Use of gonadotrophin releasing hormone (GnRH) vaccines for behavioural and reproductive control in managed Asian elephant *Elephas maximus* and African elephant *Loxodonta africana* populations

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Abstract

Because of their size and reproductive anatomy, surgical castration is not a practical option in adult elephants. However, similar to other species in human care, the effects of gonadectomy are desired in specific situations. This may be for contraceptive purposes, or for behavioural or veterinary management of elephants in human care or wild elephants managed in small reserves. Research into non-surgical contraceptive measures for wild and domestic animals has resulted in an array of hormonal and immunological options to downregulate gonadal function. Driven by the production-animal industry, commercial gonadotrophin releasing hormone (GnRH) vaccines are readily and cheaply available. This immunocontraceptive is effective in both males and females as it inhibits gonadotrophic hormone release and, thus, downstream stimulation of testicles and ovaries. Here, available studies from the past 10 years on the GnRH vaccine application in male and female elephants are reviewed. Furthermore, we add our own experience gathered from the treatment of male African elephants *Loxodonta africana* and female Asian elephants *Elephas maximus* in human care. The GnRH vaccine offers a viable approach for various management purposes. It should be noted that the GnRH vaccine was not primarily designed as a reversible contraceptive. Therefore, its use must be well justified on an individual basis and the effects closely monitored.

Introduction

Asian elephants *Elephas maximus* and African elephants *Loxodonta africana* are the largest terrestrial mammals and have been living close to humans for thousands of years. Domesticated (tamed) elephants have been used traditionally in religious ceremonies and warfare, as working labour and, at the time of writing, as tourist attractions and animal ambassadors in zoological facilities. The close relationship with humankind, both in terms of elephants in captivity and human–wildlife conflicts with wild elephants, has resulted in the need for castration/contraception methods to control behaviour and fertility. Three possible reasons for the need of gonad function downregulation are: (1) aggression control (unwanted sex-hormone driven behaviours), (2) population control (contraception) and (3) control of reproductive pathologies (usually medical reasons in female elephants).

Because of the intra-abdominal location of the testicles in male elephants (i.e. caudal to the kidneys) and the enormous size of the female elephant reproductive tract, gonadectomy is only possible with significant risk to the animal if not performed in very young individuals (Foerner *et al*., 1994). Furthermore, specialized equipment and skilled surgeons are required, making this procedure impractical in terms of animal safety, time and costs.

Thus, those who care for these animals have been searching for non-surgical ways to ‘castrate’ elephants. Injectable gonadotrophin releasing hormone (GnRH)-based immunocontraceptive vaccines have been studied in numerous domestic animal and wildlife species (Kirkpatrick *et al*., 2011). The vaccines are commercially available, and appear to be a cost-effective and minimally invasive option for the control of gonad function.

The GnRH vaccine is a prepared inoculant designed to induce an immune response against the endogenous GnRH. Several different formulations have been trialled in elephants and other wildlife (as reviewed in detail in Bertschinger & Lueders, 2018). GnRH is secreted in the hypothalamus and is the beginning of a hormonal chain reaction, the hypothalamic-pituitary-gonadal (HPG) axis. The secretion of GnRH triggers the release of further hormones – luteinizing hormone (LH) and follicle stimulating hormone (FSH) – from the pituitary gland. As their common name suggests, these hormones act on the gonads (testicles, ovaries) and upregulate their function. In females, this involves the growth, maturation (FSH) and ovulation (LH) of follicles, and the subsequent secretion of oestrogens and progesterone. In males, the gonadotrophins are responsible for the development of sperm (FSH) and the secretion of testosterone (LH).

The GnRH vaccine injection triggers antibody production by introducing a foreign protein to the body's immune system. In response to an injection of the protein-conjugated, exogeneous GnRH, antibodies will neutralize the GnRH produced naturally in the body. By binding onto the endogenous GnRH, the antibodies prevent GnRH action on pituitary gonadotrophs, because it is now unable to bind to the receptors in the pituitary. Subsequently, LH or FSH is no longer released, and the testicles or ovaries will not be stimulated and, therefore, will become inactive.

Originally, commercial GnRH vaccines were developed for male piglets and cattle, because of the welfare issues arising in relation to surgical castration (Bonneau & Enright, 1995; Dunshea *et al*., 2001). The use of GnRH vaccine in elephants has only been studied fairly recently. In this article, we summarize the existing studies on male and female elephants, and provide some preliminary results from ongoing research.

