

Use of anti-gonadotropin-releasing hormone vaccines in African elephants (*Loxodonta africana*): A review



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Dates:

Received: 24 Oct. 2017

Accepted: 30 May 2018

Published: 30 July 2018

How to cite this article:

Bertschinger, H.J. & Lueders, I.,
2018, 'Use of anti-
gonadotropin-releasing
hormone vaccines in African
elephants (*Loxodonta
africana*): A review', *Bothalia*
48(2), a2320. [https://doi.
org/10.4102/abc.v48i2.2320](https://doi.org/10.4102/abc.v48i2.2320)

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Background: Androgen-related aggressive behaviour and musth cause serious problems in captive African elephant bulls and often lead to human and animal injuries, and damage to property.

Objectives: To review the work carried out with anti-gonadotropin-releasing hormone (GnRH) vaccines to control androgen-related behaviour and fertility in captive and free-ranging elephant bulls and the induction of anoestrus in elephant cows.

Method: In the first study, an anti-GnRH vaccine from Pepsican was tested in six bulls (four captive and two free-ranging). Once the vaccine Improvac® became available, the effect on behaviour, the reproductive organs and semen quality was tested. Improvac® was also used to attempt induction of anoestrus in elephant cows.

Results: The first study proved that aggressive behaviours are significantly associated with increased faecal androgen concentrations. Musth ($n = 1$) and aggressive behaviour ($n = 2$) were down regulated and correlated with a decline in faecal androgen concentrations. Aggression and musth could be controlled with Improvac® (600 µg), but were more consistent when the dose was increased to 1000 µg administered every five to six months. The same dose down regulated testicular function and bulls ($n = 17$) were rendered infertile within 12 months after commencement of treatment. Initial attempts to induce anoestrus with 600 µg in free-ranging elephant cows gave inconclusive results, but 1000 µg in captive cows delivered five-monthly was successful.

Conclusion: The treatment of elephant bulls with Improvac® resulted in the successful down-regulation of androgen-related behaviour and sperm production in captive and wild elephant bulls of various ages (≤ 34 years). Preliminary studies to induce anoestrus in cows with Improvac® appear to be successful.

Introduction

Musth in elephant bulls

In the wild, age, social ranking and reproductive behaviour, the latter being androgen-dependent, are the three most important factors that determine the likelihood of a bull being able to mate with cows in oestrus. Hollister-Smith, Poole and Archie (2007:287–296), determined the paternity of calves born in Amboseli over a 30-year period. The authors showed that African elephant bulls (*Loxodonta africana*) that were 45 years and older sired 50% of all calves. They also showed that 75% of progeny were sired by bulls that were in musth at the time of mating. Although physical stature and strength are known to affect ranking, older bulls in musth are more likely to be afforded the chance to breed than non-musth bulls. The physiological signs of musth are (1) swelling of the temporal glands accompanied by thick mucoid exudates and (2) during the height of musth, urine dribbling (Ganswindt et al. 2010:506–514). Watery exudates from the temporal glands are seen in both sexes and all ages of elephants and are associated with excitement and frights (Bertschinger et al. 2008:257–328). Musth is accompanied by extremely high blood levels of testosterone (Kaewmanee et al. 2011:379–383). In free-ranging African elephants, the first signs of musth occur at about 25 years of age. In the absence of older dominant bulls, however, musth may occur at an earlier age. This is significant for bulls that are raised in captivity or translocated to reserves where there are no adult bulls present. In the wild, regular musth cycles, which are bull specific, only occur from about the age of 35 (Eisenberg, McKay & Jainudeen 1971:193–225; Ganswindt et al. 2010:506–514).

Aggression in elephant bulls

Testosterone is not the only hormone controlling aggressive behaviour in animals. In laboratory animals, the wiring for dominance and behaviour is established early in perinatal life (Yu & Shi

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2009:44–49). Testosterone acts by stimulating existing pathways from puberty onwards. Very likely, the same applies to elephant bulls. Not all bulls in musth with blood testosterone levels of ≥ 100 nmol/L behave aggressively towards humans, objects such as vehicles or other animals. Life experiences and the influence of adult elephants certainly play an important role in these intelligent animals. In bulls that are raised in captivity under hands-on conditions, the keepers or grooms take on the role of the dominant individuals. Many bulls, in particular, start to challenge this hierarchy as they approach puberty. During this period, testosterone concentrations start increasing, and bulls become more assertive, difficult to handle and, sometimes, aggressive. Following a number of incidents in South Africa and Zimbabwe, the so-called problem bulls were euthanised. In larger reserves, African elephant bulls seldom pose a problem, but in small reserves, encounters with bulls in musth are probably more frequent. Reportedly, lone elephant bulls learn to chase tourist vehicles. Inevitably, when accidents happen, the elephants and not the drivers are deemed to be at fault (Bertschinger & Sills 2013:85–107).

The need for a method to control musth and aggressive behaviour in free-roaming ‘problem bulls’ and captive, hands-on elephant bulls to avoid animal welfare relevant practices is thus clear. Rarely, surgical castration has been performed in Asian elephants in Western zoos. Because of the intra-abdominal location of the testicles, this procedure is not without risk to the animal. Castration was performed on a young bull in Zimbabwe (currently kept in South Africa). This animal, although in his 20s now, has remained in direct contact for more than 18 years without problems (R. Hensman, *Adventures with Elephants*, June 2010, pers. comm.). For free-ranging bulls, gonadectomy would require experienced veterinarians and heavy equipment. Therefore the method is not cost-effective and, of course, is irreversible. Earlier attempts to control musth and aggressive behaviour in elephant bulls using anti-androgens (Niemuller, Brown & Hodges 1998:1018–1029) and the gonadotropin-releasing hormone (GnRH) agonist leuprolide (De Oliveira et al. 2004:70–76) were met with limited success. The development of commercial anti-GnRH vaccines to control reproduction and associated behaviour in domestic and some wildlife species offered the opportunity to test two different formulations in elephant bulls in southern Africa.

