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The use of pZP and GnRH vaccines for contraception and control of behaviour in African elephants

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The use of antigens to control of fertility is known as immunocontraception or immuno-fertility control. A number of antigens have been employed in the past with varying degrees of success. In the past the most commonly used antigen have been porcine zona pellucida (pZP) proteins to control fertility of females. It was first investigated as a possible contraceptive for use in humans but owing to possible destruction of ovarian follicles in the ovary resulting in permanent sterility the use has never been implemented in this species. Other methods have looked at the use of sperm antigens to control both male and female fertility but the results have been disappointing, particularly in the female. Another approach is to use hormones as antigens. Because hormones are endogenous substances and sometimes haptens, they need to be conjugated to foreign proteins in order to make them antigenic. One hormone in particular, which has been used successfully as a vaccine to control reproduction in domestic animals, is GnRH[9]. This paper reports on a project that was carried out in three phases to investigate the use pZP vaccine to contracept some elephants in the Kruger National Park (KNP) and, following on this initial work, a number of projects in private game reserves. The use of a GnRH vaccine to control aggressive behaviour in elephant bulls is the briefly discussed.

Contraception of elephant cows with pZP vaccine

Zona pellucida proteins isolated from ovaries of pigs (pZP) have been shown to be effective for the immunocontraception of horses[3,6,10] and a range of wild and zoo herbivores[4,5,7,8]. In these species the vaccine was shown to be safe and, where tested, also reversible. The potential use of pZP vaccine for contraception of free-ranging African elephants has been recognised for some time.

In order to understand how pZP vaccine works as a contraceptive we need to look at the process of fertilisation of the egg by a sperm. This is explained in Fig 1 (A-C). Blocking of all sperm receptor sites relies on antibody concentrations that are sufficiently high to achieve this and should the concentrations fall below a critical level, which happens over time, the cow will once again be fertile. pZP only targets the zona pellucida of the cow and has no direct effect

on behaviour. Because the cow does not fall pregnant she will continue to show an oestrous cycle, which is 15-17 weeks long. This means that she will come on heat 2-3 times a year.

Kruger park: Phase 1

This phase was designed to establish homology between porcine and African elephant zona pellucida proteins. In 1995, during the last cull that took place in the KNP, ovaries were collected from several elephant cows. After fixation ovarian blocks were embedded in paraffin and sectioned. Sections were then processed for immunocytochemistry. They were

Fig 1: A - When the egg (oocyte) is ovulated into the Fallopian tube it is surrounded by a capsular layer known as the zona pellucida capsule.
B- Before fertilisation can take place the sperm binds to one of thousands of receptor sites on one of the zona proteins. The sperm then undergoes the so-called acrosome reaction.
C - Only once the sperm has undergone the acrosome reaction can it penetrate the zP-capsule and then a single sperm fertilises the egg.
D - The antibodies formed in response to the pZP vaccine recognise and cover all sperm receptors on the ovulated elephant egg. The binding of sperm is blocked as is fertilisation and thus pregnancy

first treated with rabbit-anti-pZP (primary antibody) followed by the secondary antibody labelled with colloidal gold and finally enhanced with silver. Histological examination of the sections showed distinct immunogold staining of the zona pellucida of oocytes in primary, secondary and tertiary follicles. The results were confirmed in samples examined with TEM. This proved that there are shared epitopes between the ZP proteins of the two species and that antibodies to pZP should recognise elephant zona pellucida proteins (Fayrer-Hosken *et al.*, 2000).

Kruger Park: Phase 2

Phase 2 was designed to establish a PZP vaccination regime for African elephants. Two elephant cows (both at zoos in the USA) were vaccinated with 400 µg of pZP and 5mg of synthetic trehalose dicorynnomycolate adjuvant. Boosters (400 or 600 µg) were administered after 4 weeks and 10 months, respectively. The antibody concentrations achieved after the second booster in these cows were comparable to those of horses that had been successfully contracepted with pZP vaccine (Fayrer-Hosken *et al.*, 2000).

Kruger Park: Phase 3

The objective of Phase 3 was to test the contraceptive efficacy of the pZP vaccine in a sample of free-ranging elephant cows in the KNP. In 1996 41 cows (21 treatment and 20 controls) were selected for the first trial. Selection criteria were a small calf (< 1 m high) and a negative trans-rectal ultrasound pregnancy result. The Treatment Group was vaccinated with 600 µg of pZP and the same adjuvant as described above. The controls were injected with placebo. All treated cows were radio-collared and 6 weeks later they were located and darted with a booster from a helicopter using dropout darts. The procedure was repeated 6 months after the primary vaccination.

