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Introduction – virally vectored immunocontraception in Australia

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In Australia, pest vertebrates continue to generate major economic and conservation damage in the agricultural, pastoral and environmental sectors. Wild house mouse (*Mus domesticus*) plagues periodically cause major economic loss and social distress (Caughley *et al*. 1994; Singleton *et al*. 2005), while European rabbits (*Oryctolagus cuniculus*) and European red foxes (*Vulpes vulpes*), introduced in the late 1850s and 1860s, continue to directly affect both agricultural and pastoral productivity as well as the survival of our unique flora and fauna (Saunders *et al*. 1995; Williams *et al*. 1995; McLeod 2004; Saunders and McLeod 2007). A cost-effective, humane solution to their broad-scale management is still required.

Since the myxomatosis epizootic in Australia in 1950–51, CSIRO Wildlife Research had been researching the ecology of wild rabbits, their social behaviour and the epidemiology and insect vectors of the myxoma virus (see references in Williams *et al*. 1995). By 1984 it was concluded that the wild type virus and the rabbit populations had reached equilibrium and that further research on myxoma virus should aim to increase its efficacy. To begin to achieve this, an understanding of the genome of the myxoma virus was essential: Russell and Robbins (1989) characterised part of the genome of the myxoma virus and demonstrated its close homology to vaccinia virus. Since it had already been shown that a recombinant vaccinia virus could express inserted genes of the rabies pathogen (Kieny *et al*. 1984), Russell and Robbins' (1989) results opened the possibility that the myxoma virus could be similarly used to carry foreign genes that enhanced its virulence in rabbits.

Contemporaneously, there was another strong group in CSIRO working on the endocrine control of marsupial reproduction and development. In February 1987, a local press report of Steve Robbins' group's current research on myxoma virus included a final comment, which said 'we could put a sterilising gene in the virus'. Reading this, one of us (HTB), leader of the marsupial reproduction group, realised that a way to curb rabbit fecundity had suddenly become possible: if such a recombinant myxoma virus could infect wild rabbits, they would either succumb to myxomatosis or, if they recovered, they would henceforth be sterile and so not replenish the population. The first reproductive agent suggested for insertion was the gene for the hypothalamic decapeptide gonadotrophin releasing hormone (GnRH) or an analogue, which would immunise the rabbit against its own hormone and, through downstream effects, castrate it. Thus the idea of a 'virally vectored immunocontraceptive' (VVIC) was born.

However, two funding agencies rejected the proposal on the grounds that it was far too risky and 'blue sky', especially because there are only very minor species differences in the small GnRH molecule and there was the possibility of non-target species being affected if the carrier virus cross-infected other species. However, a more cogent criticism of the proposal came from within CSIRO Wildlife Research itself: Ian Parer, who had much experience of wild rabbit behaviour pointed out that most of the effective reproduction in a warren is contributed by the dominant males and females (Mykytowicz 1959) and that castrated and ovariectomised rabbits lose their social status in the warren and are replaced by intact subordinate members, so the GnRH approach would be ineffective.

The idea then lapsed until July 1988 when, at a symposium in Kyoto on Development of Preimplantation Embryos and their Environment, Jurien Dean presented a paper on the expression of a mouse zona pellucida gene (ZP3). His group had synthesised a mouse-specific peptide of ZP3 containing a B-cell epitope, which provoked a strong immunological reaction in female mice, rendering them sterile for many months (Millar *et al*. 1989). The possibility for the use of ZP3 in VVIC was apparent: it obviated the objections to GnRH in being species-specific and, more importantly, would neither compromise the endocrine condition of reproductive females nor their social status. Jurien Dean offered to provide his mouse *ZP3* gene clones, and so began a long and generous collaboration with the project.

Through the rest of 1988 and 1989 the idea was discussed among various international colleagues, who observed that the biggest challenge would be to induce a sufficiently strong and sustained immune reaction to the antigen and urged the recruitment of a reproductive immunologist. Alternative reproductive proteins, such as the uterine protein associated with implantation in the rabbit, uteroglobin, the gene for which had already been cloned (Bailly *et al*. 1983), were suggested.

In 1989, the Australian Government provided the seed money as part of two initiatives to begin developing the concept of virally vectored immunocontraception in two species: the rabbit and the fox. In the first, molecular, immunological and reproductive biology studies commenced immediately to isolate and characterise rabbit reproductive antigens, initially sperm surface antigens, as candidates for gene cloning. Concurrently, studies were conducted in the field by surgically ligating the oviducts so that the rabbit's endocrine condition was not altered (Twigg *et al*. 2000; Williams *et al*. 2007). Further work continued to define

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sites within the myxoma genome that could be used to generate recombinant viruses expressing foreign genes (Jackson and Bults 1992) and, subsequently, to test a recombinant myxoma virus expressing a foreign antigen, the influenza virus haemagglutinin (Kerr and Jackson 1995). With the new knowledge of the myxoma virus, field strains from different parts of the country were characterised by their molecular structure and compared to determine their virulence (Saint *et al*. 2001). This disclosed for the first time that all field strains were related to the original strain released in 1950 and none to the more virulent Lausanne strain that had been used for all subsequent releases. Kerr *et al*. (2003) showed that the virulence of myxoma strains from other regions could be very different when tested in local wild rabbits; in other words, evolution of host and virus was being played out independently in each locality.