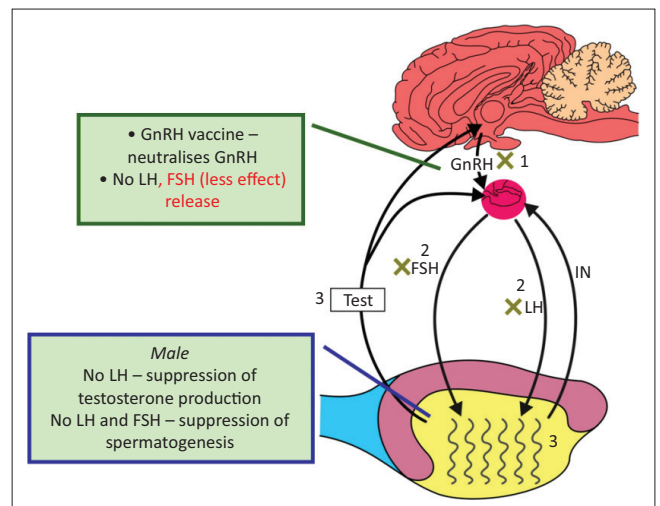
Ethical considerations

The project was approved by the Animal Ethics Committee of the University of Pretoria (Permit No. V016-12).

Anti-gonadotropin-releasing hormone vaccines

Mode of action

The hypothalamic hormone, GnRH, controls the release of follicle stimulating hormone (FSH) and luteinising hormone (LH) from the pituitary gland. Simplified, FSH largely regulates spermatogenesis, and LH controls testicular



Source: Modified from Bertschinger, H.J. & Sills, E.S., 2013, 'Contraceptive applications of GnRH-analogs and vaccines for wildlife mammals of southern Africa: Current experience and future challenges', in E.S. Sills (ed.), *Gonadotropin-releasing hormone (GnRH). Production, structure and function*, pp. 85–107, Nova Science Publishers Inc., New York, (eBook) LH, luteinising hormone; FSH, follicle-stimulating hormone; Test, testosterone; IN, inhibin.

FIGURE 1: Endocrine control of testicular function and mode of action of anti-gonadotropin-releasing hormone (GnRH) vaccines. Anti-GnRH antibodies neutralise endogenous GnRH (1); down-regulate LH (2) and FSH (2) release; spermatogenesis (3) and testosterone synthesis and release (3).

testosterone secretion. The control mechanisms are intricate and involve feedback mechanisms (Figure 1). Anti-GnRH immunocontraceptive vaccines specifically target GnRH as a result of anti-GnRH antibodies, which are formed in response to treatment. The antibodies neutralise endogenous GnRH, which leads to a lack of stimulation of the hypophysis and thus no secretion of FSH and LH and, as a result, down-regulation of gonadal function. The anti-GnRH vaccines have the potential to be used in both sexes to control reproduction and associated behaviour.

Anti-gonadotropin-releasing hormone vaccine in other species

Anti-GnRH vaccines were first developed for the immunocastration of cattle (D'Occhio 1993:345). One of the main reasons for further development of these vaccines was to control boar taint in pork as an alternative to surgical castration (Dunshea et al. 2001:2525–2535). In stallions, similar vaccines have been used to control androgen-related behaviour and testicular size (Dowsett et al. 1996:228–235; Janett et al. 2009:88–102; Malmgren, Andresen & Dalin 2001:78–83; Turkstra et al. 2005:247–259). In this respect, the anti-GnRH vaccine Improvac® (registered for use in pigs; Zoetis Animal Health, South Africa) has been shown to be highly effective in young stallions (Birrell 2017:1–49). The same vaccine was also successfully employed to control the oestrous cycle and thus fertility in mares (Botha et al. 2008:548–554; Schulman et al. 2012:1–3). Reversibility was demonstrated in 47 of 51 mares, with a return to normal cyclicity within two years of last treatment (primary and one booster). Anti-GnRH vaccines have also been used in a number of other species to control fertility or androgen-related behaviour. Examples are sheep (Janett et al. 2003:291–299), rats and dogs (Ferro et al. 2004:73–86), cats (Levy et al. 2004:1116–1130), feral swine (Killian et al.

2006:378–384), camels (Ghoneim et al. 2012:1102–1109), goats (Godfrey 1996:41–54), white-tailed deer (Miller, Johns & Killian 2000:266–274) and bison (Miller, Rhyan & Drew 2004:725–730).

