REVIEW OF LITERATURE

A. INTRODUCTION

The recent growth of the Indian population has been unprecedented. It stands currently at over one billion and is expected to touch 2 billion by 2035 assuming an average growth rate of 2%. Even though curtailing population growth is a major national concern, meanwhile substantial numbers of infertile couples in India have an equally great concern (Seshagiri, 2001). Male reproductive function in the general population has gained more attention due to the occurrence of several biological problems affecting the male genital tract and has increased during the last 50 years (Toppari et al., 1996). Infertility is an equally important national problem concerning reproductive health and the infertile couples has to be treated by medically assisted reproductive technology (MART) for procreation (Seshagiri, 2001). “Infertility is defined as failure to conceive after 12 months of unprotected sexual intercourse with same partner” (Rubenstein, 2006). It is a worldwide problem affecting people of all communities, though the cause and magnitude may vary with geographical location and socioeconomic status. It is a problem faced by couples rather than individuals. When efforts to have children are unsuccessful, feelings of helplessness, frustration and despair are common and it can be a major life crisis for many couples. Standard semen analysis has long been the primary laboratory test to find out male fecundity. Male sterility still poses a diagnostic problem and remains difficult to treat. Subfertility is the another condition which is characterized by sperm concentration less than 15*10^6/ml, less than 50% showing forward progression motility, and normal morphology in less than 20% (WHO, 1999). A precise understanding of the
functional competence and production of mammalian spermatozoa is essential to create advancement in treating clinical features of infertility.

Semen is produced as a concentrated suspension of spermatozoa, stored in the paired epididymides, and fluids secreted from the accessory reproductive organs. About 90% of semen volume is made up of secretions from the accessory organs (Weiske, 1994), mainly by the seminal vesicles, prostate and minor contributions from the bulbo urethral (Cowper’s) glands and epididymides. semen has two major characters:

1. The total number of spermatozoa: This reflects sperm production by the testes and the patency of the post-testicular duct system.
2. The total fluid volume contributed by the various accessory glands: this reflects the Secretory activity of the glands.

Based on the semen quality like sperm count, morphology, and motility the infertile condition is classified and nomenclature as follows. (Eliasson et al., 1970)

1. Aspermia: No semen/retrograde ejaculation/ ejaculation failure
2. Asthenozoospermia: Percentage of progressively motile (PR) spermatozoa below the lower reference limit.
3. Asthenoteratozoospermia: Percentages of both progressively motile (PR) and morphologically normal spermatozoa below the lower reference limits.
4. Azoospermia: No spermatozoa in the ejaculate.
5. Teratozoospermia: Percentage of morphologically normal spermatozoa below the lower reference limit.
6. Oligozoospermia: Total number of spermatozoa below the lower reference limit.
7. Oligoteratozoospermia: Total number of spermatozoa and percentage of morphologically normal spermatozoa, below the lower reference limits.

8. Oligoasthenozoospermia: Total number of spermatozoa and percentage of progressively motile (PR) spermatozoa, below the lower reference limits.

9. Oligoasthenoteratozoospermia: Total number of spermatozoa and percentages of both progressively motile (PR) and morphologically normal spermatozoa, below the lower reference limits.

B. PREVALENCE/INCIDENCE OF INFERTILITY

The prevalence of infertility is varying from different geographical location, but it is estimated that globally, 60–80 million couples suffer from infertility every year (Poongothai et al., 2009). However, according to a study by WHO, the incidence of infertility in India is not yet clear. It is estimated that approximately 13 to 19 million couples are infertile (Sharma et al., 2005). 15% of the couples in the US at reproductive age are unable to conceive naturally (Fernandez et al., 1991). According to Johnson, (1983) it is estimated that 2 to 3 million American couples are unable to have offspring. Belsey, (1976) reported that 10% of Sub Saharan Africa couples are infertile. Approximately 10% of the couples in Sweden and one in seven couples in the UK are infertile (Osser et al., 1992).