In the second initiative, with its emphasis on the need to control pest species, especially the European red fox, the Australian Nature Conservation Authority (more recently Environment Australia, Department of Environment and Heritage, and now Department of Environment and Water Resources) received special funds for work on foxes and, in April 1990, organised a workshop in Canberra on fox control. The concept of VVIC was presented by the scientists from CSIRO and although it was the most costly and most high risk of several options, funding was provided for an initial investigation of fox reproductive antigens and possible viral vectors, in conjunction with field studies on more conventional methods of fox control. By the end of 1990, several fox sperm antigens had been isolated (Beaton *et al*. 1994, 1995) and a captive fox colony had been established in Canberra to enable testing of the antigenicity of the isolated proteins. Blood samples from foxes shot or trapped in Western Australia were also screened for fox-specific viruses (Robinson *et al*. 2005).

With the two new groups of reproductive immunologists, the pre-existing myxoma virus group, and the reproductive endocrine group, a critical mass of active and highly motivated young scientists in complementary disciplines had been brought together to test the concept of immunising wild mammal species against their own reproductive antigens, using a species-specific virus as the vector. In addition there was the very experienced team of field ecologists testing what proportion of a rabbit population would need to be sterile in order to reduce the natural rate of increase.

The research on both rabbit and fox continued and in November 1990 two papers were presented at the 2nd International Conference on Fertility Control in Wildlife in Melbourne (Tyndale-Biscoe 1991), which excited much interest among animal welfare groups. This was followed in December by the establishment by the Australian government of the Cooperative Research Centre (CRC) for Biological Control of Vertebrate Pest Populations, with funding of AU$12M over seven years. This represented the largest and most integrated commitment of research effort to fertility control for wildlife in the world. Its principal aim was to explore alternative methods of biological control for the European rabbit and the European red fox, both being major vertebrate pests in Australia. From 1995, additional funds were provided by the Grains Research and Development Corporation of Australia to include similar studies on the wild house mouse, with ecology and reproduction research based at CSIRO and virology research at the University of Western Australia.

The overarching concept driving the work of the CRC was that the genes for proteins that are critically involved in fertilisation or implantation can be inserted into a virus that infects the target species; the inserted genes would then be expressed in an animal infected with the recombinant virus and the infected animal would simultaneously raise antibodies to the virus and the reproductive protein; thus fertilisation or implantation would be prevented, without affecting the animal's sexual activity or social status in the population. Four central questions derived from this concept:

(1) *Ecology and behaviour.* What proportion of a wild population must be sterilised in order to reduce significantly the rate of growth of the population?
(2) *Reproductive immunology.* Can gamete-specific proteins be presented to the animal in such a way as to provoke an effective and long-lasting immune response that interferes with fertilisation or fetal development?
(3) *Virology and molecular genetics.* Can recombinant viruses or bacteria that express the genes encoding the gamete proteins be constructed, and can they act as vectors to immunosterilise the proportion of the wild population of the target species identified in the first question?
(4) *Risk analysis.* Can this be achieved in a way that does not put other species at risk?

The papers in this special issue review the results, outcomes and implications of the research undertaken over the last 15 years to address these questions. The successes and failures of the research programs for rabbits (van Leeuwen and Kerr 2007), foxes (Strive *et al*. 2007) and mice (Redwood *et al*. 2007) and the ecological aspects and potential role in vertebrate pest management of releasing VVIC are considered by McLeod *et al*. (2007). The risks associated with the inadvertent release of a VVIC transported out of Australia are modelled in a case study for the wild mouse (Williams 2007) while Henderson and Murphy (2007) review the regulatory aspects that would be pertinent to the potential release of a vectored fertility control agent in Australia and New Zealand. These authors emphasise the need for international debate on proposed products for release – this was always pertinent and remains so now that the only work on vectored fertility control is occurring on possums in New Zealand (Cowan *et al*. 2006; Grant *et al*. 2006*a*, 2006*b*). For possums, the vector being engineered to carry a fertility control agent is the nematode, *Parastrongyloides trichosuri*, which occurs naturally in Australia – although it is highly desirable that it be effective in New Zealand, an escape of this engineered nematode has the potential to affect brushtail possum populations in Australia, a highly undesirable outcome. This topic is also discussed in the final paper in which vectored delivery of a disease vaccine for European rabbit conservation and management in Spain (Angulo and Bárcena 2007) is reviewed. In their approach, a recombinant myxoma virus expresses part of the RHDV capsid protein such that it will transmit and protect wild rabbits from both diseases. The steps taken to commercialise this product for use in Europe currently do not involve discussions with other countries, such as Australia, in which the release of such a product could adversely affect efforts to minimise rabbit numbers and their damage to the environment.

In summary, 20 years ago the concept of VVIC was novel and untested but potentially capable of providing a long-term solution to the control of vertebrate pest species. The VVIC concept required research of a high order in four disparate disciplines by a team of people with a wide range of expertise collaborating to achieve a common goal. It was a good example of how to conduct difficult research that had an uncertain outcome but which could potentially be of great public benefit. Funding bodies had the courage to support an untried concept at a level that enabled the key questions to be tested rigorously so that the results were unequivocal. Had the research delivered an effective VVIC, it is a moot point whether such a self-disseminating agent would then have been approved for release for use in rabbit, fox or mouse control in Australia (Gilna *et al*. 2005). However, public attitudes to the use of GM technology have changed over time, with a recent study showing that positive attitudes are now more prevalent (Fisher *et al*. 2007). That the concept did not fulfil the early promise is no reflection on the quality of the research but on the intrinsic nature of the problem itself: long-term control of vertebrate pest species still remains a challenge to the imagination and skill of scientists of many disciplines.

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