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

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ABSTRACT

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

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

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INTRODUCTION

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

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

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

MATERIALS AND METHODS

Elephant bulls

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

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

Thembo: wild and free-ranging on

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

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

*While immobilised for relocation into captivity to Moketsi, tractable after 3 weeks.

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

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

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

GnRH vaccine and adjuvants

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

Vaccination protocol

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

Collection and storage of faecal and serum samples

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

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

Table 2: **Serum testosterone concentrations of 5 elephant bulls.**

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

Faecal steroid analyses

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

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

Serum testosterone

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

Collection of behavioural data

Frequency of aggressive behaviour was assessed daily during Stages 1, 2, 3, 4, 5 and 7 (Kinkel); Stages 1, 4 and 7 (Thembo) and during Stages 1, 2 and 7 (Imire bulls) using behavioural traits relating to aggression, musth and dominance as previously described[19,43,45]. In total, 38 different behavioural traits related to aggression were used. Some of the more important ones were: head high, chin tucked in, shaking head, ear-flapping, waving, forward trunk swing, pushing, kicking, charging/advance towards, grabbing, throwing or destroying objects, tusking, rumbling and urine dribbling. The monitoring frequency of the Imire bulls and Thembo was lower because of distance constraints. Grootvoet was free-ranging and mostly solitary so that behaviour towards other elephants could not be monitored. Observations allowed the classification of bulls as being in musth or non-musth, compare frequencies of aggressive behaviour before and after immunisation, determining the dominance hierarchy between bulls and detection of other possible behavioural and physical changes. A male in full musth exhibits temporal gland secretion and urine dribbling, or evidence of recent urine discharge[43].

Data analysis

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

RESULTS

Vaccination

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

Behaviour and GnRH vaccination

The frequencies of aggressive behaviour in relation to the stages of the study are shown in Fig. 1. Grootvoet was the only bull in musth at the time and before the primary vaccination. He went out of musth 10 days after the 1st vaccination and his aggressive behaviour ceased

