Vaccines for immunological control of fertility in animals

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Summary
Fertility control has gained considerable momentum as a management tool to regulate populations of captive and wild animals and to control aggressive behaviour or improve meat quality in livestock. Anti-fertility vaccination (immunocontraception and immunocastration) is a humane alternative to methods that rely on surgical or chemical sterilisation and lethal control. Two types of experimental immunocontraceptive vaccine have been registered for field use in animals. They contain either porcine zona pellucida (PZP) proteins extracted from pig ovaries or synthetic conjugated gonadotrophin releasing hormone (GnRH) peptides. These vaccines require repeated injections and are limited to captive or small populations of free-ranging wild animals. Alternative immunocontraceptive vaccines are actively being developed either to improve efficacy or enable large numbers of wild animals to be targeted. Some employ live genetically modified viruses to deliver immunocontraception and have proved successful under laboratory conditions. The relative merits, risks, social acceptability and regulations controlling the use of existing and novel animal immunocontraceptives are reviewed.

Keywords
Animal contraception – Ethics – Genetically modified organisms – Legislation – Risk.

Introduction

Fertility control, either by surgical, chemical or immunological means has been demonstrated to be a viable alternative to lethal methods of controlling animal populations (9, 21, 38). The appropriate approaches to controlling fertility depend on the target species and intended outcome and include consideration of factors such as relative efficacy and safety, economic value, delivery mechanism, non-target and environmental effects and social acceptability (5, 9, 25, 58). Each approach raises ethical (48) and social acceptability issues (11).

In contrast to vaccines that protect against infectious diseases, anti-fertility vaccines aim to induce immune responses (antibodies or cellular immunity) against molecules naturally present in an individual. These include proteins or chemicals involved in the production of gametes and sex steroids, release of viable sperm or eggs, fertilisation, implantation and subsequent embryo development (20). Immunological approaches to controlling fertility lead to three different outcomes, depending on the reproductive functions that are affected. These outcomes are contraception, castration or interference with gestation (5, 25, 60). Immunocontraception describes the prevention of fertilisation, either by blocking the release of sperm or eggs or their subsequent interactions in the female reproductive tract. It can also be considered to include immunological interference with receptiveness of the uterine environment to embryo implantation in females. Immunocastration on the other hand describes autoimmune responses that directly affect the structure and function of the gonads in either sex, thus interfering with their ability to produce gametes and sex steroids. The third target for

immunological interference with reproduction is post-implantation embryo development and survival (maintenance of pregnancy).

Immunocontraception and immunocastration can be applied to both sexes. Immunocontraception ideally is reversible once vaccination ceases and should not affect levels of circulating sex hormones or libido in any discernable way (1). Immunocastration, apart from infertility, causes a reduction in sex hormone levels and alterations to metabolism. It is generally applied in cases where modification to behavior such as aggression control is required (19, 43, 65). Interference with pregnancy applies only to females and is linked to health risks for the mother and fetus, particularly late in gestation. It can also appear as an undesired side effect in some other contraceptive approaches and raises ethical issues comparable to those associated with chemical or surgical termination of pregnancy (2, 60).

Anti-fertility vaccines are intended to break immune tolerance to 'self antigens' and so pose a real risk of inducing unintended long-term autoimmune disease (69). Many potential target molecules are either directly required for physiological activities other than reproduction or have features in common with molecules with different functions. Vaccines against fertility must therefore selectively target antigens to minimise the risk of immunological cross-reactions and avoid unintended interference with other physiological processes (61).

Non-immunological fertility control

Conventional methods of regulating fertility in a wide range of animals include physical separation, chemical contraception, surgical sterilisation and barrier methods (5, 9, 21, 42). All these approaches have certain advantages, but also suffer from drawbacks (24, 57). Physical separation, whilst highly effective, can affect the behaviour of animals, particularly males. Surgical sterilisation (castration and hysterectomy) and contraception (tubal ligation) are highly effective for captive animals when irreversible infertility is required. However, this approach generally requires veterinary skills and since it involves invasive procedures, causes pain, discomfort and carries the risk of infection in the short term. They are not readily applicable to wildlife. Long term, castration prevents sex steroid production and affects metabolism. Tubal ligation is highly effective where there is a need to retain hormonally-dependent social hierarchies, but is not effective for controlling undesirable sex-related behaviour. Chemical contraception or castration (using oral or injectable implants) can be a highly effective and

