8 Summary and Conclusions

8.1 Aflatoxin toxicity

The hitherto reported varied manifestations of aflatoxin toxicity in male reproductive system include drastic reduction in the semen parameters like motility, viability and count, reduction in the fertility rate, generation of multinucleate giant spermatids, formation of pale vacuolated epithelial cell, etc. Since several manifestations of aflatoxin toxicity in the male reproductive system have already been reported, the observations presented in this thesis are based on leads from the previous studies from our lab. This consist of one research paper already published (Faisal et al. 2008, *Reproduction*, 135: 303-310) and the material under preparation for two more manuscripts. In the paper published, it has been reported that aflatoxin treatment produces, among other abnormal morphologies of the sperm, extrusion of one or more outer dense fibers and the associated microtubule doublets which is novel. The extrusion occurred at either of two points, the head-midpiece junction (connecting piece) and the midpiece - principal piece junction. The extrusion begins with the development of a weak patch in the membrane and the mitochondrial sheath. This was traced to abnormal development of mitochondrial sheath at the respective points. Since this extrusion affected motility of the spermatozoa, it was concluded that aflatoxins could be a potential risk factor affecting the reproductive health in men chronically exposed to the aflatoxins through dietary route in developing and 3rd world countries and one of the underlying mechanisms could be spermatotoxicity in general and the extrusion of ODFs and the associated microtubule doublets, which is the subject of interest in the present context.

The other new finding is indication of a newer role to the epididymis viz., management of defective spermatozoa in the lumen. Innumerable studies have found small to large percentages of spermatozoa to be defective when exposed to toxicants. These spermatozoa are not only unviable but can affect the viability of the normal spermatozoa. Processes such as phagocytosis by luminal macrophages and endocytosis by the epithelial cells of the epididymis are known, which are believed to remove such defective spermatozoa. But in instances where large bulks of spermatozoa are
defective, these processes would not be adequate. There has been search for a more powerful mechanism of management of such defective spermatozoa. A newer modality was found in this research. In the lumen of the cauda epididymidis of the treated animals, spermatozoa in different degrees of disintegration were found embedded in a dense matrix. This matrix is a mechanism not only to seclude the defective spermatozoa from the normal ones but also to provide for the disintegration of the former. Thus, it is a provision for management of large bulks of defective spermatozoa. The pattern of disintegration of defective spermatozoa revealed different courses of disintegration of the major parts of the spermatozoa, viz., head, mid-piece and the principal piece.

Evidence was also obtained regarding the source of this dense material. We found the principal cells of the initial segment of epididymis to copiously produce and release electron-lucent, roughly spherical bodies, the aposomes, in apocrine manner. The aposomes were seen to arrive into the lumen of the epididymis and initially present loosely scattered bodies. The spermatozoa were found to lie amongst the aposomes. The aposomes were seen to associate among themselves by fusion and form into conglomerates. Thus, the aposomes lose their identity and form the dense matrix. The aposomes disintegrated, liberating their content of small membranous vesicles, the epididymosomes. The spermatozoa were seen to be entangled in the dense matrix. The epididymosomes, have been already reported to part-take in epididymidal sperm maturation, are here in ascribed with a newer role of management of large bulks of defective spermatozoa.

8.2 **Quassia amara**

*Quassia amara*, as different preparations is a traditional herbal remedy for several ailments including malaria. Quassin, a triterpinoid lactone, is the active secondary chemical in this plant, is currently under investigation for anti-malarial property. There are already some leads for the male reproductive toxic effect of *Q. amara*. Therefore, *Q. amara* methanolic extract and quassin were subjected in this research for testing the male reproductive toxic effects in a systematic study. The methanolic extract of stem bark of *Q. amara* and the pure compound quassin produced toxic manifestations in the spermatogenic compartment of the testis, spermatozoa and the epididymis. The major targets in the seminiferous epithelium was indicated as the
Sertoli cell, which in the treated animals accumulated cytosolic as well as mitochondrial vacuolation and also dense lysosomal bodies. This affected the Sertoli cell-germ cell junctions, causing inappropriate division and differentiation of the germ cells and also their premature release from the Sertoli cell. However, 35 day treatment of the materials did not produce noticeable change in the dark- and light spermatogonia, indicating that *Q. amara* may not affect the stem cell compartment. It suggests that, if used in therapy, *Quassia* / quassin, if treated for this short duration may not affect male fertility permanently.

The treatments produced dose-dependent decrease in the counts and motility and increase of abnormal cauda epididymal spermatozoa. In the epididymis, all cell types were affected suggesting impaired support to the epididymal sperm maturation. Features such as swelling of the Golgi apparatus of principal cell, hypertrophy and hyperplasia of apical cell and narrow cell, increase in abundance of basal cells and macrophages and appearance of the abundant lipofuscin in the principal cell cytoplasm and their acquisition and further processing by the basal cell and macrophages are among the several reflections of histopathological changes. Retention of the cytoplasmic droplets (CD) by the cauda epididymal spermatozoa and several newer manifestations in the CD, various levels of coiling of the flagellum including around the head, etc., made interesting observations.

One of the novel observations consisted of lasso-shape of the sperm. The etiology of this shape was traced to the retention and swelling of the CD followed by the failure of the volume regulation in it causing flexure of the sperm flagellum at the midpiece-principal piece junction, resulting in fusion of two limbs of the flagellum.

Ubiquitination is believed to play a critical role in removal of dead and/or defective spermatozoa in normal and, more importantly, under circumstances when such spermatozoa are produced in large numbers due to genetic defects or toxic manifestations. We hypothesized that such spermatozoa would be removed during epididymal processing which would involve expression of some specific genes concerned in the ubiquitination pathway. Thus, in the present study, the transcriptional profile of selected genes involved in the ubiquitin proteolytic pathway in the testis and
epididymal segments of quassin-treated rats were analyzed adopting semi-quantitative RT-PCR. As ubiquitination and DNA damage are correlated, the level of DNA damage of the treated rat spermatozoa were also studied. It is found that all the three genes, \textit{Ubb}, \textit{Ube2C} and \textit{Psmb8} showed marked increase in their level of expression in the treated rats compared to the corresponding controls and the treatment induced ‘slight damage’ and ‘damage’ and in rare cases ‘high damage’ to the sperm DNA.

Thus, this study shows that quassin is causative of sperm DNA damage and defective spermatozoa, and in such cases, the expression of specific genes concerned with the ubiquitination pathway is increased, implying that ubiquitination-proteosomal degradation is involved in the processing of dead/defective spermatozoa. Also, the study reveals that \textit{Quassia amara} / quassin, when used as therapeutic, can potentially jeopardize male reproductive health.