Use of anti-gonadotropin-releasing hormone vaccines in elephant bulls

First anti-gonadotropin-releasing hormone vaccine study in elephant bulls

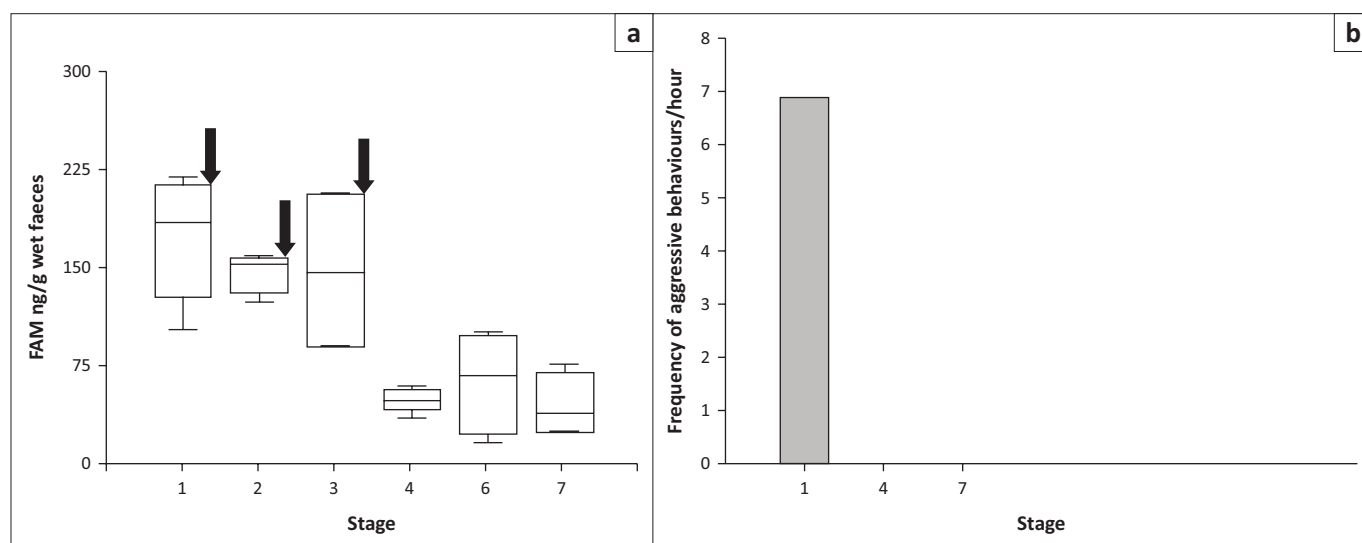
In 2003, the first study to control aggressive behaviour and musth in African elephant bulls with an anti-GnRH vaccine was carried out on four captive and two free-ranging bulls in southern Africa (De Nys et al. 2003:17, 2010:8–15). Only one of the four captive bulls was showing aggressive behaviour at the onset of treatment. The three others had shown periods of aggressive behaviour previously. One free-ranging bull was in musth and the other one was aggressive and had destroyed a number of the facilities on the reserve. A modified GnRH-tandem-dimer-ovalbumin conjugate vaccine (Pepscan Systems; Lelystad, Netherlands) combined with Covaccine™ (Covaccine B.V.; Utrecht, the Netherlands) as adjuvant was used. Three captive and two free-ranging bulls were treated three times at intervals of three to seven weeks. A fourth captive bull was first treated using a different adjuvant (Montanide ISA 51VG, Seppic; Paris, France), but owing to incomplete injection on account of high viscosity, a fourth treatment using Covaccine™ was administered. Faecal androgen concentrations were monitored on consecutive days prior to treatment and at intervals after each vaccination. A validated assay, known to reflect blood testosterone concentrations in African elephant bulls, was used to monitor faecal androgen concentrations (Ganswindt et al. 2002:27–30; Palme & Möstel 1994:111–117). Behaviour was also recorded and related to faecal androgen concentrations. Aggressive behaviour before vaccination was positively correlated with faecal androgen concentrations.

The behaviour of the captive aggressive bull improved after the fourth vaccination. Both free-ranging bulls showed an improvement in behaviour. Signs of musth ceased 10 days after the first vaccination and aggression in the other bull ceased after the third vaccination (Figure 2b). Furthermore, at commencement of the study, faecal androgen concentrations were significantly higher in the two aggressive bulls than in the three non-aggressive bulls. Figure 2a shows the response of the faecal androgen concentrations to the anti-GnRH vaccine in the non-musth free-ranging aggressive bull Thembo. Overall, the results were encouraging. The three non-aggressive captive bulls in Zimbabwe (Imire Rhino and Wildlife Conservation) remained tractable for the duration of the four-month study. Monitoring of these bulls continued after the end of the trial. One bull (age 27 years) entered musth 10 months after the last vaccination. During this musth period, he killed a rhino bull on the same property and was subsequently euthanised. The two other elephant bulls (ages 28 and 18 years) received no further treatments, but became problematic in 2007 and 2009, respectively, when they were treated with Improvac®. After resumption of five-monthly treatments, no further problems were reported. The two bulls are currently 38 and 28 years old, and although musth should have occurred by now, the anti-GnRH vaccine appears to have suppressed this state completely.

The free-ranging aggressive bull (Thembo, age 18 years) was moved to a sanctuary in 2005 where treatment with the Pepscan vaccine was resumed and retreated every five to six months. Since 2006, he has been treated regularly with Improvac®. He is currently 35 years old and remains tractable.

Additional bulls treated with Improvac® vaccine in southern Africa

Since the first trial in 2003, more than 45 additional bulls, mostly captive, but some wild, were treated with



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

FIGURE 2: (a) Box and whisker graphs showing the grouped concentrations of faecal androgen metabolites (FAM) for free-ranging aggressive bull Thembo before (Stage 1), during (Stages 2 and 3) and after (Stages 4, 5 and 6) anti-gonadotropin-releasing hormone (GnRH) immunisations. (b) Frequency of aggressive behaviours before (Stage 1) and after (Stages 4 and 7) anti-GnRH immunisations.

Improvac® to control aggressive behaviour and musth (Bertschinger et al. 2009:71; Bertschinger & Sills 2013:85–107). Indications for treatment were always the presence of one or more bulls on the property showing bull-related dominant and aggressive behaviour, which sometimes resulted in serious injuries to elephant groomers. The initial dose tested was 600 µg GnRH protein conjugate (= 3 mL Improvac®). The primary treatment was followed by a booster five weeks later and with follow-up boosters every six months, or earlier if required, using the same dose. Some sanctuaries, however, reported bulls reversing earlier with this dose. As a result, the dose was empirically increased to 1000 µg (5 mL) of GnRH protein conjugate with intervals of five to six months between boosters. The increased dose has produced much more consistent results.