reversible method for animal applications (5) and oral delivery of chemical contragestatives has also been used with mixed success to control populations of overabundant bird species (87). In humans, the technology for safe female chemical contraception is well advanced (7), but remains elusive for males (29). Chemical contraception can be associated with metabolic side effects and severe pathology in some species following prolonged use (18, 55). In livestock, the presence of steroid metabolites in the animals can affect meat quality (40, 71, 76) or growth rates (34). The barrier method and intrauterine devices are effective and have been used with high-value animals (dogs, monkeys and camels) but have not been widely adopted (82). It has been recognised that alternative forms of contraception need to be made more widely available both for human (58) and animal use (24).

Immunological fertility control

Adjuvant vaccines

Most vaccines that have been used for routine fertility regulation in captive or small groups of free-ranging animals are comprised of various formulations of proteins or peptide antigens mixed with adjuvants. The most effective antigens fall into three main groups:

– those derived from the gonadotrophin releasing hormone (GnRH), the luteinizing hormone releasing hormone (LHRH) and the follicle stimulating hormone (FSH)

– the structural glycoproteins forming the zona pellucida (ZP) that surrounds the oocyte

– the ovary and/or placental hormones, e.g. luteinizing hormone (LH) and human chorionic gonadotrophin (hCG) (19, 27, 30).

Many other antigens (natural and recombinant proteins and synthetic peptides), most notably derived from sperm, also have immunocontraceptive potential based on a large number of experimental studies, but so far appear less efficacious than hormonal or ZP antigens (31, 60). Vaccines based on GnRH and pig ZP (PZP) have proved practical to use in a range of captive and wild animals where direct injection or darting is feasible, whereas hCG vaccines are restricted to primates (25). Immunisation against GnRH prevents LH and FSH synthesis and leads to atrophy of the ovaries and testes. This generally induces reversible castration in males and females, although it can lead to irreversible infertility in rats (49) and lesions in the hypothalamus in pigs (54). However, immunising against the beta subunit of hCG or PZP also causes reversible contraception by preventing fertilisation and gamete development in the case of PZP (8) or early embryo

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survival in the case of hCG (3, 73, 78). All these self-antigens require potent adjuvants and repeated immunisations to generate their effect and overcome immune tolerance. The antigens and some adjuvants in current use (particularly Freund's complete, Freund's modified and other oil-based formulations) are known to be irritant and are associated with side effects that vary significantly in their nature and intensity, depending on the species and sex targeted (27, 33, 56).

Virally vectored immunocontraception

A relatively recent development is the use of genetically modified viral vectors to produce anti-fertility biological control agents. In this approach, termed virally vectored immunocontraception (VVIC), the gene for a fertility antigen is inserted into the genome of a replication competent virus. The recombinant virus then expresses the antigen in infected animals and induces a contraceptive autoimmune response (80). The viral vectors can either be used for the direct inoculation of individuals or they can be administered in a way that allows the vectors to be transmitted (self-dissemination) among free-ranging wildlife and pest animals. These vaccines are intended to supplement current control practices of shooting, trapping and poisoning for wide-scale problem species. VVIC has yet to be tested outside laboratory and quarantine containment, but it shows considerable promise as a biological control for wildlife (31, 32).

Virally vectored immunocontraception has been tested with varying degrees of success in laboratory trials in a range of species; vaccines tested include recombinant vaccinia virus in rats and foxes, myxoma virus in rabbits, canine herpesvirus in foxes, and ectromelia virus and murine cytomegalovirus in mice and rats (32). The approach has proven highly successful in the laboratory and infertility rates approaching 100% have been achieved in naïve mice infected with recombinant viruses expressing mouse ZP3 after only a single inoculation (35, 44, 67). Sterility, as well as reversible long- and short-term, single shot contraception, without the need for adjuvants, appear feasible using this approach.

Nematode-vectored immunocontraception

New Zealand Landcare Research and AgResearch are developing a parasitic nematode (*Parastrongyloides trichosuri*) for biological control of possums in New Zealand (17). The ability to produce transgenic *P. trichosuri* has been established (28) and it appears amenable for use as an immunocontraceptive vaccine vector in the field as the parasite is specific to possums and is able to establish rapidly in wild possum populations (17).

