) on FAM concentrations of a problem bull (highly aggressive but not in musth) in

FIGURE 3 a: Association between frequency of aggressive behaviours and faecal androgen metabolite concentrations in five African elephant bulls. b: Effect of three GnRH vaccinations (black arrows) on faecal androgen concentrations in an initially aggressive but non-musth African elephant bull (modified from De Nys et al., 2010)

FIGURE 4 Mechanism of action of the pZP vaccine

one of the game reserves (De Nys et al., 2010). The bull in question, Thembo, is now in captivity and has been treated with a GnRH vaccine for the past 12 years. He is 32 years old and, since the start of treatment, has never shown musth or aggressive behaviour. Since our initial trial in 2003 we have treated at least 40 bulls successfully to control aggressive behaviour and musth. In 2006, the GnRH vaccine Improvac® (Zoetis, South Africa) was registered for use in pigs in South Africa, and, after we had established that it was also effective, it was routinely used to control aggressive behaviour and musth in elephant bulls (Bertschinger & Sills, 2013).

Until 4 years ago, however, we did not know what effect GnRH vaccine would have on fertility of African elephant bulls. To investigate this, we studied the effect of repeated Improvac® treatments on 11 captive and 2 free-ranging bulls (8–35 years of age) on semen quality and the internal reproductive organs. The bulls were examined and then treated twice with 5 ml (1,000 μg) Improvac® (Day 0 and 35) and then boosted with the same dose every 5 months. Follow-up examinations were carried out at 6-monthly intervals for 2–3 years. After 6 months, testicular size had reduced significantly, and by the end of the study, they were only 40% of the original area. 6 months after the first vaccination ejaculates either contained dead sperm or were aspermic (Lueders et al., 2014). The trial clearly showed that the GnRH vaccine can be used as a contraceptive vaccine and at a fraction of the cost of vasectomy, which is a major surgical procedure in the elephant requiring highly specialized equipment. Currently, we are monitoring some of the bulls to establish reversibility of the method on fertility. Furthermore, the method is being used in two game reserves for population control. Given the long intercalving interval in elephants (4–6 years), the results are not available yet.

During the period 2011–2015, we have also used Improvac® to control problem free-ranging male giraffes on six game reserves in South Africa. One adult bull (8 years old) and five 3- to 4-year-old pubertal males were treated. Typical problems associated with such males are that they attack motor vehicles, people on foot and giraffe calves that are sometimes their own offspring. Each male was darted intramuscularly with two doses of Improvac® (800 μg each) 5 weeks apart. All males responded well with problem behaviour ceasing after
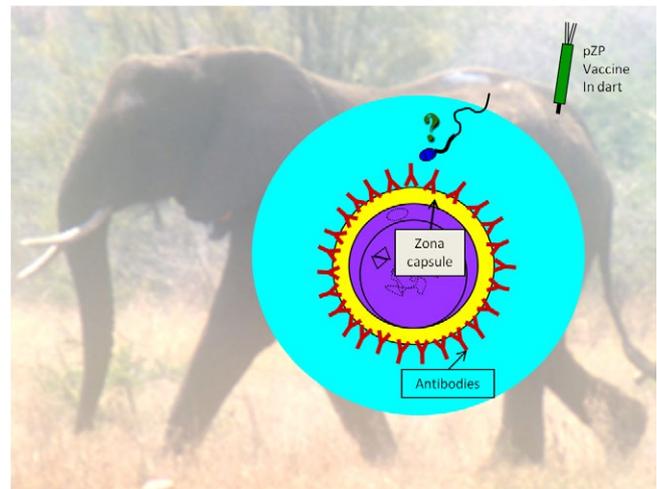
the primary immunization. So far, follow-up treatment has not been necessary in any of the males (unpublished data).

4.2 | pZP immunocontraception

The zona pellucida capsule consists of 3 or 4 glycoproteins some of which are essential to the fertilization process. Before a sperm can penetrate the zona capsule, it must bind to a sperm receptor site. This process induces the acrosome reaction which, in brief, enables the penetration process. If a female is treated with the pZP vaccine, she produces antibodies which bind to zona proteins surrounding the oocyte. The process is believed to block sperm-zona binding and thereby prevents fertilization from taking place (Fig. 4). As long as antibody titres are sufficiently high, fertilization will be prevented but, because reproductive hormones are unaffected by this process, the female will continue to cycle normally. The origins of the pZP contraceptive vaccine date back to 1973 when researchers demonstrated that antibodies to pZP proteins could inhibit sperm-zona binding. In 1989, the first paper on contraception of domestic mares with pZP vaccine was published (Liu, Bernoco, & Feldman, 1989). 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 in other wildlife species (≈100 species; Kirkpatrick, Lyda, & Frank, 2011). The vaccine can be delivered remotely and is both safe and reversible.

Although pZP immunocontraception was first tested on elephants in the Kruger National Park in 1996 (Fayrer-Hosken, Grobler, van Altena, Bertschinger, & Kirkpatrick, 2000; Fayrer-Hosken et al., 1999), it was only from 2000 that the method was routinely used for population control of African elephants in The Makalali Game Reserve (Delsink et al., 2006, 2007). When used in smaller reserves where elephant cows can be individually identified, the method is 100% successful. It is safe to the cow, safe during pregnancy, has no effect on behaviour (Delsink et al., 2013) and is reversible, at least in the medium term (Bertschinger et al., 2012). The vaccine is made in our laboratory

and is now supplied to 22 private and five national or provincial game reserves where, in total, approximately 700 cows are being treated for population control.

5 | CONCLUSIONS

Contraception is an ideal solution for population control of free-ranging wildlife. Contrary to methods such as culling and removal of animals, both of which reduce population density, it does not stimulate reproductive rate. Here, we presented one hormonal and two immunological methods that have been used for population and androgen-related behaviour control in some African species. The successes have mostly been good but it should be remembered that no method meets all the properties of an ideal contraceptive. Furthermore, although we have a range of effective methods, there is a need to critically investigate possible side effects in each species where they are applied. Apart from the obvious health issues, possible effects on social and territorial behaviour are very important and often neglected.

CONFLICT OF INTEREST

All authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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