C. ETIOLOGY OF MALE INFERTILITY

The etiology of the male infertility is multifactorial and little is known about the causative factors leading to decrease in spermatogenesis. In men, the main causes of infertility are Oligozoospermia, Asthenozoospermia, teratozoospermia and azoospermia, which account for 20–25% of cases (Egozcue et al., 2000; Hargreave, 2000). The
nonobstructive azoospermia has a strong genetic basis where there is an excess existence of autosomal abnormalities (Hargreave, 2000). A reduction in the male fertility potential may be due to congenital or acquired conditions such as Urogenital abnormalities, infections of the genital tract, genetic abnormalities, endocrine disturbances, testicular failure, immunologic problems, cancer, systemic diseases, altered lifestyle, and exposure to gonadotoxic factors (Nieschlag, 2000). In addition to these causes, another important category is ‘unexplained male infertility’ (UMI) and idiopathic male infertility. UMI is reserved for infertile men with infertility of unknown origin with normal semen and in which female infertility factors have been ruled out. Its prevalence ranges from 6 to 27% (Moghissi and Wallach, 1983; Sigman et al., 2009). Idiopathic male infertility is a condition in which fertility impairment occurs spontaneously or due to an obscure or unknown cause (Hamadaa et al., 2011). In addition to this, still the pathophysiology of idiopathic condition is an enigma but in the past few years modern diagnostics to investigate male infertility have progressed making it possible to understand the cause of decrease in spermatogenesis.

**C. 1. GENETIC BASIS OF MALE INFERTILITY**

Male factor infertility is a complex disorder that affects a large sector of the population. A variety of physiological processes like hormonal homeostasis, spermatogenesis, and sperm quality are influenced by genetic factors (Katherine et al., 2010). Most common genetic factors related to male infertility are mutation in Cystic Fibrosis Transmembrane conductance Regulator (CFTR) gene which results in cystic fibrosis (CF), a common fatal autosomal recessive disorder. More than 95% of CF men have abnormalities in the structures derived from the Wolffian duct (Dork et al., 1997).
The second type of mutations that result in congenital absence of the vas deferens (CAVD) is found in 2% of men presenting with infertility. It is a common cause of azoospermia associated with low semen volume and acidic pH (Pauer et al., 1997). The third type is Klinefelter syndrome, affecting up to 7-13% of azoospermic men, whereas limited sperm production is commonly found in men with a mosaic pattern of Klinefelter syndrome (47, XXY). Structural alterations of the autosomes (Robertsonian and reciprocal translocations, inversions, duplications and deletions) may instead be present in patients with less severe alteration of the spermatogenesis (Peschkaet et al., 1999; Gekas et al., 2001).

C.2. CHROMOSOMAL ANOMALIES CONFINED TO SPERMATOZOA

Aneuploidy in spermatozoa is the direct result of constitutional genetic anomalies or they may be caused by errors during the meiotic phases induced by the altered testicular environment (Gekas et al., 2001). In this altered testicular environment in fact, determine the alteration of the testicular genetic and epigenetic control, which giving rise to a greater disposition to errors in the cells during meiosis. (Vegetti et al., 2000; Calogero et al., 2001; Burrello et al., 2004). Recent studies have shown that an alteration in the intra-testicular microenvironment, such as that which occurs in patients presenting damage to the spermatogenesis, which is negatively influenced on the mechanisms of chromosomal segregation during meiosis and results in the formation of aneuploidal gametes. Earlier studies showed that patients with primitive testiculopathy produce a greater number of spermatozoa with aneuploidies compared to subjects with normal parameters of seminal fluid, in spite of the presence of a normal karyotype (Vegetti et al., 2000; Calogero et al., 2001).
C.3. Y - CHROMOSOME MICRODELETION.

