VI. SUMMARY

A study was undertaken to characterize 25 bovine mastitis *S. aureus* (SA1 to SA25) isolates for *bap* (Biofilm associated protein) gene, analyze proteins of *S.aureus* SA16 and *S. aureus* SA2 grown under BF and FC mode with reference to their immunogenicity and cross reactivity, and for experimental evaluation of *S.aureus* BF based vaccine in rabbits.

The *bap* specific PCR analysis of 25 bovine mastitis *S. aureus* isolates indicated 40 per cent of isolates were ‘*bap*’ positive that showed an amplicon of 971 bp.

The SDS-PAGE analysis of protein profiles of *S.aureus* SA16 (BF vaccine and homologous challenge strain) and SA2 (heterologous challenge strain) grown under BF and FC mode indicated that the protein profiles of *S.aureus* SA16 and SA2 grown under BF mode had differed from *S.aureus* SA16 and SA2 grown under FC mode by 56 per cent with unique expression of 26.69, 30.59, 34.02, 40.77, 51.77, 57.22, 65.09, 102.72 and 114.21 kDa and repression of 19.03, 22.61, 25.25, 28.6, 33.3, 38.09, 39.91, 62.45, 79.14 and 149.02 kDa. The unique proteins of 51.77 and 57.22 kDa were detected only in *S. aureus* SA16 BF cells, but not in *S. aureus* SA2 BF or free cell proteins. The polypeptides of 37.05, 54.39 and 94.91 kDa were expressed in BF and FC of both the strains with more prominent expression of 37.05 kDa protein in BF cells.

The western blot analysis of proteins with reference to their immunogenicity and cross reactivity revealed that six immunogenic proteins of 30.59, 34.02, 37.05, 54.39, 94.91 and 114.21 kDa and three immunogenic proteins of 30.59, 34.02 and 37.05 kDa
were detected in *S.aureus* SA16 BF and *S.aureus* SA2 BF respectively upon probing with hyper immune sera raised against *S.aureus* SA16 BF. These proteins were not detected either in BF proteins or in FC proteins when probed by FC HIS. This indicates the superiority of BF proteins which are capable of inducing better antibody response compared to FC proteins. Further, the immunogenic proteins of 30.59, 34.02, 37.05 kDa were observed in *S.aureus* SA2 BF proteins, indicating the cross reactivity of BF proteins. But, such immunogenic cross reactivity was not observed when blot was probed with FC HIS.

Mastitis was induced in lactating rabbits by inoculating both *S.aureus* SA16 and SA2 strains with $10^4$, $10^5$, $10^6$ and $10^7$ cfu/ml of bacterial suspension at the base of the teat. Induction of mastitis was indicated by gross lesions of mammary glands, increased SCC and CMT positivity. All these relevant indicators of mastitis showed maximum values at 48 hrs after inoculation. These results showed that both the strains had induced mastitis in rabbits and that $10^4$ cfu/ml of bacterial load was found optimum for challenge infection in vaccinated rabbits.

Pregnant rabbits immunized with *S.aureus* SA16 BF and FC vaccines were evaluated for the gross lesions of mammary glands, SCC, CMT and serum IgG level by ELISA after homologous and heterologous challenge.

The statistical analysis to evaluate BF and FC vaccines with respect to development of mastitis indicated that the mean percentage of mammary glands showing lesions at 48 hrs after challenging and mean SCC and percentage of CMT positive
mammary glands after challenge was less in BF vaccinated compared to FC vaccinated rabbits.

The indirect ELISA was standardized for the detection of post-vaccinal IgG antibodies in sera samples from rabbits vaccinated with *S.aureus* BF and FC vaccines by optimizing *S.aureus* BF antigen concentration at 1.25 μg/ml and a serum dilution of 1:100. Further, serum IgG level detected by ELISA was significantly higher in BF vaccinated rabbits than FC vaccinated ones. Cross protection conferred by BF vaccine was noticed based on challenge studies using homologous (*S.aureus* SA16) and heterologous (*S.aureus* SA2) strains. In conclusion, the experimental vaccination trials in pregnant rabbits with *S.aureus* BF and FC based vaccines indicated the superiority of *S.aureus* BF vaccine to the FC vaccine against experimentally induced mastitis using *S.aureus* isolates from bovine mastitis.