Improvac® vaccine is now being used as a behavioural management tool rather than as an experimental drug. Consequently, monitoring of behaviour is carried out by owners or managers who are likely to have been subjective. Nonetheless, the number of serious incidents was reduced to zero and tractability was improved to such a degree that sanctuaries preferred to continue with vaccinations. Where the collection of blood samples has been possible, serum testosterone concentrations have either been very low or undetectable. Some examples are shown in Table 1.

Does Improvac® affect the fertility of treated bulls?

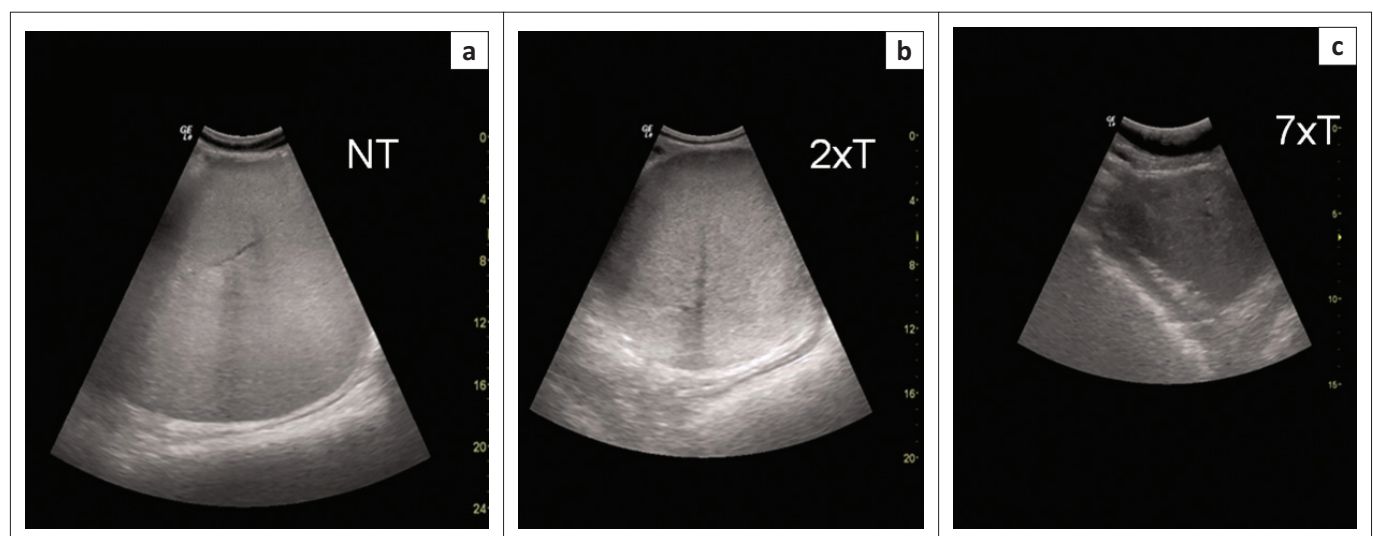
This question was addressed in a study carried out recently in South Africa (Lueders et al. 2017:1–21). A prospective group of 11 bulls (Group 1; nine captive and two wild) aged eight to 36 years, after the initial primary and first booster (five weeks later), were treated every five to seven months with Improvac® (1000 µg) by deep intramuscular injection (i.m.). Prior to vaccination and six-monthly, the internal genital organs were examined by means of trans-rectal ultrasound, semen was collected and evaluated, and serum testosterone concentration was measured over a period of three years. Another six bulls aged 19–33 years, which had been treated for 5–11 years were subjected to a once-off examination as described above (Group 2; four captive and two wild). Behaviour of Group 1 bulls was monitored subjectively throughout.

There was a marked effect of the vaccine on all variables measured. Mean testicular diameter in Group 1 decreased from 13.3 cm × 15.2 cm at the beginning to 7.6 cm × 10.2 cm after more than five treatments (Figure 3). Mean sizes of the ampullae, prostate and seminal vesicles also decreased significantly. Percentage normal sperm decreased from 33.8% to 93.8% prior to vaccination to only 0.7% – 26.0% after three treatments and no normal sperm were found after four or more treatments. At the same stage, very few or no sperm

TABLE 1: Examples of the effect of anti-gonadotropin-releasing hormone vaccine treatment on serum testosterone (T4) concentrations (vaccine changed to Improvac in 2006).

Bull	Date	T4 nmol/L	Date	T4 nmol/L	Date	T4 nmol/L	Date	T4 nmol/L	Date	T4 nmol/L	Date	T4 nmol/L	Date	T4 nmol/L
Thembo	BT 2003	27.10	2005	0.00	2005	0.80	2005	1.30	2007	0.00	2008	0.00	2014	0.00
Makavhuze	BT 2003	34.6	2014	0.01	-	-	-	-	-	-	-	-	-	-
Toto	BT Jul 12	32.4	Feb 13	0.05	-	-	-	-	-	-	-	-	-	-
Mana	BT Jul 12	32.4	Feb 13	0.05	-	-	-	-	-	-	-	-	-	-
Sharu	BT Jul 12	61.3	Feb 13	0.05	-	-	-	-	-	-	-	-	-	-
Michael	BT Jul 12	6.4	Feb 13	0.11	-	-	-	-	-	-	-	-	-	-
Sapi	BT Jul 12	26.4	Feb 13	0.16	-	-	-	-	-	-	-	-	-	-

BT, before treatment.



