

DISCUSSION

Prospects for managing African elephant population growth by immunocontraception: a review

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Abstract

Immunocontraception has been proposed as a tool for managing African elephant populations threatening to 'outgrow' a wildlife reserve. To date, however, the only immunocontraceptive technique tested on elephant cows is porcine zona pellucida (pZP) vaccination, in which solubilized pZP is injected together with an adjuvant to induce formation of circulating antizona pellucida antibodies, which block fertilization. A review of the literature on the use of pZP vaccination in free-ranging mammals reveals that the contraceptive efficacy ranges between 22% and 100% (15 trials, 2 in elephants). A pZP vaccine can be delivered by dart, but at present more than one inoculation is needed to ensure contraceptive antibody titres. Initial studies in elephants suggest that pZP vaccination is safe, even in pregnant animals, does not pass through the food chain and is reversible, at least in the short term. However, little is known about possible long-term side effects. Elephants are social animals that live in matriarchal herds, and inhibiting individual fertility and herd growth may have unforeseen longer-term consequences on behaviour and social structure. There is also a fear that immunization may favour weaker animals by preferentially sterilizing individuals capable of mounting a vigorous immune response, or that animals may become resistant to vaccination. In short, while pZP vaccination appears to be a promising tool for controlling elephant population growth, questions about the long-term side effects need to be answered before use on a large scale can be recommended.

Résumé

L'immuno-contraception est un des moyens proposés pour la gestion des populations d'éléphants qui menacent de dépasser les capacités d'une réserve de faune. A ce jour pourtant, la seule technique d'immuno-contraception testée sur des femelles éléphants est le vaccin porcin contre la zone pellucide (pZP), une injection de pZP soluble et d'un adjuvant pour induire la formation d'anticorps contre la zone pellucide, qui bloque la fertilisation. Une révision de la littérature traitant de l'utilisation de la vaccination pZP chez des mammifères en liberté révèle que l'effet contraceptif a une efficacité qui va de 22% à 100% (15 essais, deux chez l'éléphant). Un vaccin pZP peut être administré par fléchette, mais pour le moment, il faut plus d'une inoculation pour garantir le taux d'anticorps contraceptifs. Les premières études montrent que la vaccination pZP est sans danger pour les éléphants, même pour les femelles enceintes, qu'elle ne passe pas par la chaîne alimentaire et qu'elle est réversible, en tout cas à court terme. Cependant, on sait peu de choses de ses effets secondaires à long terme. Les éléphants sont des animaux sociables qui vivent en groupes matriarcaux, et le fait d'empêcher la fertilité

individuelle et la croissance du groupe peut avoir à long terme des conséquences imprévues sur le comportement et la structure sociale. On peut aussi craindre que l'immunisation puisse favoriser des animaux plus faibles en stérilisant de préférence les individus capables de manifester une réponse immunitaire vigoureuse, ou que les animaux deviennent résistants au vaccin. En brève, si le vaccin pZP semble un outil prometteur pour contrôler la croissance des populations d'éléphants, il faut d'abord répondre aux questions sur les effets secondaires à long terme avant de pouvoir en recommander l'usage à grande échelle.

Introduction

In 1967, it was decided that the Kruger National Park (KNP) elephant population should be restricted to approximately 7000 individuals, the estimated carrying capacity of the park (Van Aarde et al. 1999; Whyte et al. 1999). As a direct consequence, in excess of 17,200 elephants were culled or relocated between 1967 and 1996 (Van Aarde et al. 1999). However, in 1995 the park stopped culling as a result of public disapproval (Cumming et al. 1997; Fayrer-Hosken et al. 1997, 1999; Van Aarde et al. 1999), and the number of elephants has been rising ever since. Worryingly, some studies have concluded that the resulting high elephant densities will lead to habitat destruction (Cumming et al. 1997; Fayrer-Hosken et al. 1999; Whyte et al. 1999; Fayrer-Hosken et al. 2000) and threaten the survival of other species (Cumming et al. 1997; Whyte et al. 1999), thereby reducing biological diversity (Cumming et al. 1997; Van Aarde et al. 1999; Whyte et al. 1999). Although this conclusion is disputed (Van Aarde et al. 1999), KNP and other wildlife reserve managements now widely accept that a high elephant density may negatively influence a park's other flora and fauna, and they are investigating measures to maintain stable 'optimum' elephant populations (Van Aarde et al. 1999).