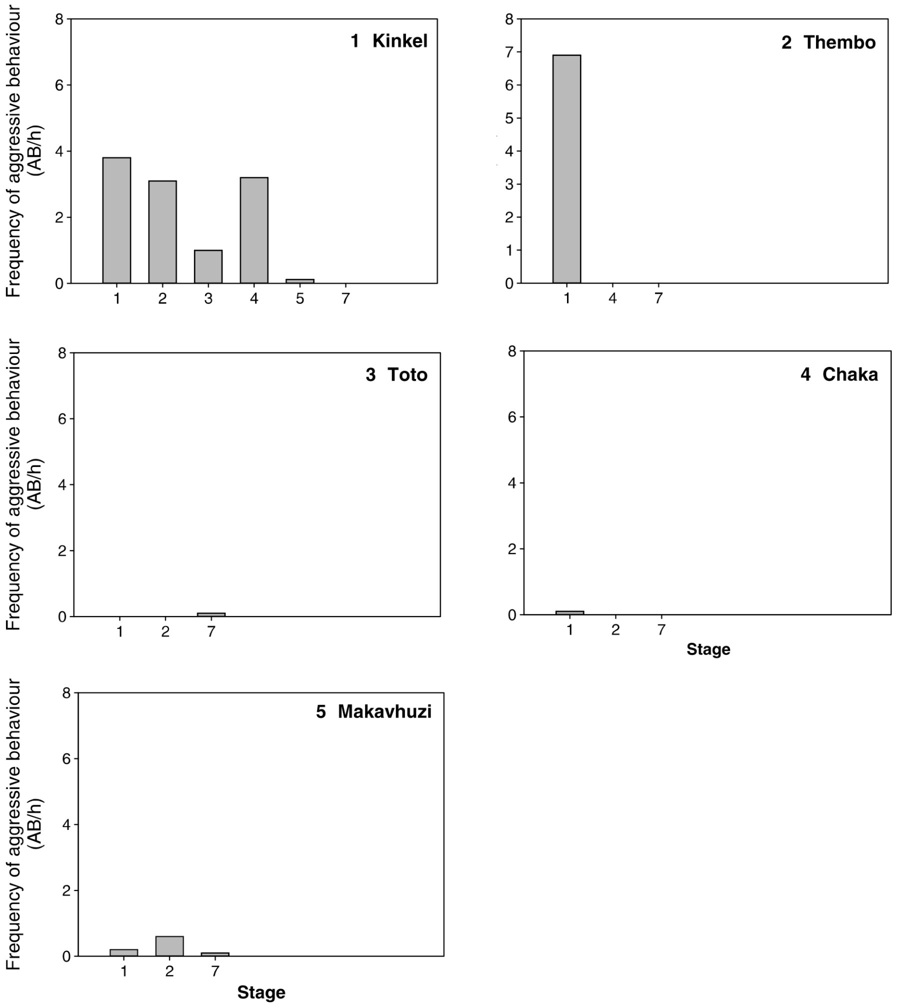

Fig. 1: **Frequency of aggressive behaviour** (number of AB per hour) **as a function of the stage of immunization for Kinkel, Thembo, Toto, Chaka and Makavhuzi.** (Stage 1, before primary vaccination; Stage 2, after primary vaccination; Stage 3, after 1st booster; Stage 4, after 2nd booster; Stage 5,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

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

Faecal androgen metabolites (FAM)

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

Figure 3 shows the within bull effects of a GnRH vaccination on FAM concentrations. Significant differences between stages were observed in Kinkel ($P < 0.05$), Thembo ($P < 0.001$), Toto ($P < 0.05$) and Chaka ($P < 0.05$). Only Stages 1, 5 and 7 were taken into consideration for Kinkel

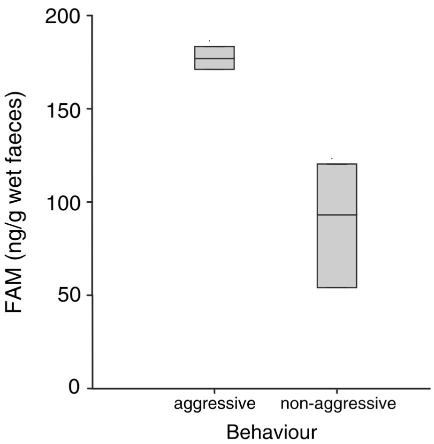

Fig. 2: **Grouped concentrations of faecal androgen metabolites** (FAM) **of aggressive** (n = 2) **and non-aggressive** (n = 3) **non-musth bulls during Stage 1** (before primary vaccination).

due to the lack of complete administration when Montanide ISA 51 was used for the first 3 vaccinations. No significant differences were found in Makavhuzi and Grootvoet. The faecal sample collected from Grootvoet 3 months before the 1st vaccination (in full musth), however, had a FAM concentration (209 ng/g) approximately 3 times higher than the samples collected during Stages 1, 2, 3 and 4 (62 ± 24 ng/g).

Serum testosterone

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

Faecal cortisol metabolites (FCM)

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

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

DISCUSSION

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

When non-musth bulls were divided into aggressive (Thembo and Kinkel) and non-aggressive groups (Toto, Chaka and Makavhuzi) during Stage 1, behaviour was well correlated to FAM concentration. The mean FAM concentration of the non-aggressive bulls was significantly lower than the mean of the aggressive bulls. During this stage both aggressive bulls had permanent mucoid TGS without urine dribbling which is equivalent to the 1st and last stage of full musth (bulls