Other immunological approaches

Several studies have explored the use of bacteria as delivery vectors for immunocontraception (13, 50, 75, 88), although efficacy has been disappointing. DNA vaccines (14, 59, 62, 86), recombinant plant viruses (26) and recombinant bacteriophages (1) have also been trialed in mice and primates with some success.

Risks of exposure to anti-fertility vaccines for human handlers

The long-term safety and reversibility of hCG, FSH and GnRH immunocontraceptive vaccines have been directly assessed in humans. Various formulations of hCG and FSH vaccines have also undergone fertility trials in humans (19, 60), although clinical human trials with a GnRH vaccine were restricted to male patients with advanced carcinoma of the prostate (77). The potential safety issues for humans at risk from unintentional exposure to most contraceptive vaccines being used in animals can only be inferred from primate or other animal studies. As some immunocontraceptive antigens (particularly those based on FSH and GnRH) have the potential to affect fertility in both males and females, precautions should be taken to minimise the risk of accidental exposure to these agents when administering them to animals. However, repeated or prolonged exposure by accidental injection would be required in most cases to induce long-term effects in human handlers. A recent comprehensive coverage of the relative merits of currently available chemical and immunological agents for animal contraception, including the procedures and precautions that should be taken when handling each of these products has been made available on the website of the AZA Wildlife Contraception Center at Saint Louis Zoo in the United States of America (USA) (http://www.stlzoo.org/downloads/CAGrecs2006final.htm).

The use of PZP antigen may pose an additional risk to human handlers as they must be given using either Freund's complete or modified adjuvants (27). Adjuvants such as these contain a combination of mycobacteria and mineral oil that have been reported to cause severe localised lesions in people following accidental self-inoculation (84). The PZP antigens themselves are also associated with the appearance of adverse side effects in some species, including T-cell mediated ovarian damage (oophoritis) and inhibition of steroid production (6, 64, 68, 74, 81). In other species, there do not appear to be any significant PZP-related side effects in somatic tissues after PZP immunisation (8).

Regulatory issues

The various national regulatory frameworks that govern the use of animal contraceptives are complex (39). Each

country has its own set of regulations and legislations, although many international arrangements are also in place, and researchers or practitioners are required to adhere to their own jurisdiction's guidelines.

Vaccines against GnRH (Vaxstrate™ and Improvac™) have been registered for control of libido and sex steroid production as well as increased growth in livestock such as pigs, sheep and cattle in Australia (19, 25). Another GnRH vaccine (GonaCon™) is registered as an experimental wildlife vaccine by the United States Food and Drugs Administration (FDA). It is undergoing a number of field studies in the USA, principally in white-tailed deer, by the National Wildlife Research Center (NWRC) in the United States Department of Agriculture (USDA) Wildlife Services (20, 25, 51, 52). A vaccine derived from whole solubilised PZP (SpayVac™) is also registered as an investigational new animal drug by the FDA for use in captive zoo animals and certain wildlife applications (27).

There are considerable regulatory and social acceptability issues linked with the use of live genetically modified immunocontraceptive virus vaccines. These include the perceived risks associated generally with the use of genetically modified organisms and concerns about species-specificity, efficacy, safety, choice and international implications for trade and biodiversity (4, 45). The presence of strict regulatory controls governing any intended registration of VVIC products has meant that this technology has not yet been approved for use in any country outside containment facilities for handling genetically modified organisms.

Ecological issues

It has been proposed that the widespread use of immunocontraception in wildlife may lead to the development of genetic resistance or heritable immune dysfunctions that reduce the ability of animals in the target populations to combat diseases (15, 16, 46, 57). However, most debate on applying immunocontraceptive products to wildlife relates to the relative environmental impacts of population control using sterility versus lethal methods and the theoretical risks of affecting non-target species in the case of viral-vectored immunocontraceptive agents (10). The actual impacts of behavioural modifications in wildlife and the risk to non-target species still need to be determined as few long-term data sets are available, although most authors concede that fertility control methods such as immunocontraception so far appear likely to provide significant environmental benefits over lethal control (9, 10, 11, 13, 38, 52, 80). Positive benefits of immunocontraception have been reported to include weight gain and increased longevity in populations of wild horses treated with PZP (38) and immunocontraception

with GnRH in bison is being explored as a possible means of controlling the transmission of brucellosis to adjoining populations of cattle in the USA (52).