A microdeletion is defined as a chromosomal deletion that spans several genes but is not large enough to be detected using conventional cytogenetic methods. Y chromosome microdeletions are a frequent cause of infertility in males (Schlegel, 2004). Some genes located on the Y chromosome are suggested as candidates for the conditions like azoospermia and oligozoospermia (Ozdemir et al., 2007). Y-chromosomal microdeletions occur due to intrachromosomal recombination between homologous sequences. Long arm of Y-chromosomes composed of many Y specific repetitive sequence block (Kawaguchi et al., 2001; Skaletsky et al., 2003). These repetitive sequences assembled in eight palindrome structures (P1-P8) which are highly symmetrical. The AZFb and AZFc deletions occur between palindromes whereas the AZFa deletion is the result of intrachromosomal recombination (Kamp et al., 2000). High resolution karyotyping map of Y-chromosome showed that more than just 0.2% of azoospermic men had defects of the Y chromosome, and more than a few percent of severely infertile men had a genetic basis for infertility condition. (Sherman et al., 2002; Ristanovic et al., 2007). Recent studies have shown that, among infertile males Y chromosome long arm have been recurrently deleted in AZF a, AZF b and AZF c regions (Samli et al., 2006). The most frequent AZFc deletion is associated with milder azoospermia and oligozoospermia (Choi et al., 2004). Complete deletions of AZFa, AZFb and AZFb+c regions are associated with cases of more severe azoospermia (Ferlin et al., 2007). Yq deletions and its clinical significance have been debated for a long time because of the large variability in the frequency of deletions reported by different
researchers, because Yq deletions have also been reported in “fertile” men (Krausz, 2006).

C.4. ENDOCRINE CAUSE OF MALE INFERTILITY.

The successful and complete male germ cell development is dependent on the balanced endocrine interplay of hypothalamus, pituitary and the testes. (De Krester, 1979). The proper production and maintenance of spermatogenesis generally requires the presence of FSH, LH and Testosterone. The adult testis has two important functions; namely, the production of spermatozoa and the secretion of testosterone, are both dependent on stimulation by the pituitary gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which are secreted in response to hypothalamic gonadotropin-releasing hormone (GnRH). FSH binds with receptors in the Sertoli cells and stimulates spermatogenesis, while LH stimulates the production of testosterone in Leydig cells, which in turn may act on the Sertoli and peritubular cells of the seminiferous tubules and stimulates spermatogenesis (O’Donnell, et al., 1994; Robert et al., 2002). The failure of the pituitary to secrete FSH and LH will result in disruption of testicular function leading to infertility. Testosterone, estradiol and inhibin control the secretion of gonadotrophins through feedback mechanism. (Weinbauer and Nieschlag 1995). Disturbance in these hormone levels impairs the normal spermatozoa production and results in causing different endocrine abnormalities. Hypogonadotropic hypogonadism (HH) is a condition in which there is a low serum gonadotropin measurements accompanied by low serum testosterone and/or oligo/azoospermia. In case of HH, FSH and LH levels are increased with decreased levels of testosterone were recorded (Scott et al., 2006). Another important endocrine cause of male infertility is an
androgen resistance syndrome (AR). AR can be classified on the basis of the step in androgen action that is impeded by the individual mutations. 5 alpha-Reductase deficiencies is an autosomal recessive enzyme defect that impairs the conversion of testosterone to dihydrotestosterone. A variety of disorders influence the androgen receptor that mediates the action of both testosterone and dehydrotestosterone (Griffin et al., 1982). Rajender et al. (2007) reported that a C3693T missense mutation in the AR gene in a familial case of complete androgen insensitivity syndrome (CAIS), resulting in the replacement of a highly conserved leucine residue with phenylalanine (L859F) in the ligand-binding domain (LBD) of the receptor. Mutations in the androgen receptor (AR) gene cause an array of abnormal sex differentiation phenotypes in humans, ranging from mild through partial to complete androgen insensitivity.

Another important endocrine cause of male infertility is congenital adrenal hyperplasia (CAH), (White and Speiser, 2000) in which the deficiency of an adrenal enzyme 21-hydroxylase (21-OH CAH) and it is evident that, in all its forms involved in the biosynthesis of cortisol. The incidence of 21-hydroxylase deficient is 1 in 15,000 births (White and Speiser, 2000). Abnormal adrenal steroid production inhibits the release of gonadotropins by the hypothalamic-hypophyseal axis, resulting in failure of normal testicular maturation leads to infertility in men (Kwak et al., 1993). Hyperprolactinemia is the usual known cause of male infertility, the main causes are pituitary or suprasellar tumors, hypothyroidism, and intake of certain medications. These factors may deplete the dopamine levels in hypothalamus or block the action of dopamine on the pituitary (Wong and Jones, 1984). The effect of hyperthyroidism and hypothyroidism on female reproduction are well established, limited data exist
concerning the impact of thyroid disorders on sperm quality in adult males. In a few of
the male infertility cases sex hormone binding globulin (SHBG) is found to be increased
in male hyperthyroidism leading to a rise in circulating levels of total testosterone and to
a decrease in the metabolic clearance rate of testosterone (Monson, 1988). However, the
plasma level of free testosterone is not significantly different from normal, whereas
testosterone has been reported recently to be low. Serum free estradiol levels are
increased out of proportion to the rise in the SHBG, and increased peripheral conversion
of androgen to estrogen has been reported. Serum progesterone levels are also elevated in
these patients. Basal levels of gonadotropins are usually normal, with LH and FSH
responses to exogenous GnRH significantly greater than those of controls (Krassas et al.,
2009).