Source: Modified from Lueders, I., Young, D., Maree, L., Van der Horst, G., Luther, I., Botha, S. et al., 2017, 'Effects of GnRH vaccination in wild and captive African elephant bulls (*Loxodonta africana*) on reproductive organs and spermatogenesis', *PLoS One* 12(9), e0178270, 1–21. <https://doi.org/10.1371/journal.pone.0178270>

NT, No treatment; T, Treatments.

FIGURE 3: Trans-rectal ultrasound images showing the relative size and appearance of the testis of the same bull (a) before, (b) after two treatments, six months, and (c) after seven treatments, 24 months.

were present. The period required to reach infertility was approximately 12 months. Serum testosterone concentrations were below baseline in all captive bulls six months after start of treatment, whereas in wild bulls this was only reached after 12 months. Group 2 bulls had small internal genital organs and very little or no seminal fluid could be recovered, which contained no sperm. Serum testosterone concentrations were at baseline or undetectable.

Side effects of Improvac® vaccine

Other species

Improvac® is registered for pigs where extensive safety and efficacy studies were performed before registration. It is also used extensively as an extra-label drug in mares and stallions to control sex hormone-related behaviour. In other species, injection site reactions, depending on the anti-GnRH vaccine, can include acute inflammation with swelling and pain and abscesses (white-tailed deer, Curtis et al. 2008:68–79; horses, Botha et al. 2008:548–554; Dowsett et al. 1996:228–235). This is dependent on the species and especially the vaccine used. Vaccines consist of the specific antigen(s) and an adjuvant. The latter helps to mount an immune response to the vaccine antigen and is also largely responsible for the side effects. Theubet et al. (2010:459–467) observed a febrile reaction accompanied by reduced food intake after vaccination of calves with Bopriva® (Zoetis). This resolved within one to three days. Short-lived febrile reactions are common to many vaccines and are indicative of a good immune response. The same observations were made in mares (Botha et al. 2008:548–554) and stallions (Birrell 2017:49) treated with Improvac®.

Injection site reactions in elephant bulls

Injection sites reactions are sometimes seen in elephants. Lueders et al. (2017:1–21) reported an incidence of 3.4% in a total of 84 vaccination events. These reactions were stiffness or lameness and occasionally localised swelling, which resolved spontaneously and did not require treatment. One particular elephant sanctuary regularly observed stiffness in the injected leg in two bulls and one cow (S. Hensman, Adventures with Elephants, December 2016, pers. comm.).

Behavioural effects in elephant bulls

De Nys et al. (2010:8–15) clearly demonstrated the positive relationship between aggressive behaviours and faecal androgen concentrations. Using regular treatments of Improvac®, musth was prevented for 4 to 15 years in ≥ 45 captive African elephant bulls (Bertschinger & Sills 2013:85–107). Mounting behaviour between captive bulls also ceased after treatment with Improvac (Lueders et al. 2017:1–21). Treated bulls seldom attempt to mount cows in oestrus, although they do show interest. Contrary to Europe and North America, breeding of African elephants in captivity is seldom required in southern Africa and is in fact banned according to the National Norms and Standards for the

Management of Elephants in South Africa (2008:39) for elephant sanctuaries. Thus, reproductive behaviour and fertility of captive elephants is not required in South Africa. Where there are two or more bulls in a captive facility, it is important to either treat all bulls or carefully select the bulls for treatment according to age and size. If neglected, problems may arise as a result of potential changes in the hierarchy because untreated bulls may challenge down-regulated conspecifics.

In the wild, however, there are other considerations. Between musth periods, bulls are calmer, socialise with other bulls and often encounter cows during their daily movements, but do not specifically seek them out. In musth, this changes with bulls becoming more solitary, leaving their home range, and they travel large distances to seek out cows in oestrus. Essentially down-regulated bulls likely behave like inter-musth bulls and most certainly they will be less assertive and dominant towards bulls of similar size and possibly smaller stature. When treating individuals in a population of wild bulls, similar to captive bulls, hierarchy also needs to be considered. Doughty et al. (2014:88–96) recorded a change in hierarchy after the 43-year-old dominant bull was treated with Improvac®. They suggest that the second oldest of nine 9–21 year-old untreated bulls became the dominant bull in the population. The treated bull, however, was still able to cope with challenges from other bulls. In contrast, three adult bulls were treated with Improvac for seven years on Karongwe Game Reserve and no hierarchical or dominance problems were noted between them or untreated younger bulls. In another study, a 15-year-old bull had separated from his natal herd, but following Improvac® he reverted to his herd associated behaviour (Lueders et al. 2017:1–21).

Additional positive aspects of anti-GnRH treatment are effects on fence-breaking (reported above) and damaging of trees. In the same study, one wild and one captive bull frequently broke fences prior to treatment. This behaviour ceased after two treatments. Two adult wild bulls (> 30 years) developed the habit of excessively damaging trees on a reserve in KwaZulu-Natal. Following two Improvac® treatments, the incidence of tree damage was markedly reduced. About six months after discontinuation of treatment, the behaviour returned, but has reduced once again after further treatments (M. Toft, Wildlife Veterinarian, November 2017, pers. comm.). Owners and managers should, however, take note that the improved behaviour was based on a small sample size ($n = 4$). Responses to vaccination may vary individually and are strongly dependent on age, social factors and life experiences of the individual.