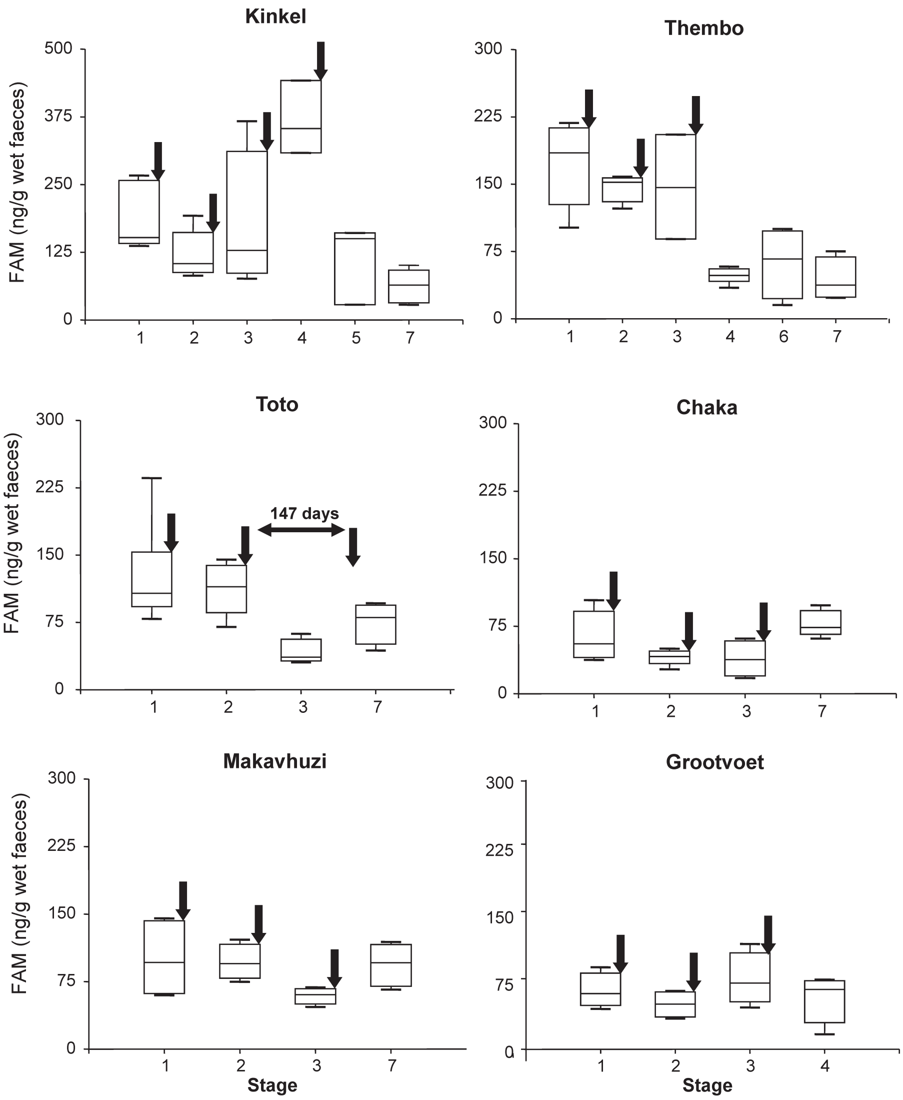

Fig. 3: **Grouped concentrations of faecal androgen metabolites** (FAM) **for each of the 6 bulls. The boxes represent the median and the upper and lower quartile values of samples collected at different stages of vaccination. The whiskers show the range. Black arrows indicate vaccinations.** (Stage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

entering or leaving full musth[17,18]). Kinkel's behaviour only improved after the 3rd booster vaccination which was the 1st time vaccine delivery was complete. This was accompanied by cessation of TGS and a significant lowering of FAM concentrations during Stages 5 and 7. Thembo's behaviour, accompanied by a cessation of TGS, improved after the 1st booster. A significant decrease in FAM concentration was also seen. At the same time he was taken into captivity where he has remained for the last 6years. He is revaccinated (since 2006 with the commercially available GnRH vaccine Improvac, Pfizer Animal Health, Sandton, South Africa) every 6 to 8 months, is tractable and used for education purposes and elephant-back rides.

Of the 3 non-aggressive bulls, Toto also showed a significant decrease in FAM after the 1st booster (Stage 3). The other 2 other bulls (Chaka and Makavhuzi) showed no significant changes, with concentrations having been low from the onset of the trial. They remained non-aggressive for the remainder of the observation period and for another 6 months thereafter when Chaka came into musth. At the time, he and the 2 other bulls had not been revaccinated since the 2nd booster.