Effectively, there are only two ways to actively manage the size of an animal population: increase the rate of removal, usually by death, or reduce the rate of addition, usually births (Kirkpatrick and Rutberg 2001). Currently, the numbers of elephants in the larger southern African parks are too large for relocation to be considered practical and culling is subject to an ongoing ethical debate (Whyte et al. 1999). For these reasons, attention has recently focused on the development of techniques for reducing the birth rate, usually by contraception.

Similar problems with overabundant wild or feral species in protected reserves have previously arisen in the USA. Once hunting and trapping ceased to be considered acceptable by the public, non-lethal ways of

controlling population growth were sought and studies on non-invasive contraceptive techniques were initiated (Kirkpatrick and Rutberg 2001). Although a number of different approaches were examined, namely 1) non-hormonal chemicals, 2) steroid hormones, 3) non-steroid hormones, 4) barrier methods and 5) immunocontraception, the first four were, for various reasons, found unsuitable (Kirkpatrick and Rutberg 2001). As a result, immunocontraception was singled out for further investigation. Since some forms of immunocontraception, notably porcine zona pellucida (pZP) vaccination, have proven reliable and safe for controlling population growth in ungulates and horses, they were obvious candidates when starting contraception studies in elephants.

Review and discussion

Immunocontraception

Immunocontraception is based on the same principles as disease prevention by vaccination. In this instance, however, vaccination stimulates the immune system to produce antibodies against endogenous molecules that play an essential role in either gamete production or fertilization, rather than against micro-organisms (Fayrer-Hosken et al. 2000; Kirkpatrick and Rutberg 2001). Although there may be many possible target molecules for immunocontraception, the two that have received most attention are zona pellucida (ZP) proteins and gonadotrophin-releasing hormone (GnRH). GnRH is the hypothalamic neuropeptide that controls the reproductive endocrine system; successful vaccination against GnRH powerfully inhibits reproductive function, essentially returning treated animals to a pharmacological prepuberty (Stout and Colenbrander 2004). Vaccination against ZP proteins makes use of the fact that ZP, an extracellular matrix surrounding the oocyte (Kirkpatrick and Turner 1991; Muller et al. 1997; Barber and Fayrer-Hosken 2000b), plays critical roles in regulating sperm binding, penetration and fertilization (Barber and Fayrer-Hosken 2000a). Although

the exact mechanism of infertility induced by ZP vaccination may differ between species and individuals, it appears that antibodies raised against ZP proteins either directly block sperm–ZP binding or disrupt ZP formation and thereby indirectly inhibit the ability of sperm to bind and penetrate (Muller et al. 1997; Miller et al. 2000; Kirkpatrick and Rutberg 2001); in either case the end result is temporary infertility (Barber and Fayrer-Hosken 2000a; Powel and Monfort 2001). On the other hand, because ZP vaccination blocks fertilization, vaccinated females should continue to experience regular ovarian cycles, including oestrus and ovulation (Barber and Fayrer-Hosken 2000a).

Porcine zona pellucida is currently the active ingredient of choice for ZP vaccination of wildlife species because it can be harvested in large quantities from the ovaries of slaughtered pigs and the antibodies induced recognize ZP epitopes in many target species (Fayrer-Hosken et al. 1997, 1999; Barber and Fayrer-Hosken 2000a, 2000b). Initial evidence that pZP vaccination might be an effective contraceptive for elephants was provided by an immunohistochemical study demonstrating that antibodies raised against pZP in rabbits also recognize epitopes in elephant ZP (Fayrer-Hosken et al. 1997, 1999, 2000). Recent studies have examined the possibility of producing synthetic subunit ZP vaccines. They would offer better biosecurity by lowering the risk of transmitting micro-organisms between species and could more specifically target molecules involved in sperm–ZP binding and therefore reduce the risk of inducing generalized ovarian destruction.