GnRH Vaccine Application in Elephants

The application of GnRH-based immunocontraceptive vaccines to individual elephants may become desirable in captivity or smaller game reserves in range countries for a number of reasons (Table 1). When in human care, the rationale for administering GnRH vaccines is mainly behavioural modification in males; and the most common application in mature, non-reproductive females is control of reproductive-tract pathologies. Although several different vaccines have been used in elephants and other wildlife (Bertschinger & Lueders, 2018), the most commonly applied vaccine in elephant studies is a commercially available formulation produced by Zoetis®, under the brand name Improvac® (GnRH analogdiphtheria toxoid conjugate, available in different concentrations: 150 µg ml^{-1} in Europe; 200 µg ml^{-1} in Africa) or Improvest® (200 µg ml^{-1} in North America). Several aspects of the GnRH vaccine used in both genders of African and Asian elephants have been studied.

Table 1. Proposed applications of the gonadotrophin releasing hormone (GnRH) vaccines in male and female Asian elephants *Elephas maximus* and/or African elephants *Loxodonta africana* to suppress gonadal function and sex steroid-related behaviours

	Application For	Comment
AFRICAN/ASIAN RANGE COUNTRIES		
Elephant bulls in human care	1. musth/aggression control	1. reducing testosterone-driven aggression
	2. animal welfare	2. to avoid separation/food deprivation during musth
	3. intraspecies aggression	3. to reduced injury/conflict between bulls
Wild elephant bulls	1. musth/aggression control	1. human–elephant conflict mitigation
	2. contraception	2. population control
Wild African elephant females	1. contraception	1. population control
ZOOLOGICAL INSTITUTIONS		
Elephant bulls in human care	1. musth/aggression control	1. bulls difficult with conspecifics/keepers
	2. contraception	2. over-represented bulls
	3. intraspecies aggression	3. establishing bachelor groups
Elephant females in human care	1. ovarian downregulation	1. leiomyoma treatment in Asian elephants
	2. contraception	2. over-represented females/discontinuation of breeding because of medical issues

GnRH vaccine use in male elephants

Musth is a state of high testosterone secretion, occurring annually in most mature male African and Asian elephants. It is characterized by increased aggression, temporal-gland secretion, urine dribbling, restlessness and heightened sexual activity (Sukumar, 2003; Hollister-Smith *et al*., 2007). This phase may last up to several months and is especially problematic for males in human care that are handled in unrestricted contact (‘hands-on’). Many tourist or temple bulls in Asian range countries are chained, and deprived of food and water during this phase as it becomes too dangerous for the human handlers to approach musth bulls (Jainudeen *et al*., 1972). Similarly, wild African elephant bulls may become a threat to tourists, other animals or inventory (e.g. fences, vehicles, buildings) in small game reserves. Testosterone downregulation and prevention of musth by injection may be desirable in these situations.

Testosterone secretion

Studies have shown that musth in elephants appears to be under the control of the HPG-axis (Lincoln & Ratnasooriya, 1996). Injection with exogenous GnRH induces a surge of LH secretion, followed by the release of testosterone (Somgird, Sripiboon *et al*., 2016) Thus, it is not surprising that immunization against GnRH led to lowered testosterone and to cessation of musth in elephants in the available studies (De Nys *et al*., 2010; Rajapaksa *et al*., 2010; Lueders *et al*., 2014, 2017; Somgird, Homkong *et al*., 2016). The effect of the GnRH vaccine on blood serum (Somgird, Homkong *et al*., 2016) or faecal androgen (Lueders *et al*., 2017) levels was measured. Results suggest that an initial inoculation followed by a booster 4–6 weeks later with a minimum of 600–1000 µg of GnRH conjugate was enough to suppress testosterone levels for at least 5 months. Regular booster vaccinations every 6–12 months are recommended to keep the androgen levels low in adult bulls (Lueders *et al*., 2017). Baseline testosterone is associated with reduced sexual activity and less dominance in most mammals. The effects caused by the GnRH vaccine may be useful for the management of elephants; for example, it would be possible to leave younger bulls with their maternal herds for longer, keep bachelor groups together in zoological institutions or be able to use hands-on management for bulls in range countries without the recurrence of musth (De Nys *et al*., 2010).

Male behaviour

In male elephants, dominance is related not only to size and age but also by the state of musth (Hollister-Smith *et al*., 2007). Thereby, older bulls appear higher up in the social hierarchy with fluctuations influenced by testosterone concentrations. Suppression of testosterone secretion by GnRH vaccines may alter social structures in wild and captive male groups. To date, there are only two published reports expanding on behavioural changes in wild males after GnRH vaccination. A wild African elephant bull of 43 years of age was behaviourally monitored post vaccination and, despite being the oldest bull, he was only second in rank (Doughty *et al*., 2014). However, in this study, the authors admit that the treatment may not have been conducted correctly, because the bull was seen in musth and siring offspring even after GnRH vaccination (Doughty *et al*., 2014).