Twelve months after the primary vaccination 19 Treatment and 18 Controls were located, immobilised and examined for pregnancy. One cow in the Treatment Group was excluded as the results showed that she was already pregnant at the time of the primary vaccination. Of the remaining Treatment Group 8 (44 %) were pregnant compared to 16 (89 %) in the controls. Although the difference was significant the results were disappointing compared to those achieved in free-ranging horses. A second trial with 10 vaccinated cows was thus planned. This time the vaccination intervals between the primary vaccination and first booster and the first and second boosters were 2 and 4 weeks, respectively. The results showed a

significant improvement with only 2 (20 %) cows falling pregnant. The trials also showed that the vaccine is safe to use in pregnant elephants, that contraception can be maintained by administration of an annual booster and, if a booster is not used, that cows return to fertility. These are important qualities when selecting a contraceptive method for wildlife species

Private Game Reserves

Since the Kruger Park project we have been using the pZP vaccine on elephants in private game reserves. The vaccination protocol has changed in that we now make use of modified Freund's for the primary and incomplete Freund's adjuvant for the booster vaccinations. This has resulted in a 100 % efficacy in the cows that have passed the critical inter-calving period since vaccination was first introduced. The reserves, number of cows and the year in which vaccination was implemented are Makalali (n = 23; 2000), Mabula (n = 4; 2002), Shambala (n = 4; 2004), Phinda (n = 19; 2004) and Thaba Tholo (n = 8; 2004). The vaccine used during 2003 and 2004 was produced by our laboratory where we manufacture about 1 500 doses a year. The number of doses can be increased if necessary to about 10 000/annum or more.

Makalali is the only private reserve where the project has been running long enough to provide data on the efficacy of the vaccine and possible effects on behaviour. Twenty-three cows have been vaccinated. The protocol employed for most of the cows was a primary vaccination followed by two boosters at 3-4 weeks intervals during the first year. A single annual booster for each cow followed this. Darting was performed from the ground during the first two years of vaccination but in 2003-2004, 17 and 21 cows were darted from the helicopter. At no time was immobilisation of animals necessary to perform vaccinations. Total time taken for helicopter vaccination was about 30 min and the herds settled down much quicker (1-2 days) then if darted from the ground. The success rate has been encouraging to say the least. Ten cows have passed the 53-month inter-calving period of the reserve with no early calving indicating 100 % reproductive control so far.

The behavioural observations and the effects of pZP vaccination on herd structure at Makalali will be reported by Delsink *et al.* at this Workshop.

This coming year we will treat the first elephant cows with a so-called one-shot vaccine. This means that the vaccination protocol for the first year can be reduced to a single darting. This will make a big difference to the cost of contraception and will make it feasible to treat much larger populations of elephants. Even without the one-shot vaccine it is possible to contracept large populations.

In conclusion we feel that it is important to emphasise the following points:

- The pZP vaccine can be used successfully to contracept African elephants
- The Vaccine is safe during pregnancy and has no negative effect on birth or calf raising
- It has no side effects other than occasional lumps at the site of vaccination
- It is reversible
- Other than an increased incidence of heat no behavioural side effects were seen
- Administration of the vaccine is carried out remotely by darting and does not require immobilisation

Control of aggressive behaviour in African elephant bulls using

GnRH vaccine

Captive and free-ranging elephant bulls can sometimes constitute a serious management problem when displaying aggressive behaviour with or without musth. They can endanger the lives of both other animals and people. Trained elephants usually have to be removed from work programs and necessitate restraint to such an extent that it becomes a welfare issue. As there is no practical way to control musth as yet, culling often constitutes the only resort for free-ranging problem bulls. Musth and aggressive behaviour seem to be related to high concentrations of testosterone. The aims of this project were to test the effects of a GnRH vaccine on testosterone secretion and behaviour, with a view to using the vaccine to control musth and aggressive behaviour in elephant bulls.

Fig 2 shows the endocrine control of testicular function in the male and also indicates the level at which the GnRH antibodies work. Because endogenous GnRH released from the Hypothalamus is neutralised the release of LH and FSH by the pituitary will also be down-regulated. This means that the vaccine should work in both males and females.

Fig 2: Endocrine control of testicular function in the male and site of action of anti-GnRH antibodies

The GnRH vaccine that we used is GnRH-tandem-dimer conjugated to ovalbumine (Pepscan Systems, Netherlands) used together with Covaccine adjuvant[9]. We have treated 10 bulls so far and each bull was vaccinated with 2 mg GnRH, 3 times at 3 weeks intervals by means of darting or a pole syringe. Faecal epiandrosterone using an EIA was used to monitor testosterone secretion and faecal samples were collected prior to, 3 weeks after each and 4 months after the last vaccination. Behaviour was monitored during the same periods when faecal samples are collected.

Two of the bulls were in musth at the time of the primary vaccination and another 5 bulls were acting aggressively. Three bulls were part of a safari exercise and were neither in musth or aggressive at the time. Faecal epiandrosterone assays have only been performed on the first 5 bulls. The effect of GnRH vaccination on epiandrosterone was unclear partly because the concentrations in 3 of the bulls were low from the start. Two of the bulls did show a lowering of epiandrosterone. The assays on the remaining bulls still need to be carried out. From a behaviour point of view the 5 bulls that were showing aggressive behaviour without distinct musth became much calmer. One trained bull that was non-aggressive at the start came into musth approximately one year after the last vaccination. He was given a booster but because the owners did not have facilities to control him properly until the vaccine may have taken effect, he had to be shot. Musth ceased in the one bull three weeks after the first vaccination while we are still waiting for the results on the second musth bull. Although it is premature to draw any definite conclusions on the use of GnRH vaccine in elephant bulls the results look promising.

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