Social and ethical issues

The ethical principles underpinning the rights, welfare and fertility control of captive and free-ranging animals have been debated (53, 85). It is beyond the scope of this paper to address the rights of animals to live their lives without human interference (66), whether animals should have freedom to express normal behaviour (23), or whether animals suffer from imposed castration or contraception (83). It is, however, understood that separation of males from females is the preferable means for controlling fertility in livestock and zoos. However, animal welfare issues and the ethics of imposed fertility control of free-living and captive wildlife will be discussed.

The use of immunocastration and immunocontraception has been recognised as providing animal welfare benefits relative to alternative procedures (63, 66, 70, 80). In production animals, the animal welfare benefit is the provision of alternatives to the on-farm surgical removal of an animal's gonads, usually undertaken without anaesthetic. In free-living wildlife, fertility control is recognised by animal welfare proponents as preferable to most lethal methods of controlling populations (63, 66).

Production animals

In the pig industry, to avoid boar taint due to fat androstenone in the meat, male piglets are routinely castrated without anaesthetic during the first fews weeks of life. European Commission Directive 2001/93/EC still allows castration of piglets less than seven days of age without anaesthesia, even though research has shown that pain associated with castration is not affected by age (79). There have been campaigns by the Royal Society for the Prevention of Cruelty to Animals (RSPCA) in the United Kingdom (UK), and others, to ban the practice. Alternatives include no castration and early slaughter, surgical castration under general or local anaesthetic, immunocastration and sex-sorting of sperm for the selective production of female pigs (41). Immunocastration, using vaccination against GnRH (Improvac™ CSL, Australia) has been shown to avoid the pain associated with surgical castration, suppress androstenone and improve the yield of lean meat in vaccinated pigs compared to those surgically castrated (36).

Immunocontraception with another GnRH vaccine (Vaxstrate™. Arthur Webster, Australia) has been applied

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commercially as an alternative to surgical speying of heifers. This procedure is applied to stop them becoming pregnant whilst they are being fattened for slaughter under free-ranging conditions. Although originally promoted for the animal welfare benefits inherent in avoiding surgical speying without anaesthesia, the vaccine is no longer commercially available due to the additional cost associated with mustering of cattle for booster vaccinations, the cost of the vaccine itself and the lack of effect in stopping a proportion of heifers from cycling and becoming pregnant (12, 37).

Zoo animals

Population management in zoos is a particularly difficult issue as there is an imperative to allow for natural behaviour in captive animals, including reproduction, and to conduct successful breeding programmes while not producing surplus animals (83). Immunocontraception with PZP has been used succesfully in a range of zoo species. However, safety concerns preclude its use in pregnant animals and long-term effects have been described that include disruption to the reproductive endocrine system in some species. The time taken to return to fertility also varies between species (27).

Free-living wildlife

There are a range of ethical issues associated with the differing strategies used to control free-living animals in most countries and three different ethical positions on wildlife population control have been taken (66). These are the Animal Rights position, i.e. an animal's right to control its own life should not be interfered with in any way; the Environmental or Natural position, i.e. animal populations can only be controlled by predation or hunting and; the Animal Welfare position, i.e. populations should be controlled by non-lethal methods such as immunocontraception.

Another ethical position has been taken in countries with large numbers of introduced, non-indigenous animals that are serious pests. These pests include rabbits (*Oryctolagus cuniculus*), foxes (*Vulpes vulpes*) and cats (*Felis domesticus*) in Australia and ferrets (*Mustela putorius*), stoats (*Mustela erminea*) and possums (*Trichosurus vulpecula*) in New Zealand. The large populations of non-indigenous species have severe negative impacts on native plants and animals. The control of these populations is undertaken for two reasons: their economic impact on agricultural production, and their damage to the environment. The large numbers

of animals involved, the widespread use of non-target specific toxicants and the use of lethal disseminating disease agents has led to considerable debate on the ethics of controlling pest animals (15, 45, 63, 72, 80). A contentious ethical position for controlling these populations has been advocated that combines the principles of ecocentrism and animal welfare (22, 47). Ecocentrism holds that ecosystems have their own intrinsic values and moral standings which can be equal to or greater than individual species, including humans. Under this framework, the conservation value of the indigenous ecosystem (native plants and animals) requires the control of the non-indigenous populations and overrides the animal rights ethic of equal consideration of the interests of all sentient beings (72). However, it is considered that an animal welfare ethic should also be applied and control of non-indigenous sentient animals should be undertaken by the most effective and humane methods available, such as immunocontraception.