An important part of both the GnRH and the pZP vaccines, and indeed vaccines in general, is the adjuvant. The adjuvant enhances the efficacy of vaccination by stimulating the immune system to produce larger concentrations of antibodies against the target antigen. Not surprisingly then, the efficacy of both GnRH (Stout and Colenbrander 2004) and pZP (Lyda et al. 2005) vaccination varies markedly depending on the adjuvant used. On the other hand, effective adjuvants are often ‘aggressive’ and may induce significant injection-site swellings, including abscess formation, or systemic reactions such as fever and anaphylaxis. For example, although Freund’s complete adjuvant (FCA) (Muller et al. 1997; Kirkpatrick and Rutberg 2001; Lyda et al. 2005) is a highly effective accompaniment to pZP vaccination it has been associated with significant adverse reactions, such as injection site and systemic granulomatous in-

flammation (Harrenstien et al. 2004). FCA also has a second specific disadvantage in that it can trigger false positive tuberculosis test results in vaccinated animals (Lyda et al. 2005). For these reasons, more recent pZP vaccination studies have concentrated on less aggressive adjuvants, such as Freund’s incomplete adjuvant (FIA), Freund’s modified adjuvant (FMA) (Lyda et al. 2005) or a synthetic trehalose dicorynomycolate (S-TDCM) adjuvant (Fayrer-Hosken et al. 1997, 1999; Bertschinger et al. 2003).

Brief history of ZP vaccination

Zona pellucida vaccine was first patented as a contraceptive agent in 1976, and the first field trials in free-ranging feral horses followed in 1988–1989 (Kirkpatrick et al. 1997; Kirkpatrick and Rutberg 2001; Kirkpatrick and Turner 2003). In the early years, studies focused predominantly on whether ZP immunization was contraceptive, and how many inoculations were required to achieve infertility. Subsequent studies investigated other aspects of immunization such as long-term side effects on health and behaviour, and more efficient delivery methods (Kirkpatrick and Rutberg 2001; Miller et al. 2001).

The first elephants to be treated with pZP were zoo animals. The initial study aimed to establish an effective dose and inoculation protocol, and the results were sufficiently promising for the South African National Parks Board, KNP and the Humane Society of the United States to design a field trial to examine the safety and efficacy of pZP vaccination in wild elephants (Fayrer-Hosken et al. 1997, 2000; Kirkpatrick and Rutberg 2001; Bertschinger et al. 2003). While the results of the field trial, at least in terms of short-term safety and efficacy, were also promising (Fayrer-Hosken et al. 1997, 2000), there are still considerable hurdles to negotiate. For example, Whyte (2003) predicted that to stabilize a large elephant population, 75% of all breeding females would need to be continuously contracepted; even this assumes no compensatory improvement in fertility among non-contracepted animals. Moreover, repeated immunization of large numbers of elephants may be financially impossible for many parks or conservation agencies. Nevertheless, research into more efficient vaccination protocols and the effects on behaviour continues (Delsink et al. 2003), because immunocontraception may be an affordable management option for smaller parks with 100 cows or fewer (Bertschinger et al. 2003).

Prerequisites for an immunocontraceptive

To objectively assess the suitability of putative contraceptives for use in wildlife species, it is essential to be clear about the prerequisites to which a 'gold standard' contraceptive should conform (Frayne and Hall 1999; Kirkpatrick and Rutberg 2001). Here we will examine pZP vaccination, and in particular initial results from elephant studies, in terms of whether the following criteria for an acceptable contraceptive are met (Kirkpatrick and Turner 1991; Kirkpatrick and Rutberg 2001):

- contraceptive effectiveness of at least 90%
- the capacity for remote delivery with no (or minimal) handling of animals
- reversibility of contraceptive effects
- safety for use in pregnant animals
- absence of significant health side effects
- no passage of the contraceptive agent through the food chain
- minimal effects upon individual and social behaviour
- low cost

Contraceptive efficacy of at least 90%

Reported contraceptive efficacy of pZP vaccination varies considerably, although results can be difficult to compare because they are often expressed differently, for example, as a percentage of animals failing to become pregnant or as a percentage reduction in the pregnancy rate between a treated and a control population (table 1). In non-elephant species, contraceptive efficacy of pZP vaccination has varied dramatically with reports of between 78% and none of the treated animals giving birth during the treatment period (Kirkpatrick et al. 1990, 1997; Turner et al. 1992, 1996a, 1996b; McShea et al. 1997). The two elephant studies reported to date have recorded post-vaccination pregnancy rates of 44% and none among immunized cows (Fayrer-Hosken et al. 2000; Delsink et al. 2003).