Environmental Factors: Humans are exposed to various environmental agents that may
be hazardous to their reproductive capacity. Environmental hazards to male reproductive
function were revealed 30 years ago, when pesticide manufacturers and agricultural
workers in contact with the nematocide, 1, 2-dibromo-3-chloropropane (DBCP), suffered
from severely impaired spermatogenesis, leading to infertility (Eaton et al., 1986; Slutsky
et al., 1999) because male reproductive function is known to be highly sensitive to
several chemical and physical agents generated by industrial activities. These industrial
toxicants damage the testis and results in reduction of sperm density, abnormal sperm
morphology and impaired production of androgen (Bonde, 1996). Circulating steroid
hormone level is also altered, by endocrine-disrupting chemicals (EDC) which
mimicking the effects of endogenous hormones, specifically endocrine disrupters, and
antagonize the actions of endogenous hormones by modifying steroidogenic enzyme
expression activity (Edward et al., 2005). Semen analysis allows the male reproductive function to be evaluated directly and correlate the interaction between exposure to environmental agents and fertility rates. Due to the widespread use of such chemicals, and their potential for leakage into the environment, constitute a recognized hazard to male fertility. In addition to these life style factors like excess alcoholism also affect the hypothalamin-pituitary-gonadal (HPG) axis. Alcohol is the second most addictive substance after nicotine. Alcohol is reported as direct testicular and Leydig cell toxin (Lipsett, 1980; Van et al., 1975). Guo et al., (2006) showed that excessive alcohol consumption had the potential to decrease an already low percentage of sperm with normal morphology. Martini et al., (2004), also found a significant reduction in seminal volume, sperm concentration, percentage of motile spermatozoa, and a significant increase of the non motile, viable gametes among men with habits of alcohol and smoking. Great numbers of leukocytes in the seminal fluid were found in alcohol users than in normal (Close et al., 1990). Alcohol use affects the hypothalamin-pituitary-gonadal (HPG) axis, where FSH, LH testosterone and E2 levels were significantly increased and thereby decreasing in the semen volume, sperm count, motility and morphology of normal sperm (Muthusami and Chinnaswamy, 2005).

C.5. HEAT AND STRESS

Elevation of scrotal temperature to normal core body temperature results in complete failure of spermatogenesis in man and most mammals (Mieusset and Bujan, 1995). The study conduct on human exposed to occupational and lifestyle had shown an adverse effect on semen quality. Occupational exposure to radiant heat in bakers, welders, furnace workers, ceramics workers, etc. can induce such effects due to
prolonged sitting and thus reduction in airflow around the scrotum (Mieusset and Bujan, 1995). Infertility is also very important reason for stress and anxiety in male and female, thus affecting the couple’s sexual relationship and relationship as a whole. Harlow et al., (1996) demonstrated that infertility can cause stress in the couple and this stress can increase the levels of prolactin and cortisole which itself can be an attribute to infertility.

**C.6. Influence of drugs on male fertility**

Erectile dysfunction in a young man represents a major problem for the patient and his partner. Although, the prevalence of ED in otherwise healthy men is about 5% at 40 years; it is usually associated with many marital and social troubles (Broderick, 1995). Many drugs have been reported to cause erectile dysfunction. Central neurotransmitter pathways, including serotonergic, noradrenergic, and dopaminergic pathways involved in sexual function, may be disturbed by antipsychotics, antidepressants and centrally acting antihypertensive drugs. Beta-adrenergic blocking drugs may cause erectile dysfunction by potentiating alpha-1 adrenergic activity in the penis. Thiazide diuretics have been reported to cause erectile dysfunction (ED), but the cause is unknown. Spironolactone can cause erectile failure as well as decrease in libido and gynecomastia (Wolfe, 1979; Lue, 2000; Thompson et al., 2005; Jackson et al., 2006). Thus sedentary lifestyle, smoking, alcohol abuse, diabetes mellitus, hypertension, dyslipidemia and obesity are considered as a possible risk factor in causing ED (Feldman et al., 2000).

