

Rabbit-Stat V — Progress Towards a Disseminating Viral Vectored Immunocontraceptive Vaccine for Rabbits

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The use of myxoma virus as a control agent for the European rabbit represents one of the most successful and best known examples of biological control of a vertebrate pest species. As described in the previous talk the effectiveness of myxoma virus appears to be decreasing. In addition there are general concerns from sections of the public about the acceptability of many lethal based control procedures. There are thus a number of motivating factors favoring the development of new, novel control procedures. One option is to decrease fertility rather than enhance mortality. Previous attempts at fertility control using various chemical agents in a range of vertebrate species have met with mixed success (review Bomford) for different reasons, one of which has been inability to target a sufficient portion of the population.

Modern biotechnology may provide a solution through the concept of viral vectored immunocontraception (VVIC) which seeks to use genetic engineering to combine recent developments in vaccine technology and reproductive biology and produce an immunocontraceptive vaccine delivered by a genetically engineered myxoma virus. Briefly, genes encoding proteins that perform essential roles in reproduction, such as the components of the egg to which sperm must bind in the initial steps in fertilization, are engineered into the myxoma virus to yield a recombinant virus. When this virus subsequently infects rabbits and replicates to produce more virus particles, the reproductive antigen is also produced. This can mount an immune response in the infected animal and produce antibodies that interact with both the natural protein as well as that produced by the virus. This interference prevents the protein fulfilling its normal role in reproduction thus producing infertility. If the population is thus sufficiently reduced, the deleterious effect(s) of the pest species may be concomitantly reduced.

A critical step in this process is selection of the reproductive protein(s) to be targeted. Initially we focused on sperm proteins essential in fertilization because sperm are highly immunogenic in both males and females. However, studies showed that whilst strong immune responses can be provoked in serum, these antibodies cannot enter the reproductive tract at high levels and thus cannot access the sperm. This led us to focus on proteins in the outer coating of the oocyte – the zona pellucida – with which sperm interact in the obligatory initial steps of fertilization, in particular the ZPB protein. We established that serum antibodies could enter the developing follicle within the ovary and bind to the native proteins in the zona pellucida. These antibodies reduced fertilization in *in vitro* fertilization assays. Active immunization studies with recombinant proteins reduced fertility by approximately 80%. This was particularly hopeful because two major field studies in which varying proportions of females in wild rabbit population had been surgically sterilized suggested that this level of infertility would be required to have an impact on rabbit population dynamics. However, infection studies using recombinant virus expressing the ZPB protein produced only 25-30% infertility.

We are currently testing strategies to modify the immune response through the use of immune modifying molecules and by the use of other proteins and combinations of proteins with the intention of increasing infertility. In addition, study of ovarian pathology in immunised animals has shown that there is a time dependent reduction in the primordial follicle pool. If this reduction is of sufficient magnitude then animals could not return to fertility because primordial follicles are not replaced after birth. This would provide lifelong, irreversible infertility.