Effects on growth rate

Table 2 shows growth rates (measured by shoulder height) of African elephant bulls treated with Improvac® for one to three years. Age at first treatment had no significant effect on annual growth and varied from 1.5 cm to 9.5 cm a year (unpublished data). In Table 3, the change in shoulder

TABLE 2: Age, height increase and average annual increase in 11 Group 1 bulls from the first to the final examination.

Bull	First examination		Last examination		Height increase (cm)	
	Age (years)	Height (cm)	Age (years)	Height (cm)	For period	Per year
Nduna (C)	8	210	9	212.0	2.0	2.0
Gambo (C)	10	224	13	237.0	13.0	4.3
Shaka(C)	15	230	18	269.0	39.0	13.0
Clyde (C)	17	240	20	268.5	28.5	9.5
Mukwa (C)	22	292	25	310.0	18.0	6.0
Thaba (C)	22	290	25	308.0	18.0	6.0
Duma (C)	23	297	25	306.0	9.0	4.5
Harry (C)	24	290	27	294.5	4.5	1.5
Namib (C)	24	274	27	287.0	13.0	4.3
Mashatu (W)	15	247	17	263.0	16.0	8.0
Jabulani (W)	34	302	36	319.0	17.0	8.5

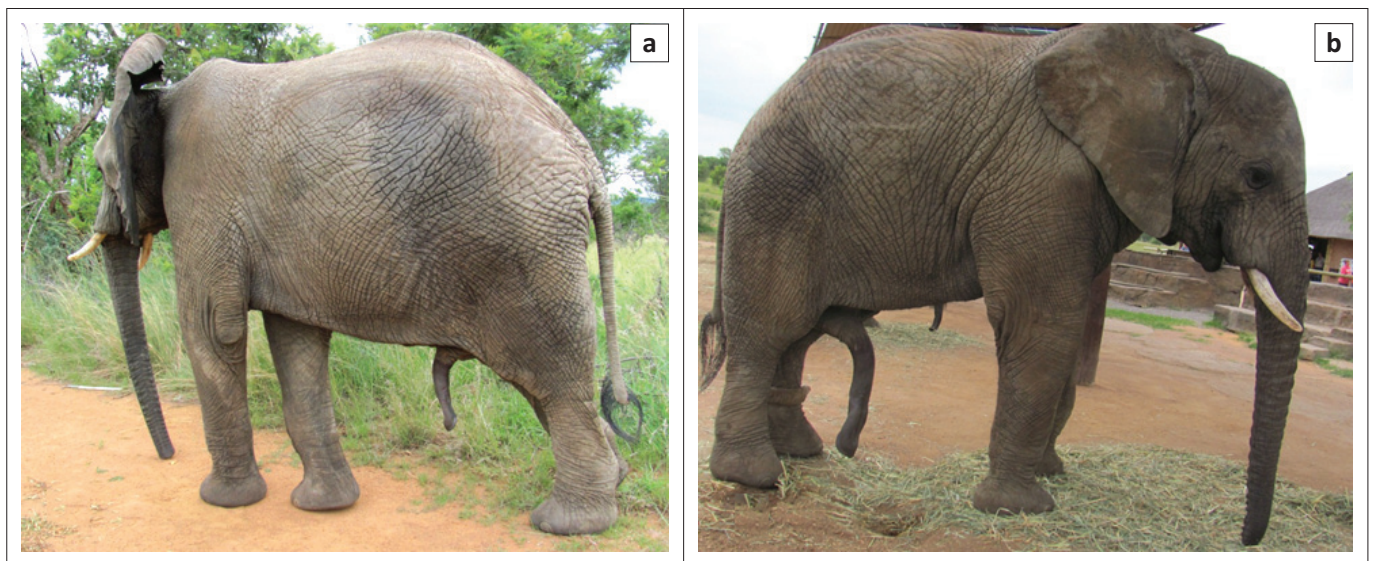
Source: Modified from Lueders, I., Young, D., Maree, L., Van der Horst, G., Luther, I., Botha, S. et al., 2017, 'Effects of GnRH vaccination in wild and captive African elephant bulls (*Loxodonta africana*) on reproductive organs and spermatogenesis', *PLoS One* 12(9), e0178270, 1–21. <https://doi.org/10.1371/journal.pone.0178270>

C, captive; W, wild; cm, centimetres.

TABLE 3: Change in shoulder height, left and right tusk length over 4 years in four captive bulls during ongoing treatment with Improvac® vaccine at Elephant Whispers.

Name	Year of first treatment	First measurement (2014)				Second measurement (2018)				Mean annual increase	
		Age (years)	Height (cm)	Left tusk (cm)	Right tusk (cm)	Age (years)	Height (cm)	Left tusk (cm)	Right tusk (cm)	Height (cm)	Tusk (mm)
Thembo	2003	30	325.5	34.65	20.20	34	334.0	41.34	25.79	2.13	154
Shawari	2008	21	263.0	14.96	16.93	24	298.0	23.03	24.25	8.75	193
Ziziphus	2008	19	261.0	13.39	13.39	22	271.0	16.92	20.39	2.50	132
Medwan	2008	19	255.0	17.72	15.75	22	298.5	21.65	19.09	10.86	91

Source: A. Kotze, Elephant Whispers, April 2018, pers. comm.



