Concluding remarks

Having the remarkable new findings observed in the past few years in the area of Y chromosome, our knowledge pertaining to gene function in AZF region is still very much unclear. The probable factors could be due to experimental issues and also innate complexity of the biological structure. In technical terms the requirement of knockout animal models as the AZF structural design are exists in primate lineages and secondly, introducing the \textit{in vitro} spermatogenic cell lines in experimental approach, will help us to develop robust diagnostic assays. In addition, the biological arrangement of the AZF regions makes ambiguous, showing remarkable variation associated to the AZFc sequences and its corresponding genetic determinants. Nevertheless, AZF gene therapy approaches is still too early to anticipate, the investigation for novel AZFc molecular intrusion cross talks and their related phenotypes is very much essential for the clinical supervision of the patients.

Further, the characterization and validation of the complete AZF genes needs more consideration, the future research may be more persistent in full sequencing of Y chromosome AZF region in different population, which will eventually benefit infertile couples in due course. Thus, progress in this area is very significance for a more through outlook on the reproductive fitness of the Y chromosome. In general, screening of infertile males for specific deletion patterns or other genetic and epigenetic variations in AZFc region may disclose new clinical relevant mutations. However, combining such knowledge with functional data that affects biological
development would transform into significant conceptual progress in male reproductive genetics.

In this context, the present study has made a progress and focused on three aspects of Y chromosome AZFc deletion and male infertility. (i) Comparative analysis of semen quality and sperm dysfunction weighing against WHO values, (ii) frequency of AZFc partial deletion and its possible association with male infertility, and (iii) finally, incidence of $DAZ/CDY$ gene SNVs and its effect on spermatogenic impairment and its risk factor for infertility in our study cohort.

By comprehensive and systematic analysis, our study evaluated the semen quality and sperm functional status among infertile and normozoospermic control men. Our experimental results demonstrated that higher percentage of abnormality in odour, volume, pH and liquefaction time in infertile males compared to normozoospermic controls. Further, the detailed microscopic investigation revealed incidence of different types of infertile sub-conditions. Interestingly, age wise analysis showed that a significant reduction in sperm vitality, count, and motility in infertile cases than normozoospermic individuals across all the age groups. In addition, sperm dysfunction suggest that compared to normozoospermic and WHO reference values, the mean values for both NCD and AIT assays are indistinguishable in infertiles, thereby rule out any changes in the nuclear material decondensation and alteration in acrosome enzyme activity, respectively. However, HOS average values of infertile males are significantly differed from controls suggesting changes in the integrity of the sperm plasma membrane.
Furthermore, Y chromosome AZFc partial deletion analysis in Karnataka is the pioneering study in this population. Our study records the higher frequency of b2/b3 AZFc partial deletion in normozoospermic controls with no effect on spermatogenic impairment and severe reduction in semen characteristics are observed in infertiles, thereby exhibiting significant association. Similarly, higher frequencies of gr/gr deletion and gr/gr; b2/b3 deletion are observed among infertile subjects with variation in the sperm dysfunction. On contrary, b1/b3 and b/b3; b2/b3 deletion pattern are also observed in our study with a non-significant association on individual semen profile.

In order to compare the Y chromosome AZFc partial deletion among the homogenous and heterogeneous population, our study screened 200 Siddi tribal men, who are African decedents. To our surprise, only one individual out of 200 Siddi male showed for b2/b3 partial deletion, whereas the rest of the subjects showed no deletions for any markers indicating lower fertility impairment risk among these men. However, in absence of semen data our tribal data is solely based on experimental evidence. At this stage, it is too premature to depict any robust conclusion until further investigation that necessitates examination in larger cohort. Together, b2/b3 followed by gr/gr deletions or combination of gr/gr; b2/b3 partial deletions seems to be a risk factor for spermatogenic impairment and male infertility by altering vitality, count and motility among infertiles.

Subsequently, analysis of single nucleotide variant demonstrated the loss of DAZ and CDY1 gene copies in both infertile and normozoospermic controls with AZFc partial deletions. Precisely, DAZ3/4 + CDY1a and DAZ3/4 + CDY1b deletion
patterns are predominant and these SNV patterns are frequently observed in individuals with AZFc gr/gr and/or b2/b3 partial deletions suggesting that this SNV patterns may be considered as a risk factor for male infertility in our study cohort.

Thus, in conclusion the present study demonstrates that AZFc partial deletions are high risk factors for spermatogenic impairment and male infertility. Therefore, here we propose a two-tier approach: (i) Y chromosome AZFc partial deletion analysis by employing hotspot markers, which is followed by (ii) SNV analysis of DAZ and CDY1 specific gene copies dosage may be examined in the infertile couples in order to rule out the possible association of AZFc partial deletions in male infertility. In general, AZFc partial deletion in combination with DAZ/CDY1 gene copy dosage analysis may be seriously considered and implemented as a routine test in molecular diagnostic assays.