

## Poster G-6

### Proteomics Approach for Exploring Sperm Antigens Involved in Epididymal Maturation



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**Short Abstract:** Using proteomics approach, several spots specific to epididymal sperm were identified. Of these, some spots were cored and are being sequenced. LC-MS/MS data will be used to decipher the identity as well as presence of human homologue using in silico approach. This approach will help in identifying epididymal proteins involved in sperm maturation and fertilization which could be used as post testicular targets for contraception or as markers for infertility diagnosis.

#### Long Abstract:

The expanding population and unintended pregnancies have made it necessary to look for better and newer contraceptives methods which are safe, effective, reversible and acceptable. Contraception based on Vaccine approach would definitely be a valuable addition to the existing armamentarium of different options available for family planning. Advantage of using a vaccine based on sperm antigens is that it can be used both in males and females. The targets could be sperm antigens coming either from the testis or the epididymis. Targeting the epididymis for contraception is of interest, due to multiple advantages. Firstly intervention of post testicular maturation events would render immotile and nonfunctional sperm. Secondly, this interference would neither impair spermatogenesis nor affect testicular and endocrine function. Finally the onset of infertility as well as its reversal would be rapid as compared to targeting of testicular sperm production. In mammals, during spermiogenesis round spermatids differentiate into fully formed spermatozoa with well-defined domains, the head and the flagellum in the testis. Testicular sperm are non motile, immature and incapable of fertilizing an egg. They attain functional maturation in terms of acquisition of forward motility and ability to bind, to penetrate and fuse with the egg investments during their voyage through the epididymis. This process is termed as sperm maturation. In order to understand the basis of sperm maturation and pursue epididymis as target for contraception, there is a need to identify and characterize epididymal sperm proteins. It is well established that proteins on different domains have specific roles to perform and hence the present study aims at identifying such domain specific proteins relevant to sperm maturation in the epididymis. In the present study, testicular sperm were isolated from germ cell mixture using percoll gradient. Caudal sperm were collected and used to separate different domains of sperm such as head and flagella using sucrose gradient. All the collected samples viz. testicular sperm, caudal sperm, head fraction of

caudal sperm, flagellar fraction of caudal sperm were lysed to extract proteins. High-resolution two-dimensional gel electrophoresis was employed to separate these proteins using isoelectric focusing followed by SDS PAGE. Proteome profiles of rat testicular sperm were compared with caudal epididymal sperm as well as with proteins from different domains (head and flagellum) of caudal epididymal sperm. Analysis of these profiles was carried out using Image Master 2D-PAGE analysis software. As many as 404 proteins spots were resolved from testicular sperm and 495 from cauda epididymal sperm. On comparing, several spots specific to epididymal sperm were identified viz. AS1, AS2, AS3, AS4, AS5, AS6, AS7, and AS8. Of these, four protein spots (AS1 AS2 AS6 and AS8) were cored from 2-D gels and are being sequenced using Mass spectrometry / micro-sequencing analysis (LC-MS/MS). Peptide data obtained using LC-MS/MS will be used to decipher the identity as well as presence of human homologue of the identified proteins using in silico approach. This approach will help in identifying epididymal proteins involved in sperm maturation and fertilization. The database for the domain specific sperm proteins can serve as a reference for selection of targets for micro sequencing or generation of antibodies to understand the complex processes of sperm maturation during epididymal transit essential for successful fertilization. These proteins could then be used as post testicular targets for contraception or as markers for infertility diagnosis.