Source: Modified from Bertschinger, H.J. & Sills, E.S., 2013, 'Contraceptive applications of GnRH-analogs and vaccines for wildlife mammals of southern Africa: Current experience and future challenges', in E.S. Sills (ed.), *Gonadotropin-releasing hormone (GnRH). Production, structure and function*, pp. 85–107, Nova Science Publishers Inc., New York, (eBook)

FIGURE 4: Effect of six-monthly Improvac® treatments for 32 months (a) on the size of the extruded penis. Bull (b) was a similar age and untreated.

height and tusk length over a 4-year period in four captive bulls during ongoing Improvac® treatment is shown (A. Kotze, Elephant Whispers, April 2018, pers. comm.). The growth rates (shoulder height) of the bulls in each table are similar to those described in the literature. However, age-related shoulder heights reported here are greater than those found in Amboseli (Lee & Moss 1995:29–41) and other reserves (Shrader et al. 2006:40–48). Measurements of captive bulls reported in Tables 2 and 3 were carried out in standing animals. The two wild elephants (Table 2) were measured in lateral recumbency. Sizes reported for bulls in Amboseli and other reserves were determined using photographs or photogrammetry, respectively.

Improvac® treatment of two young (16–18 years of age) captive bulls for 32 months resulted in much smaller and shorter penises than a bull of similar age in the same facility (Figure 4; Bertschinger & Sills 2013). The difference in size appeared to be the result of penile atrophy rather than suspended growth. The mean annual tusk (both tusks) growth rate for four captive bulls under ongoing Improvac® vaccine treatment was 142 mm (Table 3). Age appeared to have no effect. There are no references to tusk growth rate in the literature. G. Steenkamp (Faculty of Veterinary Science, University of Pretoria, April 2018, pers. comm.), who studied tusk defects in captive African elephants, observed an average annual growth

Reversibility in elephant bulls

Improvac® vaccine was designed for use in male piglets and reversibility was not conclusively investigated. Early short-term treatment of African elephant bulls with Pepsan anti-GnRH vaccine resulted in reversal in at least three of the six treated bulls (De Nys et al. 2010:8–15). During the early use of Improvac®, when a dose of 600 µg was used, some bulls showed signs of behavioural reversal after five months or earlier. Hence, the dose was increased to 1000 µg (Bertschinger & Sills 2013:85–107). Despite the dose increase, bulls on long-term Improvac® treatment (> 8 years) may show minor changes in behaviour (exhibited as a reluctance to respond to commands) five to six months after the last booster (A. Kotze, Elephant Whispers, July 2013, pers. comm.), although at this stage peripheral testosterone concentrations are generally still very low. In Asian elephant bulls, anti-GnRH antibody titres start to wane around this time (Somgird et al. 2016:111–120) and, possibly, the behavioural changes are because of a few GnRH molecules that escape neutralisation. GnRH is known to induce certain sexual behaviours at the level of the brain in mammals (Kauffman 2004:794–806).

Treatment of a seven-year-old Asian elephant bull for six years appears to have resulted in permanent down-regulation of testicular function after seven years (Lueders et al. 2013:611–619). One year after the first vaccination, no sperm could be observed in the ejaculate and the diameter of the testicles had decreased by 40%. The penis stopped developing and remained at the size of a six-year-old bull. Four years after the cessation of treatment, the testosterone levels have remained at baseline (Lueders et al. 2014:611–619). Reversal of androgen production and semen quality was assessed in four African elephant bulls (two captive and two wild elephants, 18 and 30 years, and 19 and 36 years old, respectively) treated with Improvac® for 2.5–3.0 years (Lueders et al. 2017:1–21). They were examined 29 ($n = 2$) and 31 ($n = 2$) months after the last treatment. The older wild bull treated for 2.5 years had successfully reversed and come into musth at that stage. Upon electro-ejaculation, he was found to have normal semen quality and an increase in serum testosterone concentration. Since this last examination, he has come into musth a second time. Although the three other bulls only showed isolated sperm present in their ejaculates, minor increases in serum testosterone concentration – possibly the beginning of reversal – were observed. However,

3.5 years after the last vaccination, they still have not been in musth (unpublished data). Two wild bulls on Karongwe Game Reserve (T. Coetzee, Karongwe Game Reserve, November 2017, pers. comm.) treated with Improvac® for eight years have shown signs of behavioural reversal 18–24 months after the last treatment. The age of bulls at the time of their first treatment thus appears to influence reversibility, at least as far as androgen-dependent behaviour is concerned. Testicular Leydig cells, responsible for testosterone production, are far more resilient than spermatogenic cells. To date, testes of treated elephant bulls have not been examined histologically to determine short-, medium- or long-term effects at a cellular level.

Effects of Improvac® vaccine in elephant cows

Induction of anoestrus in elephant cows

The excellent results obtained with the induction of anoestrus in 100% of treated mares with Improvac® (Botha et al. 2008) led to a trial being conducted in free-ranging elephant cows on Entabeni Game Reserve. Eight cows were treated with 600 µg Improvac® and four served as untreated controls. Before and following treatments, faecal progesterone metabolite concentrations (FPMC) were monitored to assess ovarian activity (cyclic changes in FPMC). The results were inconclusive partly because the dose of Improvac® used was too low compared to what is now used in elephant bulls (5 mL) and partly because the monitoring period was perhaps too short. Some of the control cows showed seasonal anoestrus during the dry season and as such the cause of anoestrus in the treated cows could not be established conclusively (Benavides Valades et al. 2012). A study of seven Asian females (Boedeker et al. 2012; Boedeker, Schmitt & Brown 2013) resulted in suppression of the oestrus cycle. Current studies indicate that suppression of ovarian function requires more frequent and higher dosages (Boedeker et al. 2012; J. Brown, Smithsonian Institution, March 2016, pers. comm.), compared to male elephants. More recently, the successful use of another anti-GnRH vaccine (Repro-BLOC®) to induce anoestrus in a 59-year-old Asian elephant cow for medical reasons has been reported (Boedeker et al. 2012). However, a series of treatments (primary plus four boosters) were required before the cow was finally down regulated.