**C.7. OXIDATIVE STRESS**

Reactive oxygen species (ROS) are products of normal cellular metabolism. Most of the body’s energy is produced by enzymatically controlled reaction of oxygen with hydrogen in oxidative phosphorylation occurring within the mitochondria during
oxidative metabolism. This enzymatic reduction of oxygen produces energy and free radicals, which relieved them of their unpaired electron, resulting in the oxidation of lipids in membranes, amino acids in proteins carbohydrates and nucleic acids (Ochsendorf, 1999; Valko et al., 2007). The production of superoxide (O2-) and other reactive oxygen species (ROS) by mitochondria is a major cause of cellular oxidative stress which contributes many physiological and pathological conditions (Raha and Robinson, 2000). Leukocytes, particularly neutrophils and macrophages, have been associated with excessive ROS production, and they ultimately cause sperm dysfunction (Saleh et al., 2002). Many studies has been showed that life style factor like cigarette smoking can induces ROS because it contains a compounds like polycyclic aromatic hydrocarbons and smoking metabolites may act as chemotactic stimuli and thus induce an inflammatory response, recruitment of leukocyte, and subsequent generation of reactive oxygen species (ROS) and in turn tilts the delicate balance of ROS that are produced by spermatozoa for their special functions like decapitation (Richthoff et al., 2008). Thereby increased quantities of the ROS act on sperm DNA and produce a negative effect on the quality of spermatozoa. (Koksal et al., 2003).

Antioxidants are compounds which scavenge and suppress the formation of ROS, or oppose their actions (Holland et al., 1987). Among the well-known biological antioxidants, superoxide dismutase (SOD) and catalase have a significant role. SOD protects the spermatozoa against spontaneous O$_2$ toxicity. Glutathione (GSH) peroxidase, a selenium-containing antioxidant enzyme with GSH as the electron donor, removes peroxyl (ROO$^-$) radicals from various peroxides including H$_2$O$_2$ (Calvin et al., 1981). A variety of non-enzymatic antioxidants are also present in the seminal plasma, including
vitamin C, vitamin E, glutathione, urate, ubiquinone and bilirubin are considered as a chain-breaking antioxidants. Other nonenzymatic antioxidants such as vitamin E, vitamin A, haptoglobin, transferrin and ceruloplasmin are present in the plasma membrane of the spermatozoa and act as preventive antioxidants (Suresh and Sikka, 2004). These different varieties of antioxidant play an important role in protecting male germ cells from oxidative damage (Fraga et al., 1991). Concerning dietary factors, a lower intake of some antioxidant nutrients, such as vitamins A and E, Carnitines, Folate, Zinc, and Selenium, has been associated with male infertility (Paul et al., 1996; Comhaire and Mahmoud, 2003). The higher nutrient intake of vitamins C, E and β-carotene was associated with a higher sperm count and motility (Eskenazi et al., 1991). Many studies showed the improvement in semen character and sperm function status after antioxidant treatment further the improvement in the semen quality and increased fertility potential has been observed after administrating supplements with a healthy diet.

C.8. IMPACT OF ROS ON DNA

Two factors protect spermatozoa DNA from oxidative stress: the characteristic tight packaging of sperm DNA and the antioxidants in seminal plasma (Kartikeya et al., 2009). ROS induce DNA damage in the form of modification of all bases (primarily guanine via lipid peroxyl or alkoxyl radicals), resulting in the production of base-free sites, deletions, frameshifts, DNA cross-links and chromosomal rearrangements (Duru et al., 2000). ROS also can cause various types of gene mutations such as point mutations and polymorphism. Resulting in decreased semen quality. ROS is also associated with high frequencies of single- and double-strand DNA breaks (Duru et al., 2000; Aitken and Krausz, 2001). A common byproduct of DNA oxidation, 8-hydroxy-2-deoxyguanosine
(8-OH-2-deoxyguanosine), has been considered a key biomarker of DNA damage by oxidative stress (Kartikeya et al., 2009).

