Expression of putative rodent-specific contraceptive small peptides in *Nicotiana benthamiana*

Khadijeh Ghasemian, Patrick Nonnenmacher, Inge Broer and Jana Huckauf

AUF, Agrobiotechnology, University of Rostock

Khadijeh.Ghasemian@uni-rostock.de

Fertility control through immunocontraception has been proposed as a method for reducing population size. Sterility can be achieved in mammals via the induction of antibodies against Zona pellucida (ZP) glycoproteins that are located on the surface of the oocyte and mediate the gamete recognition. Oral application of the ZP antigens could be a promising alternative to control rodent pests populations if inadvertent sterilization of non-target species can be prevent. This might be achieved by limiting the vaccine to small, species specific peptides representing mainly the sperm attachment site. Plants have shown to be one of the most promising alternative pharmaceutical production platforms that are robust, scalable, low-cost and safe. The recent development of virus-based vectors has allowed rapid and high-level transient expression of recombinant proteins in plants. Nevertheless, the expression of small peptides can be a challenge. To optimize the transient expression of putative mice-specific contraceptive small ZP-peptides in *Nicotiana benthamiana* via viral MagniCON expression system, we have examined the production of two immunocontraceptive antigens that include mice-specific peptides from the mouse reproductive proteins ZP2 and ZP3. The protein comprised of a 'promiscuous' T cell epitope of tetanus toxoid (TT: 15 amino acid (aa)) followed by mZP2-peptide (21 aa) or mZP3-peptide (15 aa), a (6x)His-tag and an ER-retention signal SEKDEL. Although adequate amounts of the specific RNA were present in the infiltrated plants, no band representing a protein with the expected size of the mZP2 or mZP3 peptides (~7 kDa) could be detected by Western blot. Increasing the protein size by fusing the GFP protein to N-terminus of mZP3 antigen, analysis of the purified fusion protein revealed a dominant protein band of the expected size of ~36 kDa. Increasing the protein size by tripling of the antigenic mZP3 epitope also stabilized the peptide and led to an overexpression in infiltrated *N.benthamiana* plants. Conclusion: The increase in protein size leads to the stabilization of short ZP peptides in plant-based production systems and thus increases their overexpression.