Capacity for remote delivery with no or minimal handling of animals

Essentially, there are two ways to immunize a wild animal without needing to restrain it: oral delivery or remote delivery using an injection dart or a biodegradable 'bullet'. The major drawbacks of oral vaccine

delivery are the need to ensure that the vaccine is not destroyed by the digestive system (Muller et al. 1997; Kirkpatrick and Rutberg 2001) and difficulty in ensuring that a targeted individual receives the vaccine. The risk of inadvertently contracepting a non-target animal or species would also be unacceptably high (Kirkpatrick and Rutberg 2001). For vaccination by dart or bullet, the antigen (such as pZP) and adjuvant must be loaded to ensure effective delivery following impact (Muller et al. 1997). Even then, one of the great disadvantages in large populations requiring prolonged contraception is the need to administer multiple boosters to individual animals (Fayrer-Hosken et al. 1999; Kirkpatrick and Rutberg 2001; Pimm and Van Aarde 2001; Bertschinger et al. 2003; Delsink et al. 2003). To overcome this obstacle, recent studies have focused on developing single-administration immunization protocols (Kirkpatrick et al. 1997; Turner et al. 1997; Kirkpatrick and Rutberg 2001). The key to such protocols has been the development of biodegradable and non-toxic microspheres or pellets into which not only can the antigen and adjuvant be loaded (Muller et al. 1997; Frayne and Hall 1999; Kirkpatrick and Rutberg 2001), but for which the rate of degradation can be engineered to enable release of a dose of vaccine after a predictable delay (Kirkpatrick and Rutberg 2001; Kirkpatrick 2003). In early trials with pZP vaccine in biodegradable polymers, a single inoculation achieved anti-pZP antibody titres or degree of contraception comparable with two inoculations of conventional vaccine (Turner et al. 2001; Liu et al. 2005). Moreover, since Liu et al. (2005) were able to raise anti-ZP antibody levels in horses to contraceptive levels for at least 43 weeks, the development of an additional pellet that would release antigen nine months after introduction would allow two years of contraception to be achieved from a single inoculation (Kirkpatrick and Rutberg 2001). Proof that longer-lasting contraception from a single administration is possible was provided by a study in which contraception lasting about six years was achieved in grey seals injected with pZP packaged in liposomes (Kirkpatrick and Rutberg 2001). On a cautionary note, there are concerns that single-inoculation vaccines designed to immunize for longer periods by slow continuous release of antigen may lead to immunotolerance, instead of maintaining antibody concentrations at contraceptive levels (Kirkpatrick et al. 1997).

Reversibility of contraceptive effects

An important prerequisite for a wildlife contraceptive is reversibility. Ideally, it should be possible to allow a population to resume reproduction at short notice, such as immediately following an unexpected population crash. In theory, contraception resulting from pZP or other antifertility vaccines will be reversed automatically once circulating antibody concentrations drop below a threshold (Barber and Fayrer-Hosken 2000b). And in studies with horses, pZP vaccination for up to four years has been shown to be reversible (Turner, Kirkpatrick, et al. 1996; Kirkpatrick et al. 1997; Miller et al. 2000; Kirkpatrick and Rutberg 2001; Miller et al. 2001; Powel and Monfort 2001). Observations of pZP vaccination in elephants have also confirmed the return of fertility approximately one year after a course of three vaccinations (Whyte et al. 1998; Fayrer-Hosken et al. 2000). However, in horses vaccinated for longer periods of time, recovery of fertility was delayed for up to four years (McShea et al. 1997; Miller et al. 2000), because pZP immunization resulted in a decline in the subsequent ovulation rate (Kirkpatrick et al. 1997). It is even more sobering to consider that in some rodent and primate species, ZP vaccination has been associated with ovarian damage characterized by depletion of the primordial follicle pool and disruption of folliculogenesis, likely to result in permanent infertility (Paterson et al. 1998, 1999).