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

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

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

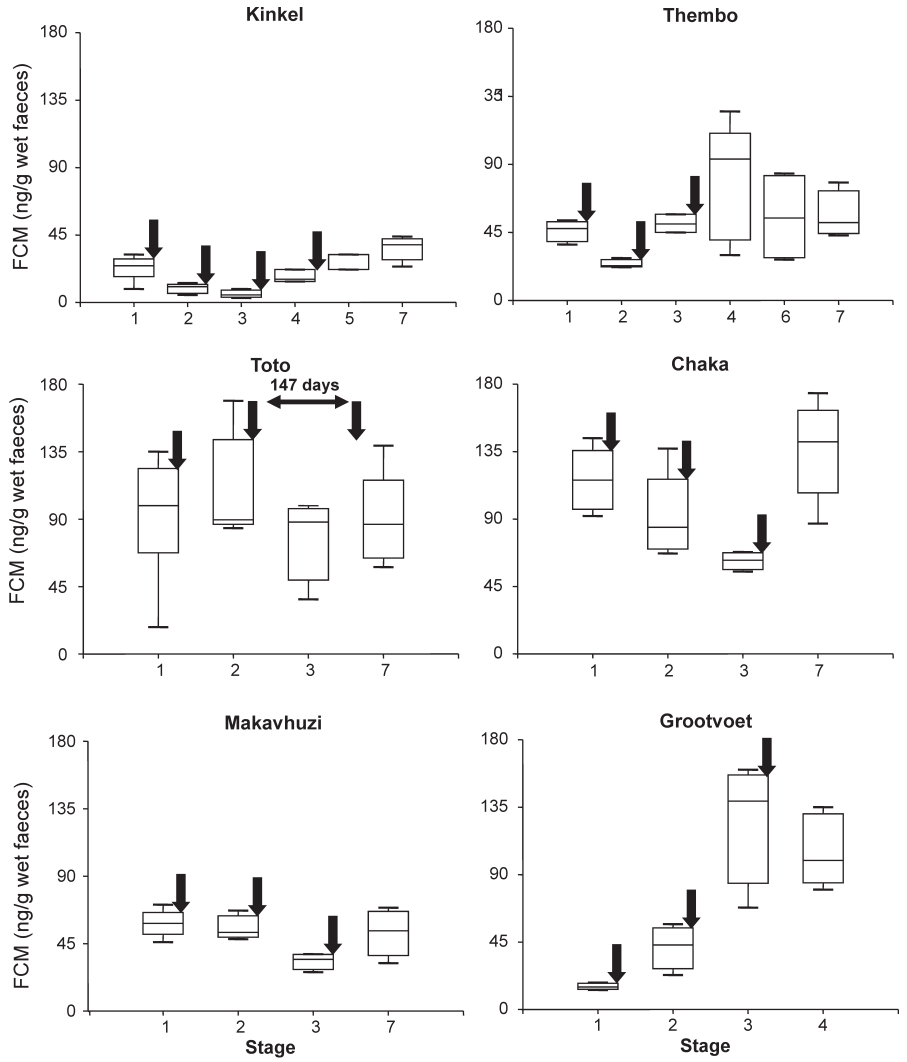

Fig. 4: **Grouped concentrations of faecal cortisol metabolites** (FCM) **for each of the 6 bulls. For further details see caption of Fig. 3**.

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

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

Despite the limitations of this study, which include low sample size, some inconsistencies in the vaccination protocol and difficulties surrounding behavioural observations of 2 bulls, the results were encouraging. Most studies with GnRH vaccine in other species failed to produce an adequate response in 100 % of treated animals. It seems that a certain number of non-responders can be expected[23,32,49,51,52]. The reasons for individual variations are not completely understood. Age has clearly been shown to influence the results with older animals showing greater individual variation, less marked responses and shorter duration of effects[9,32,49,51,52]. In horses, 2 vaccinations with the modified GnRH-tandem-dimer vaccine-Covaccine adjuvant-combination were sufficient to suppress testosterone secretion in young sexually mature pony stallions but further boosters were generally needed in older stallions[49]. In blackbuck (*Antilope cervicapra*) and springbok (*Antidorcas marsupialis*), testosterone concentrations were reduced in young but not in adult rams[51]. The authors of a recent study[23] were able to suppress testosterone concentrations in 4 of 5 stallions treated with a GnRH-protein conjugate (Equity™) for at least 6 months. The stallion that did not respond also showed the lowest antibody titres. The same stallion, however, showed a marked decrease in libido while 1 of the good responders with low testosterone concentrations demonstrated good libido. The behavioural response (libido suppression) was better than that reported by other authors[7,52]. These findings show that the control of sexual behaviour is complex and not only testosterone dependent. It is also affected by factors such as age and previous sexual experience in the horse[49]. Rather than having non-responders in our trial the results in some of the bulls were inconclusive. Two aggressive non-musth young bulls responded whereas in the non-aggressive 3 young bulls a response was difficult to assess. One of these bulls did, however, show a reduction in androgen production. The results for the sexually mature adult bull in the trial were inconclusive as he was probably going out of musth around the time of the primary vaccination.

CONCLUSIONS

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

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