In the second study on two wild bulls, a lone-ranging 35 year-old bull, which reportedly was attacking gates and fences, was not observed showing this destructive behaviour after effective vaccination (Lueders *et al*., 2017). A second, subadult wild bull, which was observed straying away from his maternal herd, was seen spending more time with the family group after vaccination (Lueders *et al*., 2017). Both bulls showed no signs of musth during the period when they were administered with regular biannual vaccinations.

In a study on African elephants managed using unrestricted contact at private facilities, behaviour was modified in certain individuals, which appeared calmer and more attentive to their handlers (De Nys *et al*., 2010). Only one subadult, captive-born Asian elephant male was treated and monitored for testosterone and behaviour (Lueders *et al*., 2014). This male (7 years if age at the beginning of the study) was regularly vaccinated for 6 years (initially monthly, then every 6 months and then yearly), and showed no testosterone production and no sexual interest or aggression. At the time of writing, this male is 19 years old and still managed using unrestricted contact and is socialized with a group of females (pers. obs). Although the last vaccine was given 6 years ago, this male's testosterone levels have remained at baseline and no sexual interest has been recorded.

The authors are in the process of carrying out an ongoing study in order to assess post-vaccination behavioural changes more objectively. Full results will be published once all the data have been collected. At the time of writing, seven captive African elephant bulls had been monitored over a 4 year period from before they were vaccinated to 1–1·5 years after the initial vaccination. The bulls were kept under different conditions, either with or without females (multiple-male groups) and were assessed by their handlers. Elephants were observed during their daily activity in the group interacting with other elephants or during the evenings inside their boma and when interacting with their handlers. The elephant handlers were asked to fill in a questionnaire every 6 months on how they rated the respective animals. Five main characteristics were assessed on a six-point scale: 1. *confidence*: no confidence to highly confident; 2. *dominance* towards humans or other elephants: submissive to highly dominant; 3. *aggressiveness* towards humans and other elephants: even tempered to highly aggressive; 4. *alertness* towards environmental changes: neutral to highly alert; 5. *sociality*: solitary to highly social. The preliminary results are summarized in Fig. 1 and show a trend towards elephants that are calmer and better in social situations, and less alert, aggressive, dominant and self-confident. No musth was observed in any bull during the observation period. One of the two oldest captive bulls showed a marked reduction in stereotypic behaviour after vaccination, although at this stage it is too early to know whether this result is significant.

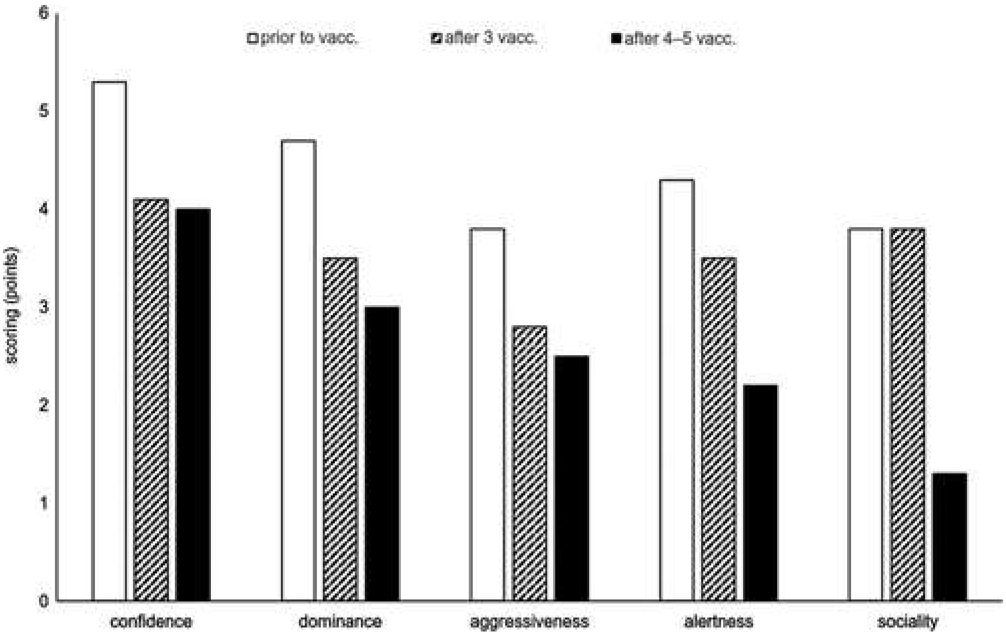

Figure 1. Trends in behavioural changes in seven captive African elephant *Loxodonta africana* bulls. Five parameters were assessed on a six-scale scoring system (0 lowest and 6 highest): prior vacc., prior to vaccination with gonadotrophin releasing hormone (GnRH); 3 vacc., after three vaccinations (8 months after the first injection); 4–5 vacc., after 4–5 vaccinations (1–1·5 years after the first injection).