Conclusions

Immunocontraception and immunocastration are developing into viable management tools for fertility regulation in both captive and wild animals. In particular, fertility control by immunological means provides an attractive and ethically supported alternative to surgical sterilisation, as it is considerably less invasive and more humane. Nevertheless, apart from PZP vaccination in some zoos and promotion of GnRH vaccines as an alternative to castration in male piglets, immunocontraceptive vaccines are only available for experimental use. The lack of penetration of existing vaccines into many markets, such as companion animals, is due to a combination of factors, including uncertainties surrounding their long-term efficacy and safety, the requirement for repeated inoculation using potentially unacceptable adjuvants and relatively high production costs. However, if these issues can be resolved, the next generation of immunocontraceptive vaccines should be able to provide consistent, long-term infertility after a single application and provide realistic alternatives to chemical and surgical methods.

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Les vaccins et le contrôle immunologique de la fécondité chez les animaux

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Résumé
Le contrôle de la fécondité a pris un élan considérable, en tant qu'outil de gestion des populations des animaux en captivité et sauvages et moyen de contenir les comportements agressifs et d'améliorer la qualité de la viande issue des animaux de rente. La vaccination visant à contrôler la fécondité (contraception et castration immunologiques) est une alternative aux méthodes basées sur la stérilisation chirurgicale ou chimique ou sur le contrôle létal des populations. Deux types de vaccins expérimentaux ont été enregistrés pour la contraception immunologique des animaux, en vue d'une utilisation sur le terrain. Ils contiennent soit des protéines prélevées de la zone pellucide (PZP) d'ovaires de truie, soit des conjugués de peptides synthétiques de l'hormone stimulatrice de la gonadotrophine (GnRH). Ces vaccins devant être administrés régulièrement, leur utilisation n'est envisageable que pour des animaux maintenus en captivité ou pour des populations limitées d'animaux sauvages. D'autres possibilités de contraception immunologique sont à l'étude afin d'améliorer l'efficacité de la méthode ou de permettre son utilisation sur des populations plus nombreuses d'animaux sauvages. Certaines de ces méthodes, basées sur l'utilisation de virus vivants génétiquement modifiés pour empêcher la conception, ont donné de bons résultats au laboratoire. Les auteurs examinent les mérites et les risques respectifs des différentes méthodes de contraception immunologique disponibles ou en voie de développement, ainsi que l'acceptation sociale et la réglementation applicable en la matière.

Mots-clés
Contraception animale – Éthique – Législation – Organisme modifié génétiquement – Risque.

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Vacunas para el control inmunológico de la fertilidad en animales

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Resumen
El control de la fertilidad ha cobrado un notable impulso como instrumento de gestión para regular las poblaciones de animales salvajes y en cautividad, y también para controlar la agresividad o mejorar la calidad de la carne en el ganado vacuno. Las vacunas anti-fertilidad (inmunoanticoncepción e inmunocastración) constituyen una alternativa suave a los métodos basados en el sacrificio o en la esterilización quirúrgica o química. Hoy en día están registrados dos tipos de vacuna inmunoanticonceptiva para una utilización experimental sobre el terreno, constituidas en un caso por proteínas de zona pelúcida porcina, extraídas a partir de ovarios de cerdo, y en el otro caso por un

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conjugado sintético de péptidos de la hormona liberadora de gonadotropina (GnRH, por sus siglas en inglés). Estas vacunas requieren repetidas inyecciones y sólo pueden usarse en el caso de poblaciones en cautividad o de un pequeño número de animales salvajes en libertad. Están en marcha procesos para obtener vacunas alternativas, que mejoren la eficacia de las anteriores o puedan administrarse a grandes poblaciones de animales salvajes. En algunas de ellas se emplean virus vivos genéticamente modificados para lograr la inmunoanticoncepción, método que se ha demostrado eficaz en condiciones de laboratorio. Los autores examinan las ventajas y riesgos, la aceptabilidad social y los reglamentos que rigen el uso de los métodos de inmunoanticoncepción animal ya arraigados o de reciente aparición.

Palabras clave

Contracepción animal – Ética – Legislación – Organismos genéticamente modificados – Riesgo.

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