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Contraceptive potential of *E. coli* expressed recombinant protein encompassing sperm associated protein and GnRH

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Statement of the Problem: Immunocontraception for wildlife management is taking on a new perspective, and many agencies charged with such management decisions are turning to fertility control as a potential humane solution. Contraceptive vaccines based on spermatozoa specific proteins aiming to interfere in sperm-egg interactions have been proposed as one of the strategies for controlling the population of various animal species. Various studies have established that spermatozoa are foreign to immune system of an animal, thus elicits a strong anti-sperm immune response. However, no sperm protein-based contraceptive vaccine has reached field application. In this direction, we have cloned, expressed and purified recombinant protein, bRNase-KK-Sp17_c-KK-TT-GnRH-GnRH (Sp17_c-GnRH₂) and evaluated its immunogenicity and contraceptive efficacy using different adjuvants.

Methodology & Theoretical Orientation: Recombinant protein, bRNase-KK-Sp17_c-KK-TT-GnRH-GnRH was cloned and expressed in BL21[DE3]pLysS *E. coli* cells. The recombinant protein was purified using Ni-NTA affinity chromatography. FVB/J mice were immunized with different combinations of adjuvants and sera were collected to evaluate the antibody titer. After immunization, female mice were put for mating with male mice and pups born per pregnant female were monitored carefully.

Results & Conclusion: Immunization of both male and female mice with Sp17_c-GnRH₂ following three injection schedules, led to high contraceptive efficacy. Interestingly, mating studies of female mice with the male mice wherein both were immunized with Sp17_c-GnRH₂ revealed complete failure of female mice to conceive. Male mice immunized with Sp17_c-GnRH₂ led to testicular atrophy and a significant decrease in the diameter and circumference of seminiferous tubules. Further, to reduce the no. of injections, when group of female FVB/J mice were immunized with Sp17_c-GnRH₂ and Squalene: Arlacel 'A' as an adjuvant, there was approximately 90% infertility. Inclusion of killed *Mycobacterium indicus pranii* in another group of female mice did not make much of a difference in case of immunogenicity. Although, there was also a significant increase in the immune response when female mice were immunized with the same antigen but along with a mixture of PCPP and alum as compared to the group immunized with only antigen and alum following a two injection schedule, but there was no significant increase in the contraceptive efficacy.

Biography

Vidisha Minhas is a PhD student at Delhi University. For the past five years, she has been working towards the production of recombinant proteins that has a role in fertilization. She is well versed with techniques like cloning, protein expression and purification, ELISA, indirect immunofluorescence, western blotting, etc. Along with her lab mates, she has cloned around 10 recombinant proteins and tested their immunogenicity and contraceptive efficacy in mice. Some of them have shown promising results and can be considered as potential candidates for development of contraceptive vaccine (CV) for managing wildlife population. She is also working on enhancing the efficacy of antigens by the use of immune potentiator and optimization of antigen delivery system.

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