In summary, these initial results point towards it being possible to suppress testosterone-driven, aggressive or dominance behaviours as well as musth in adult Asian and African elephant bulls, provided regular GnRH vaccinations are administered (Fig. 1). This castration-like effect on behaviour may be very useful for the management of bull elephants in various situations (Table 1); for example, establishing multi-male groups of different age classes or managing post-pubertal bulls within family herds for elephants in human care. In the wild, problem adult bulls may benefit from GnRH vaccination as a behaviour-management tool rather than resorting to culling or translocation.

It should be noted that the dominant behaviours of a bull elephant may only be modified to a certain degree because some learned aggressive behaviours may not be affected by testosterone levels (De Nys *et al*., 2010; Lueders *et al*., 2017). Therefore, GnRH vaccination may help to suppress testosterone peaks, but are not necessarily related to calmer behaviour.

Male fertility

Two studies describe the effects on male reproductive organs and sperm production in an Asian elephant (Lueders *et al*., 2014) and in 14 African elephants (Lueders *et al*., 2017). Reproductive organs (accessory sex glands and testicles) were measured by ultrasound and semen samples were obtained by transrectal prostate massage or, in case of wild bulls, by electroejaculation. Reproductive-organ size decreased after two injections and was significantly decreased (by up to 60%) at the end of the respective studies after 3·5 years (Lueders *et al*., 2017) and 6 years (Lueders *et al*., 2014).

Similarly, sperm production and sperm quality decreased after the second injection, but complete azoospermy was only achieved after multiple injections. In some animals, sperm production was still present after seven injections, although spermatozoa were significantly reduced in viability and showed severe morphological defects (Lueders *et al*., 2017).

No histological assessment of testicular tissue after multiple-year treatment is available for elephants to date, but ultrasound images show that sonomorphological changes take place during long-term treatment. Testicle shape changed from round to amorphous (Plate 1a) in 14 monitored males and the echotexture became inhomogeneous (Plate 1b). Diffuse vascularization of healthy testicles (Plate 1c) changed towards one dilated central vein in multiple-year vaccine males (Plate 1d), indicating reduced functionality of the organ (unpubl. data).

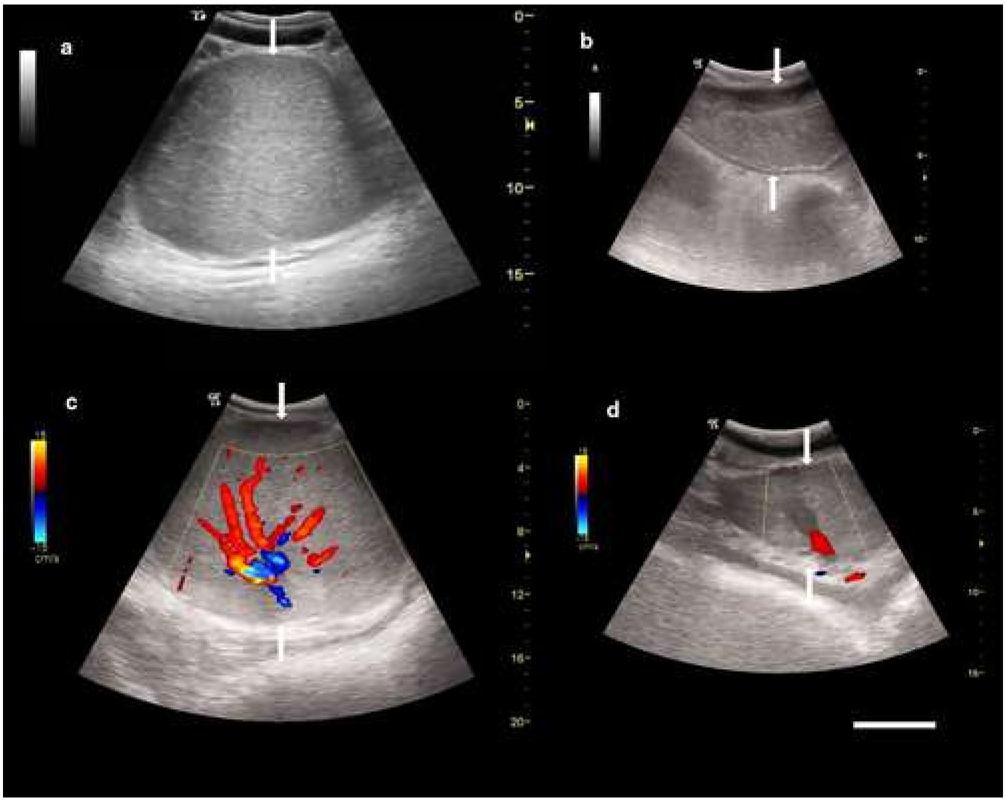

Plate 1. Transrectal ultrasound images of testicles (arrows) of two African elephant bulls *Loxodonta africana* prior to gonadotrophin releasing hormone (GnRH) vaccination (a, c) and after 3 years of continuous treatment (eight injections with 1000 µg of GnRH-protein conjugate, dosed every 5–6 months after initial injection and booster after 6 weeks) (b, d), normalized to the same scale: a, bull 1 (8–11 years of age) prior to vaccination showing a round echoic testis; b, bull 1 after 3 years of vaccination, showing marked testicle size reduction, amorphous shape and inhomogenous echotexture; c, bull 2 (26–29 years of age) prior to vaccination, showing colour flow doppler image of testicular vascularization; d, image of the testicle after 3 years showing only one enlarged central vein; white bar = 5 cm. *Imke Lueders*.