Safe for use in pregnant animals

An immunocontraceptive vaccine should be safe in pregnant animals since, in a species with a long non-seasonal gestation, it is almost impossible to avoid injecting some pregnant animals; abortion, dystocia or birth of abnormal or weakened offspring would all be unacceptable side effects. Fortunately, studies on horses, deer, burros and elephants all indicate that pZP vaccination has no visible or measurable detrimental effects on ongoing pregnancies, and harms neither the foetus nor its dam (Turner, Liu, et al. 1996; Kirkpatrick et al. 1997; Fayrer-Hosken et al. 1999, 2000; Turner et al. 1999; Kirkpatrick and Rutberg 2001; Delsink et al. 2003).

Absence of significant health side effects

Injection site reactions following pZP vaccination (Turner et al. 1996; Nettles 1997) can be serious enough to result in lameness and abscesses (Turner

et al. 1997; Fayrer-Hosken et al. 1999). However, no other dramatic effects on health have been reported and, to date, there is no evidence that porcine viruses or other microbes have been transmitted by ZP vaccines. Nevertheless, the potential risks of disease transmission by such a biological product have stimulated work on synthetic vaccines (Kirkpatrick and Rutberg 2001).

One major caveat regarding the safety of pZP vaccine is that relatively little is known about the long-term effects of repeated treatment or the associated changes in ovarian activity on overall health and behaviour (Kirkpatrick et al. 1992, 1997; Miller et al. 2001; Pimm and Van Aarde 2001). Elephants may present a particular challenge in this respect, because of their extreme longevity and complex social organization.

No passage of the contraceptive agent through the food chain

Conventional pZP vaccine does not appear to pass through the food chain (Kirkpatrick et al. 1990; Turner Liu, et al. 1996; Kirkpatrick and Rutberg 2001). The risk of a slow-release biodegradable ZP vaccine inducing contraception after ingestion is also likely to be minimal.

Minimal effects upon individual and social behaviour

One big concern in elephants is the possible effect of contraception on individual and social behaviour. The use of reproductive steroid hormones as contraceptives proved unacceptable in wildlife because of marked effects on behaviour, such as separation of treated animals from the family herd (Fayrer-Hosken et al. 2000; Kirkpatrick and Rutberg 2001). To date, there have been no reports of obvious detrimental effects of pZP vaccination on social behaviour (Kirkpatrick et al. 1997; Fayrer-Hosken et al. 2000; Kirkpatrick and Rutberg 2001; Powel and Monfort 2001). On the other hand, ZP vaccination does affect reproductive behaviour. In this respect, it is generally assumed that vaccination will not alter reproductive hormone secretion (Powel and Monfort 2001) and that treated females will therefore experience normal ovarian cycles (Barber and Fayrer-Hosken 2000). However, while some studies have indeed recorded normal oestrous cyclicality following ZP vaccination

Table 1. Overview by reference and year of pZP immunocontraception studies listed by species, duration, sample size, immunization protocol, and contraceptive effectiveness

Reference	Species/duration	Sample size		Immunization protocol	Results	
		Total	Control		Effectiveness	Effectiveness
Kirkpatrick et al. 1990	feral horses, 3 years	32	6	start: 65 µg pZP (0.5 cc) + FCA (0.5 cc) grp 1: 2x boosters: 65 µg pZP + FIA (0.5 cc) grp 2: 1x boosters: 65 µg pZP + FIA (0.5 cc)	grp 1: 50% (1 yr), 51% (2 yr), 0% (3 yr) produced foals grp 2: 62% (1 yr), 37% (2 yr), 12% (3 yr) produced foals control: 33% (1 yr), 33% (2 yr), 50% (3 yr) produced foals untreated: 45, 4% produced foals in 3rd year	
Turner et al. 1992	white-tailed deer	9	2	65 µg pZP (0.5 cc) + FCA (0.5 cc)	pZP treated 100% reduction control does 86% produced fawns	
Turner, Kirkpatrick, et al. 1996	white-tailed deer	95	52	start: 65 µg pZP (0.5 cc) + FCA (0.5 cc) booster(s): 65 µg pZP (0.5 cc) + FIA (0.5 cc) different booster regimes: 1, 2 or 3 inj.	during treatment 100% reduction control 94% (average) produced fawns	
Turner, Liu, et al. 1996	feral burros	27	11	a) 1st inj.: 65 µg pZP + FCA 2nd inj.: 65 µg pZP + FIA (3 wk) b) 130 µg pZP + FCA booster: 65 µg pZP + FIA (10/12 mo)	microspheres less successful 0% burros pregnant group a 33% burros pregnant group b 54% burros pregnant in control group	
Kirkpatrick et al. 1997	horses	127	83	start: 65 µg pZP + FCA booster 1: 65 µg pZP + FIA (4 wk)	5% pregnant in treated group 54% pregnant in control group	
	deer 2 years	30	10	a) start: 65 µg pZP + adj booster 1 (4 wk) & 2 (10 mo) 65 µg pZP + adjuvant b) start 65 µg pZP + adjuvant booster next year group b	0% pregnant group a 78% pregnant group b	
	2nd year deer	9b 74	0 6a/68b	start 65 µg pZP + adjuvant a) booster 65 µg pZP + adjuvant b) 2x booster 65 µg pZP + adjuvant	82% pregnant control 22% pregnant 39% produced fawns groups a, b 90% produced fawns before experiment	
McShea et al. 1997	white-tailed deer	28	9	a) start: 65 µg pZP + FCA (0.5 cc) booster 1 (1 mo): 65 µg pZP + FIA b) start: 65 µg pZP + FCA (0.5 cc)	group a: 0% and 10% does produced fawns (2 yrs) group b: 78% and 22% does produced fawns (2 yrs) control group: 82% and 89% does produced fawns	
	2 years			microspheres 65 µg pZP (release 4–6 wk) booster 2nd year: 65 µg pZP + FIA	both groups experienced reduced twinning	