TABLE 4: Results of treatment of elephant cows on four captive facilities with Improvac® to induce anoestrus.

Facility	Cows						Bulls present		
	<i>n</i>	Age (years)	First treatment	Ongoing	Duration	Comments	<i>n</i>	First treatment	Ongoing
Adventures with Elephants†	1	16	April 2016	yes	18 months	No oestrus	2	Aug 2013	yes
Shearwater‡	5	17–40	May 2016	yes	17 months	No oestrus No calves prior to treatment	0	-	-
Elephant Whispers§	2	16–22	Jan 2017	yes	10 months	No oestrus	4	2003 & 2008	yes
Imire¶	1	20	2009	yes	8 years	No oestrus 1 calf prior to treatment	2	2007	yes
Anonymous	5	25–34	Sept 2010	yes	7 years	No oestrus 4 cows calved prior to treatment††	5	Apr 2013	yes

†, Sean Hensman, Adventures with Elephants, Republic of South Africa; ‡, Allen Roberts, Shearwater, Zimbabwe; §, Andre Kotze, Elephant Whispers, Republic of South Africa; ¶, Reilly Travers, Imire, Zimbabwe; ||, Anonymous, Zimbabwe.

An added indication for the use of Improvac® in captive African elephant cows in South Africa and Zimbabwe is the prevention of oestrus. According to elephant housing facilities, bulls become more difficult to handle when cows come into oestrus. In addition, breeding of calves in captivity in South Africa is mostly unwanted (National Norms and Standards for the Management of Elephants in South Africa 2008:39). There are also some facilities (Shearwater, Zimbabwe, A. Roberts) where wild and captive elephants have access to one another on the same reserve. Under such conditions, captive cows coming into oestrus can create enormous problems. Following the unsatisfactory results obtained on Entabeni, the dose of Improvac® was subsequently increased from 600 µg to 1000 µg. Table 4 shows the numbers of cows treated on various facilities in South Africa and Zimbabwe. The increased dose applied as a primary, first booster after five weeks and then boosters every five to six months has been successful in inducing anoestrus and preventing breeding and pregnancies in all treated cows.

Recommendations

Indications

Improvac® treatment of captive elephant bulls is indicated as a means to control aggressive behaviour and musth. It is advisable to treat all post-pubertal bulls that are handled in direct contact, to avoid changes in hierarchy and to prevent younger untreated bulls from coming into musth prematurely. Until further research has been carried out, Improvac® should not be used as a contraceptive method for free-ranging elephants. From time to time, however, there is a need to control aggression or musth in free-ranging bulls. In such cases, careful consideration should be given to the bull population in the reserve and the consequences of treating some and not other bulls.

Administration of Improvac® vaccine

To limit injection site reactions, it is important to administer Improvac® by deep i.m. injection into the gluteal (dart gun from a helicopter) or semimembranous or semitendinous muscle masses. A dart gun, pole syringe or hand-injection can be used for the latter site. In the case of dart administration, a 60-mm needle should be used. The i.m. route is preferred to subcutaneous administration for specific reasons. Irritant substances are better tolerated in the muscles; the antibody response to anti-GnRH vaccines is better when given i.m. (Dowsett et al. 1991:183–190), and, practically speaking, it is difficult to inject elephants subcutaneously. One sanctuary has been administering the vaccine by hand injection into the neck muscles behind the ear (A. Kotze, Elephant Whispers, May 2009, pers. comm.). The injection is carried out by the groom and has been done in this manner since 2008 and, once again, local swelling is sometimes observed. Only sterile equipment should be used for administrations. Bacterial contamination is likely to result in the formation of abscesses as the local oedema caused by the vaccine is an ideal medium for bacteria to thrive in.

It should be stressed that once vaccination has commenced in captive bulls, treatment should be carried out at regular intervals and with the correct dose to avoid problems. Stop–start treatments are very likely to result in a rise in serum testosterone concentrations and an increase in aggressive behaviour. This was observed on one elephant sanctuary, and as a result of his aggressive behaviour, the bull was shot (anonymous). Since this incident, all remaining bulls have been placed on regular treatments. No further incidents have been recorded since then.

Reversal of Improvac® treatment

It should be noted that medium- to long-term treatment (≥ 3 years) of elephant bulls may result in permanent infertility. Permanent effects are more likely to occur in younger bulls (< 12 years) and especially prepubertal bulls, or after long-term regular treatments.

Conclusions

The treatment of elephant bulls with the Improvac® vaccine is able to down-regulate androgen-related behaviour and sperm production successfully in both captive and wild African elephant bulls of various ages (≤ 34 years). Reversibility of these effects after medium- to long-term use is largely unknown and should be investigated. The effects of treatment on social behaviour, dominance hierarchy and movement of wild bulls also warrant more detailed studies. Treatment of peri-pubertal bulls should be discouraged owing to possible permanent suppression of reproductive organ development following repeated Improvac® administrations. Preliminary studies in cows to induce anoestrus with this vaccine appear to be successful, but require more detailed studies.

Acknowledgements

The authors would like to thank the following for their collaboration and providing information: Adventures with Elephants (Limpopo Province), Elephant Whispers (Mpumalanga Province), Imire Wildlife and Conservation (Zimbabwe), Karongwe Game Reserve (Limpopo Province), Shearwater (Zimbabwe) and Dr Mike Toft. Funding for this study was provided by the following: University of Pretoria, International Elephant Fund, United States Fish and Wildlife Services, Technology Innovation Agency and Birmingham Zoological Gardens.

Competing interests

There are no competing interests with regard to the writing of this article.

Authors' contributions

H.J.B. wrote the article, and I.L. reviewed and contributed to the writing up of the article.

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