These gross changes visible in the ultrasound image suggest some form of testicular degeneration, which was also described for White-tailed deer *Odocoileus virginianus* after three vaccinations (Curtis *et al*., 2008). Thus, recovery of testicular function in long-term or pre-pubertal treated animals may be in question. Generally, young elephant bulls appear to have a faster, stronger response to the vaccination programme in terms of testicular changes and sperm production when compared to older, fully mature bulls (Lueders *et al*., 2017). In addition, reversibility may take longer in bulls vaccinated from a young age and over several years (unpubl. data).

Using the GnRH vaccine as a method of contraception would warrant regular revaccination to keep testosterone levels (libido) down and to interrupt sperm production. It is probable that effective contraception is only possible a year or more after the start of the vaccination programme, making this approach unsuitable for wild elephant bulls. Long-acting GnRH vaccines are under further development and may be an option for wild bulls in smaller reserves in the future, if all bulls are vaccinated at the same time.

GnRH vaccine use in female elephants

The application of the GnRH vaccine may also have its benefits for reproduction management in female elephants. The efficacy as a contraceptive in African elephants and the treatment of uterine pathologies in Asian elephants have been tested.

Ovarian activity downregulation

Downregulation of ovarian function is warranted to prevent the stimulation of pathologic conditions in the uterus of non-breeding, aged Asian elephant females. In contrast to African elephant females, captive Asian elephants are very likely to develop uterine leiomyoma (benign smooth muscle tumours) and endometrial hyperplasia (Plate 2) when not reproducing regularly (Montali *et al*., 1997; Hermes *et al*., 2004). Because elephants are long-lived species, these slow-growing, benign tumours of the smooth muscle of the uterine wall (myometrium) may become quite large. Heavily altered and enlarged uterine tissue may cause pain and pelvic obstructions. Such uteri are more often affected by chronic inflammation or pyometra (accumulation of pus). During necropsy of a 52 year old, nulliparous Asian elephant the altered uterus was found to be 370 kg, about ten times as heavy as a 'healthy' adult uterus (H. Steinmetz, pers. comm., 9 August 2018) (Plate 2). The modified endo- and/or myometrium may result in leiomyoma breaking into the uterine lumen. Usually, in continuously cycling females, increased mucous discharge is the first indication observed, coincidentally with the follicular phase and opening of the cervix. Bloody discharge may follow. Ascending infections can result in pyometra, chronic discharges of pus and/or blood, which can result in anaemia and periodic discomfort (pers. obs).

Plate 2. Example of extreme alteration of the uterus in an aged, non-breeding Asian elephant *Elephas maximus*. An isolated pathologic uterus, post-mortem finding in a nulliparous, 52 years of age female. The uterus weighed 370 kg and showed cystic-dilated endometrial hyperplasia and a heavy proliferative hyperplasia of the myometrium with pyometra: small arrows, uterine horns; large arrow, uterine body; *, right ovary freed from ovarian bursa. *Dr Maja Ruetten, PathoVet, Switzerland*.

Leiomyomata are ovarian-hormone dependent in humans and suppression of oestrogen and progesterone secretion is the most common non-surgical treatment option (Haney, 2000). The connection between ovarian hormones and leiomyoma also appears to be present in Asian elephants (Montali *et al*., 1997; Hermes *et al*., 2004). Body-weight reduction in obese, elderly elephant cows and the downregulation of ovarian function are the only treatment options. A first case report by Boedeker *et al*. (2012) showed that multiple vaccinations and a sufficient dose of GnRH protein conjugate are required in order to suppress ovarian function and thus cycle activity, as measured by progestagen and titre levels. The female was ultrasonographically monitored, and the leiomyoma stopped growing and even regressed significantly after vaccination (Boedeker *et al*., 2012).

Therefore, the GnRH vaccine has become increasingly used in Western zoos in aged Asian elephants suffering under this uterine leiomyoma. It is advisable to start treatment before the leiomyoma causes discharge/bleeding or infection in order to prevent permanent closure of the cervix while mucus, pus or blood and bacteria are already accumulated and trapped in the uterus. Closed pyometra in elephants can result in uterine rupture (Aupperle *et al*., 2008). Performing a transrectal ultrasound prior to and during vaccination is advisable.