Turner et al. 1997	feral horses	156	73	83	<p>a) start: 65 µg pZP + FCA (0.5 cc) booster: 65 µg pZP + FIA (0.5 cc)</p> <p>b) start: 65 µg pZP + FCA (0.5cc) c) 65 µg pZP + FCA (0.5cc) + microspheres d) placebo group and e) untreated group</p>	<p>a) 4.5% mares reproductively successful b) 28.6% mares reproductively successful c) 20% mares reproductively successful d) 55% mares reproductively successful e) 53% mares reproductively successful</p>
Fayrer-Hosken et al. 2003	elephants	37	19	18	<p>start: 600 µg pZP + 5 mg S-TDCM booster 1: 600 µg pZP + 5 mg S-TDCM (6 wk) booster 2: 600 µg pZP + 5 mg S-TDCM (6 mo)</p>	<p>44% pregnant in treated groupal. 2000; 89% pregnant in control group</p>
Bertschinger et al. 2003		10	10	0	<p>start: 600 µg pZP + 5 mg S-TDCM booster 1: 600 µg pZP + 5 mg S-TDCM (2 wk) booster 2: 600 µg pZP + 5 mg S-TDCM (4 wk)</p>	<p>20% pregnant in treated group after 2 yrs</p>
	2nd year	7(19)	4	3	600 µg pZP + 5 mg S-TDCM	<p>0% pregnant treated, but cyclicity maintained</p>
Miller et al. 2000	deer 6-yr study	19	11 (1 yr) 8 (2 yr) 4 (3 yr)	8	<p>start: 500 µg pZP + FCA booster 1: 300 µg pZP + FIA (4 wk) booster 2 yr and 3 yr: 300 µg + FIA</p>	<p>1 yr: 36% pregnant 2 yr: 9% pregnant 3 yr: 11% pregnant overall fertility reduction 89% (3 yr) overall fertility reduction 76% (6 yr)</p>
	only does with low antibody titre levels received boosters this means that all does were immunocontracepted					
Miller et al. 2001	deer 4-yr study	47	11 (1 yr) 8 (2 yr) 3 (3 yr)	36	<p>start: 500 µg pZP + FCA booster 1: (4 wk) 300 µg pZP + FIA booster 2: (8 wk) 300 µg pZP + FIA booster 2nd & 3rd year: 300 µg pZP + FIA</p>	<p>treated group produced 0.25 fawn/doe control group produced 1.88 fawn/doe 87% reduction (during 4-yr study) overall fertility reduction 72% (9 yr)</p>
Turner et al. 2001	feral horses	222	222	0	<p>a) 2x 65 µg pZP/FCA b) 2x 65 µg pZP/FCA + carbopol c) 1x 65 µg pZP/FCA + microspheres (no FCA) microsphere release in pulses</p>	<p>mares reproductively successful: a) 12.8% b) 10.6% c) 11.3%</p>
Delsink et al. 2003	elephants 2 yr	23	23	0	<p>start: 600 µg pZP + FMA booster 1: 400 µg pZP + FIA (3 wk) booster 2: 400 µg pZP + FIA (6 wk)</p>	<p>100% reproductive control</p>