C.9. ANATOMICAL CHANGES AS A CAUSATIVE FACTOR IN MALE INFERTILITY

The association of Varicocele with male subfertility has been repeatedly demonstrated, over the past decade. 30-40% of infertile couples, shows the incidence of vericocele (Kumpman et al., 1984: American society of reproductive medicine 2008) and it is the most commonly occur due to absent or incompetent valves in the internal spermatic vein (ISV), with subsequent reflux of blood down the vein when in the upright position engorgement of the Pampiniform plexus and venous collaterals in the scrotum lead to elevated scrotal temperature and pressure causing hypoxia, oxidative stress and lower testosterone concentration in the testis, vericocele was commonly found on the left side of the scrotum (Zorgniotti and Sealfon., 1988; Agarwal et al., 2009). This may result in decreased sperm count as well as abnormal sperm motility and morphology (French, 2008). Cryptorchidism is another clinical condition associated with male infertility. Cryptorchidism is a defect involving maldescent of the testicle. By the 12th to 14th week of gestation the testis migrates from the urogenital ridge to the level of the internal inguinal ring. The testis begin its transinguinal descent during the 26th to 28th week of gestation with simultaneous gubernaculum swelling and processes vaginalis extend into the scrotum. Testicular descent is believed to depend on an integration of factors: an increase in intra abdominal pressure, gubernaculum tension and the hormonal influence of high local concentrations of dehydrotestosterone (Kaki and Sofikitis, 1999). The incidence of cryptorchidism in the newborn is 2% to 6%. The etiology of
cryptorchidism is multifactorial, both genetically and endocrine disruption may be involved (Dohle et al., 2005). Cryptorchidism is a consequence of testicular dysgenesis. This testicular dysgenesis an reduce the fertility potential and also at risk for malignant development (skakkebaek et al., 2001).

C.10. AUTO IMMUNITY AND MALE INFERTILITY

Antisperm antibodies (ASA) are one of the main causes of infertility and the prevalence is detected in 8% -21% of infertile males (Dana and Alan, 1996; Reza et al., 2009). Occurrence of autoimmunity is due to the expression of sperm cell antigens during sexual maturation, long after the prenatal period when immunological self-tolerance is induced (Naz and Menge, 1994; Koide et al., 2000). Mechanisms that can provide the autoimmunity and ASA production are microenvironmental acceleration of Th1 immunity, enhanced secretion of proinflammatory cytokines like IL-1, IFN-γ, TNFa, reduced secretion of anti-inflammatory cytokines such as IL-10 and TGF- β. These mechanisms are associated with upregulation of major histocompacatibility molecular expression and down-regulation of immune cell apoptotic mechanism (Naz and Menge, 1994; Sakamoto et al., 1995; Pollanen et al., 1996;). The most common causes of ASA include previous genital tract infection, testicular biopsy, trauma, torsion, vasectomy, prolonged use of alcohol, smoking and environmental pollution (Broderick et al., 1989; Koide et al., 2000; Arap et al., 2007). ASA is thought to impair fertility by inhibiting sperm motility (Caron and Saling, 1991), inability of the sperm to penetrate cervical mucus (D’Cruz et al., 1991), capacitation (Bronson et al., 1982), acrosome reaction (Jaffe and Oates 1994) or they may involve the complete cascade resulting in sperm lysis (Jaffe
and Oates, 1994; Downie et al., 1997) and can also prevent implantation, and arrest embryo development (Haas, 1986; Koide et al., 2000).

In view of the above literature survey, in Mysore city though ample of male infertile cases are reported yet no systematic studies were carried out so far on the cytogenetical, biochemical and immunological basis of male infertility. Hence the present study enables to understand the type of anomalies and degree of relatedness in male infertility. The result of this work gives clear idea on precise method of diagnosis to dissect the cytogenetical, biochemical and immunological etiologies. This will be helpful in developing innovative methods of treatment and counseling on infertile population.

The key objectives of the present study include:

1) Establishment of genetic register and Pedigree analysis.

2) Chromosomal analysis through G-banding and analysis of Sex chromosomes in sperm through FISH (selected cases)

3) Semen analysis
   a. Semen quality
   b. Sperm function test

4) Biochemical analysis of semen

5) Immunological analysis