The authors studied the effect of GnRH vaccination on ovarian activity in five Asian elephant females (50–52 years) using urine-pregnanetriol (P3: a progesterone metabolite) monitoring (Niemuller *et al*., 1993) and ultrasound assessments over a period of 2–3 years. Preliminary results are given.

In the older females, acyclicity occurred after two or three monthly injections of 4–5 ml Improvac® (600–750 µg of GnRH-protein-conjugate) (Fig. 2a). Ovarian size measurements obtained from four females through transrectal ultrasound decreased from a mean of 4·2 cm × 9·3 cm to 3·4 cm × 6·7 cm. The uterine size also decreased in these females. No functional structures (follicles or corpora lutea) were recorded after 6 months of treatment. Another younger female (25 years) was only injected twice with 450 µg GnRH, but she also showed cycle cessation for 5 months, as measured by urine-P3 concentrations (Fig. 2b). However, this female returned to cyclicity, indicating potential reversibility when GnRH is not continuously injected with higher concentrations over multiple doses.

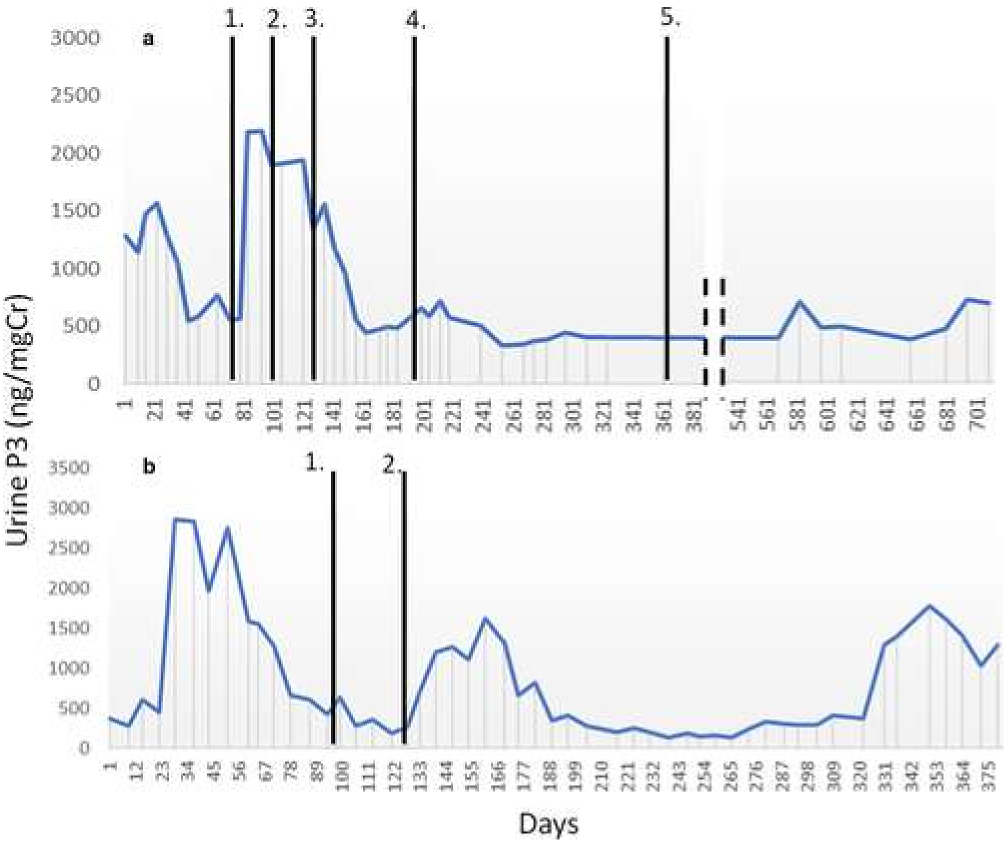

Figure 2. Examples of urine pregnanetriol profiles of gonadotrophin releasing hormone (GnRH) vaccine-treated Asian elephants *Elephas maximus*: a, long-term ovarian-cycle suppression in a female aged 50 years vaccinated monthly (1–3), 2 months after the third injection (4) and 1 year later (5) with 600 µg GnRH-protein conjugate (4 ml Improvac®); b, a female aged 25 years vaccinated only twice, 1 month apart (1, 2) with 450 µg GnRH-protein conjugate (3 ml Improvac®) and reversal back to ovarian activity after 5 months.