FCA— Freund's complete adjuvant; FIA—Freund's incomplete adjuvant; FMA— Freund's modified adjuvant; pZP—porcine zona pellucida; S-TDCM—synthetic trehalose dicorynomycolate adjuvant

(Fayrer-Hosken et al. 2000; Kirkpatrick and Rutberg 2001), others have recorded abnormalities such as:

- altered ovarian function in horses (Kirkpatrick et al. 1992) and deer (Miller et al. 2001)
- altered cyclicity in primates (Nettles 1997) and deer (Muller et al. 1997)
- reduced ovulation rate in horses (Kirkpatrick et al. 1992, 1997)
- decreased oestrogen production in baboons (Miller et al. 2001) and horses (Kirkpatrick et al. 1997)
- altered ovarian structure in primates (Nettles 1997)
- follicular inflammation in deer (McShea et al. 1997)
- acyclicity in horses (Muller et al. 1997)

The effects on cyclicity tend to become more severe with the duration of elevated anti-pZP antibody titres, but it is not clear whether failure to cycle is advantageous or disadvantageous for health and behaviour.

With regard to social behaviour, African elephants live in stable groups consisting of related adult females and their offspring. Young bulls leave the herd shortly after reaching sexual maturity and go off to live in bull groups or in solitude (Rasmussen and Schulte 1998). Adult males really interact with the matriarchal herds only when a female is in oestrus and ready for mating (Moss 1983). One obvious consequence of contraception is that the number of offspring born into a herd will decrease or stop, and as yet, it is not clear whether this will affect group behaviour (Rasmussen and Schulte 1998; Fayrer-Hosken et al. 1999). The other major change expected after ZP vaccination is an increased frequency of oestrous cycles, and therefore of interaction with adult bulls. The oestrous cycle of an elephant cow lasts 12 to 18 weeks (Rasmussen and Schulte 1998). During this cycle the cow has a 2- to 10-day period of sexual receptivity when she will show oestrous behaviour, accept mating and may conceive (Moss 1983; Rasmussen and Schulte 1998). A female elephant announces her sexual receptivity in advance through chemical, auditory and behavioural signals, increasing the likelihood that a desirable bull will present himself for mating; bulls will travel great distances to find an oestrous female (Rasmussen and Schulte 1998).

In the event of pregnancy, the cow will not cycle again for at least another two years, the length of gestation (Rasmussen and Schulte 1998). Because sexually receptive periods usually end with mating and pregnancy (Rasmussen and Schulte 1998),

repeated oestrous cycles are not a normal feature of wild elephant reproduction. It is, therefore, not clear how an increase in the number of oestrous cycles due to immunocontraception will affect male behaviour, or how much the disruption caused by more frequent bull attention will affect the matriarchal groups. In brush-tail possums, increased numbers of oestrous females led to an increase in the number of visiting males (Ji et al. 2000), whereas in deer an increase in the number of oestrous females led to a reduction in interest among the dominant males (Miller et al. 2001). The effects on female elephants of repeated oestrus and failure to produce a calf at the expected interval are similarly difficult to predict.

Low cost

For large-scale use in wildlife populations an ideal contraceptive should be low cost. Indeed, Whyte et al. (1998) warned that immunocontraception may be unsuitable for use in large elephant populations or large conservation areas because of the logistics and costs. Pimm and Van Aarde (2001) calculated that the costs of controlling the KNP elephant population by pZP immunocontraception would exceed the total management budget for South African National Parks. On the other hand, Fayrer-Hosken et al. (2001) point out that the cost and the speed of field delivery have not really been assessed in large groups of elephants, and that pZP immunocontraception has proven affordable for managing herds of horses and deer. Certainly, development of a one-shot vaccine would dramatically simplify the logistics and reduce the costs of immuno-contraception (Fayrer-Hosken et al. 2001).

