Androgens mediate diverse responses through the androgen receptor (AR) that functions as a ligand activated transcription factor. It has been reported that both testosterone (T) and dihydrotestosterone (DHT) can differentially regulate the expression of androgen responsive genes (1997; Araneo et al., 1991; Seki et al., 1991). These observations prompted the controversial idea that more than one species of cytoplasmic AR with specificity for different androgens may exist in the androgen target tissues and also that there are AR on target cell plasma membrane that modulate cellular functions through second messengers (Sheridan, 1991). Target organs for androgens include the epididymis during the transit through which the sperm acquires its motility function. It has been reported that in the rhesus monkey the caput epididymis displayed high affinity testosterone binding sites, in addition to the binding sites for dihydrotosterone (Thampan et al., 1980). Moreover the expression of the AR is reported to be low in the caput in contrast to the corpus and cauda (Ungefroren et al., 1997). These observations led us to the hypothesis that there is a testosterone receptor in the caput epididymis, which could be playing a major role in the sperm maturation function.

Our objective was to purify and characterize the putative testosterone receptor from goat caput epididymis and to examine its role in sperm maturation function. During the course of work, the putative testosterone receptor with high affinity for testosterone was purified to homogeneity. What was interesting additionally was the finding that the protein bound both estradiol and testosterone with high affinity. But N-terminal amino acid sequencing and MS/MS analysis of the protein brought out the reality that the protein was none other than serum albumin.

Albumin has been reported as one of the major components of epididymal fluid. However it is assumed not to be of epididymal origin (Dacheux et al., 2006; Fouchécourt et al., 2000). The proteins present in the epididymal fluid fine tune sperm maturation process by interacting with the sperm during their transit through the epididymal lumen. Also in vitro, albumin is known to induce hyperactivated motility in sperm and is an essential component of the sperm capacitation medium (Xia and Ren, 2009). Nevertheless albumin present in the epididymal fluid has not been assigned any specific function. This thesis tries to explore the origin and the possible functional role of epididymal albumin.
ABSTRACT

Epididymis plays a crucial role in the process of mammalian reproduction by setting a stage for the molecular events leading to the maturation and survival of sperm from the testis. The transit through epididymis is an obligatory event for mammalian spermatozoa to acquire its fertilizing ability and forward motility. The epididymis shows both structural and functional segmentation along the anterior-posterior axis. The distinct function of each epididymal segment is a manifestation of the corresponding luminal microenvironment with a dynamic protein composition. Epididymal proteins fine tune sperm maturation process by interacting with the sperm during their transit through the epididymal lumen. Although albumin was reported as one of the major components of epididymal fluid, it was never identified to be of epididymal origin. This thesis explores the origin and functional role of epididymal albumin using *Capra hircus* (goat) as the model organism.

The study was initiated with a view to isolate and to characterize the putative testosterone binding protein from goat caput epididymis. A 66 kDa protein was purified to homogeneity which displayed saturable binding sites for testosterone as well as for estradiol. Polyclonal antibody against this purified protein was raised and its specificity was confirmed. N-terminal sequencing and MS/MS analysis revealed the protein’s identity to be albumin.

Since albumin is reported to be a secretory product of liver, it was intended to explore the origin of epididymal albumin. Immunohistochemistry of the caput epididymis showed that albumin is present in the luminal epithelial cells. The primary epididymal epithelial cells in culture were also positive for the presence of albumin as demonstrated by immunocytochemistry and western blot, thus confirming the epididymal synthesis of albumin. Albumin secretion by the primary epididymal epithelial cells was confirmed by its detection in the serum free culture medium. Full length cDNA of mature albumin from caput tissue as well as primary epididymal epithelial cells in culture was cloned and sequenced. Since it was important to find whether the albumin expressed in epididymis is the same as that in the liver, the cDNA from liver was also sequenced and both were found to be the same. The translated amino acid sequence of goat epididymal albumin showed 91.1% similarity to that of bovine serum albumin.
The local synthesis of albumin in the epididymis points to its possible functional role in sperm maturation. As a first step towards this direction experiments were performed to investigate any interaction of albumin with the sperm membrane. The presence of albumin on the sperm membrane was demonstrated by Western blot. Experiments with labeled albumin using confocal microscopy and FACS analysis showed its specific binding to the sperm membrane. Albumin is reported to bind to megalin, a member of the LDL receptor super family, in kidney proximal tubular cells. Western blot of the sperm membrane lysate also showed the presence of megalin. Co-immuno precipitation was performed with sperm membrane lysate using anti albumin antibody and megalin was found to co precipitate with albumin. These data confirms the specific interaction of albumin on sperm membrane.

This thesis reports, for the first time, the synthesis and secretion of albumin by epididymal epithelial cells. It was also attempted to find the relevance of local synthesis of albumin in the epididymis. From the findings, it may be assumed that the interaction of albumin with the sperm membrane could significantly contribute to the process of sperm maturation. This thesis thus throws light on unknown regulatory mechanisms of sperm maturation events in the epididymis.