One female was euthanized at 52 years of age, 3 years after initial GnRH vaccine treatment following unrelated health issues. This female's uterus still appeared to be large (i.e. weighing 32 kg) and not reduced in size, which has been described in one elephant (Boedeker *et al*., 2012) and Indian rhinoceros *Rhinoceros unicornis* (Hermes *et al*., 2016) when ovarian activity ceases and hormone stimuli on the uterus have been missing. However, the several intramural leiomyoma observed did not exceed 2 cm in diameter and the ovaries were without functional structures. We noticed that the GnRH vaccination had no effect on cystic formation in the reproductive tract. Neither ovarian cysts nor cystic endometrial hyperplasia improved under GnRH-vaccination treatment in three of the females.

Female behaviour

In a highly social animal with clear herd structure, any alteration of behaviour is generally undesirable. The consequences on behaviour after ovarian downregulation in female herds have not been researched to date. On two occasions in captive Asian elephants, more-dominant, higher-ranking females seemed to become calmer and appeared to get along better with the rest of the herd (L. Sambrook, ZSL Whipsnade Zoo, UK, March 2016; pers. obs). How complete acyclicity of all females will affect wild elephant herds needs further investigation.

Long-term studies on the use of porcine zona pellucida (pZP) vaccine in wild African elephant females with continued cycle activity have shown a lack of behavioural modifications or change to herd structure (Delsink *et al*., 2013).

Female fertility

At the time of writing, multi-dose GnRH vaccines are deemed unsuitable for wildlife contraception (reviewed in McLaughlin & Aitken, 2011), because of the repeated localization of the individual for revaccination, which causes distress for the animal and increases the costs of drug administration.

One single-dose GnRH vaccine used in wildlife is GonaCon® (National Wildlife Research Center, Fort Collins, CO, USA), registered only in the United States as a contraceptive for White-tailed deer and feral equids. GonaCon® has been shown to have multiple-year contraceptive properties in several animal species ranging from pigs to deer and feral horses (Miller *et al*., 2004). However, this vaccine is not commercially available to date.

In southern Africa, female elephant contraception is widely and successfully achieved by the use of the immunocontraceptive pZP vaccine (Fayrer-Hosken *et al*., 2000; Delsink *et al*., 2001). Annual boosters seem to be sufficient to sustain the contraceptive properties (Delsink *et al*., 2013). A long-acting formulation of this pZP vaccine (SpayVac) has been trialled, leading to high titres of up to 7 years in two captive African elephant females (Bechert & Fraker, 2016).

With this pZP vaccine available as a proven method, the only other benefit the GnRH vaccine could add is that ovarian activity is shut down, whereas in elephants treated with the pZP vaccine oestrous cycles continue, which may cause uterine pathologies in the long run. Studies in Horses *Equus caballus* and primates suggest that long-term usage of pZP vaccine may affect ovarian function (Muller *et al*., 1997; Joonè *et al*., 2017), but most elephants seem to show continued cycles for several years (Delsink *et al*., 2013). This rather unnatural situation (elephants are usually pregnant or in lactational anoestrus with oestrus only occurring once or twice every 3–5 years) and its subsequent reproductive-tract health problems may be prevented with GnRH vaccination.

An initial study on wild African elephant females showed no complete ovarian suppression with a GnRH vaccination regime of initial injection and a booster 5–7 weeks later with a dose of 600 µg (Valades *et al*., 2012). Because other studies on Asian elephants used higher vaccination doses and more frequent vaccination intervals in the beginning, the GnRH vaccine may still be effective in African elephant females; however, the more frequent revaccination periods compared with pZP vaccines make the GnRH variant impractical for wild elephants until long-acting formulations become commercially available.

GnRH antibody titre development

Antibody titres were shown to rise after two to three injections in adult Asian elephant males (Somgird, Homkong *et al*., 2016). However, they remained elevated for only 2 months. Monthly boosters were therefore given in several studies at least at the beginning of the treatment (Rajapaksa *et al*., 2010; Lueders *et al*., 2014; Somgird, Homkong *et al*., 2016). One study in an Asian elephant female showed that a titre rise was only evoked after an adequate GnRH protein-conjugate dose of 12·5–30 mg (Repro-BLOC®) (Boedeker *et al*., 2012). These initial data suggest that a dose of at least 600–1000 μg of GnRH protein conjugate is required for the initial vaccination along with more regular boosters at the beginning of the vaccination schedule (Valades *et al*., 2012; Bertschinger & Lueders, 2018).

Although at the time of writing there are no long-term antibody titre studies available, in elephants, our experience and the published studies on behaviour and hormone levels suggest that injection intervals may be extended after initial monthly or three-monthly injections (De Nys *et al*., 2010; Lueders *et al*., 2014).

Unwanted side effects

GnRH vaccination is known to cause granuloma or occasional injection abscesses in other species (Curtis *et al*., 2008). Occasionally in the study elephants, mild swelling or stiffness in the limb in which the injection had been delivered was observed for 2–7 days (Lueders *et al*., 2017). Furthermore, when the GnRH vaccine was injected in the neck, elephants were unable to use hay nets for up to 2 days, indicating some pain or discomfort at the injection site.