Genetic selection and resistance

Immunocontraception may be selective. In theory, healthy animals with a vigorous immune response are more likely to become infertile than individuals with a weak or compromised immune system (Muller et al. 1997; Nettles 1997; Miller et al. 2001). If this is true in practice, immunocontraception would essentially favour animals with poor disease resistance, and encourage reproduction among the least 'genetically fit'. However, while Muller et al. (1997) claim that genetics play an important role in the antibody response to vaccination, Kirkpatrick et al. (1997) maintain that an individual's response to pZP is

more closely related to dose, adjuvant and route of administration than to immune competence. In the longer term, it is also possible that natural selection may favour individuals genetically resistant to a contraceptive agent, although the risk of resistance could be minimized by developing multiple vaccines with slightly different activities for use in rotation or combination (Magiafoglou et al. 2003).

Opinion and recommendations

The current challenge is to determine whether immunocontraception can be responsibly and economically used to manage African elephant populations. If pZP can be proven to satisfy all the listed criteria, it needs to be considered a realistic alternative for managing elephant population growth. Unfortunately, this is a utopian view that does not take into account the moral and social dilemmas associated with the elephant's status as a highly intelligent and sensitive keystone species, or its inhabitation of areas that are also home to millions of the world's poorest people.

In both elephants and other species, the apparent success of immunocontraception varies greatly (table 1). These differences in efficacy may be largely attributable to differences in immunization protocol; more boosters generally result in more effective contraception. However, repeated immunization of individual wild elephants would be problematic because it requires identification with a radio collar, and tracking at set intervals. Not only would this be difficult and costly, but repeated darting is likely to be stressful to the animals and may make them more wary of people or aggressive towards them. The development of a one-inoculation vaccine is thus an imperative if immunocontraception is to become a realistic proposition for medium to large elephant populations.

There are also arguments about how accurate calculations of contraceptive effectiveness really are. Some studies report efficacy in terms of a reduction in pregnancy rates or population growth rate in comparison with a control population, while others use the number of vaccinated females that give birth. While both methods have their pros and cons, the choice of one over the other is the basis of some ongoing disagreements. For example, Pimm and Van Aarde (2001) have suggested that Fayerer-Hosken et al. (2000) exaggerated the effectiveness of pZP contraception in elephants because their control group had unusually high pregnancy rates

(16/18 = 89%). In a larger sample of 813 adult cows culled in KNP between 1979 and 1994 an average of 51% (range 36–77%) of adult females were pregnant. Pimm and Van Aarde (2001) argue that this lower figure is a much more realistic basis for comparison, since it is closer to what would be predicted on the basis of the 22-month gestation and 44-month calving interval typical of African elephants.

One of the most important reasons for not yet recommending widespread implementation of pZP vaccination in elephants is the uncertainty surrounding long-term safety and reversibility: a number of studies have reported either ovarian damage (Paterson et al. 1999) or reduced or delayed return to ovarian function (Muller et al. 1987) after ZP vaccination. Clarity over the effects of longer-term administration is particularly pertinent to elephants because of their extreme longevity. Moreover, even if vaccination does not directly damage the ovaries, there are indications that long non-reproductive periods may accelerate the onset of reproductive senescence in elephant cows (Hermes et al. 2004). While it is similarly difficult to predict the effects of long periods of infertility on elephant social behaviour, it is clear that repeatedly vaccinating all the adult females in a matriarchal group would eventually lead to the collapse of that herd. Any large-scale contraceptive programme for elephants will therefore have to be carefully designed and regularly updated to avoid collapse of herds due to dwindling numbers.

Of course, the costs and logistics of immunocontraception are likely to remain the greatest obstacle to implementation in large elephant populations. And while a reliable one-inoculation immunization protocol will obviously simplify the operation and dramatically reduce costs, it is possible that elephant immunocontraception may still be viable only in smaller conservation areas, where elephant numbers are low but population growth and densities are relatively high (Slotow et al. 2005; Van Aarde and Jackson 2007).

In conclusion, available evidence suggests that pZP vaccine is an effective contraceptive for elephants that can be delivered remotely, is safe to use in pregnant animals, does not pass through the food chain, and is reversible, at least after short durations of vaccination. However, before ZP vaccination can be recommended for wide-scale use in elephant population control additional studies are needed to elucidate effects on health, behaviour and reversibility after longer periods of administration.

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