In males, a less masculine appearance, diminished muscle development (Lueders *et al*., 2014) and reduced size of the penis in younger individuals were observed (Bertschinger & Sills, 2013; Lueders *et al*., 2014). The body size and tusk growth seemed to be unaffected (Bertschinger & Lueders, 2018).

In women it is known that a lack of oestrogens after ovarian downregulation affects bone metabolism (Matta *et al*., 1988). Elephants are long-lived animals and, as yet, it is unknown whether and to what extent early ovarian hormone suppression can alter bone mineralization in females. Because GnRH receptors are not only located in reproductive organs but also in other tissues, such as the heart (Skinner *et al*., 2009), further studies on the long-term effect of GnRH vaccination on general health are needed.

Reversibility

Ideally, immunocontraceptives are reversible. However, the commercial GnRH vaccines have not been tested in this respect, especially following multiple treatments for several years. In a study on Asian elephant bulls with monthly injections for three consecutive months, testosterone levels were back to initial levels *c*. 5 months after the last injection (Somgird, Homkong *et al*., 2016). Similarly, in African elephant bulls with four injections at 3–7-week intervals, testosterone was back to normal or bulls entered musth 6 months after the last booster (De Nys *et al*., 2010). In general, our data suggest that longer treatment will cause longer reversal times or even permanent infertility. In the case of a young male in an earlier study (Lueders *et al*., 2014), the last treatment occurred more than 5 years ago, and this bull remained at baseline testosterone levels and acts as a castrate (C. Gray, African Lion Safari, Canada, pers. comm., June 2018).

Correspondingly, evidence from our tests on African elephants over a 3·5 year course of vaccinations suggests permanent damage, especially in younger individuals (Lueders *et al*., 2017). Four of these bulls (17–38 years of age) were re-evaluated 1·5 years after the last vaccination following a treatment period of 3·5 years (at least eight vaccinations per animal). Only the oldest, wild bull showed resumption in sperm production and increased testosterone levels, while the other three animals were still suppressed.

The authors are only aware of one Asian elephant female (25 years of age) which received two vaccinations that resulted in oestrous-cycle suppression for 5 months after which ovulation occurred again (Fig. 2b), suggesting a similar period of gonad function suppression for females as for male elephants.

The GnRH vaccine was not initially developed as a reversible contraception method. Although data suggest that after two to three injections reversibility is likely, age at first vaccination seems to play an important role. Younger animals may be interrupted in the development of their reproductive organs (i.e. penile atrophy), while older, fully developed animals may recover faster. Long-term application (> 6 vaccination events) may result in permanent or at least very extended infertility.

Summary and Conclusions

The existing data on the application of GnRH vaccines in elephants point towards a proper effect in suppressing gonadal function in both males and females. Massei & Cowan (2014) summarized that the efficacy of immunocontraceptive vaccines is generally dependent on species, gender, age, individual variation in immunocompetence, as well as the formulation and dose. All this seems to be reflected in the studies on elephants that have been carried out to date.

Regular booster vaccinations of the formulation that is commercially available at the time of writing, seem to play an important role for efficacy, making the use of the vaccination under field conditions more difficult. Gonadal downregulation may be especially of interest for bull behavioural management and pathology treatment in Asian elephant females, rather than for contraceptive purposes, as long as there is no long-acting option available on the market. For elephant management in captivity there are beneficial effects in terms of welfare; for example, musth suppression (males), keeping multiple males together or leiomyoma treatment (females).

Our preliminary data indicate that young elephants that are required for future breeding should not be treated with the GnRH vaccine because fertility and reproductive-organ development are affected. In these cases, reversibility is in question. At the same time, young bulls that are not designated for breeding may be vaccinated from a very young age, which will make it possible to rear and keep them within female groups for the longer term.

The use of GnRH vaccination has to be assessed on a case-by-case basis and the effects should be closely monitored (hormone measurements, behavioural changes, ultrasonographic examinations). Further studies, especially on social behaviour and long-term health under vaccination and reversibility are certainly warranted.

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Products Mentioned in the Text

GonaCon®: long-acting gonadotrophin releasing hormone vaccine (GnRH conjugate vaccine), manufactured by the National Wildlife Research Center, Fort Collins, CO 80521, USA.

Improvac®/Improvest®: GnRH conjugate vaccine manufactured by Zoetis®, Parsippany, NJ 07054, USA.

Repro-BLOC®: GnRH conjugate vaccine, manufactured by Amplicon Vaccine, LLC, Pullman, WA 99163, USA.

SpayVac®: long acting contraceptive porcine zona pellucid vaccine, manufactured by Spayvac®, Inc., Madison, WI 53705, USA.

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