Introduction and Literature Review

1.Infertility: A general idea

It is being noticed that at present not only thepopulation explosion butinfertilityhas become a major problem of human reproduction. It has been the cause for stress, anxiety, depression, and psychosomatic complications **(Pasha, 2011)**. Oxford dictionary gives a definition of stress by saying 'a state of affair involving demand on physical or mental energy'. Stress can be psychological or physical. As per mental health professional, stress is a dynamic condition by which an individual is confronted with an opportunity, constraint, or demand related to what he or she desires and for which the outcome is perceived to be both uncertain and important"**(Parray et al., 2016)**. It was also reported that most of the couples suffering from infertilitythat may be due to the most stressful and depressing period of their life. Sometime, Stress can be acted as an influencer to affect adversely the reproductive health of male and female both and it can be the prime cause of infertility **(Maroufizadeh et al., 2015)**.

According to World Health Organization (WHO), Infertility is consideredas a disease of male reproductive systemdefined by failure to conceive children naturally after twelve months of regular unprotected intercourse. Stress levels elevation has been associated with infertility **(Mendola et al., 1990)**. Few studies focused that psychological distress adversely affects the treatment of infertility patients and to major extent the outcome **(Cousineau and Domar, 2007)**. Generally two type of infertility are there. One is primary and another is secondary infertility. Primary infertility is the condition, when the man has failed to impregnate a woman. On the other hand secondary infertility applies when the man impregnated a woman, even if

the women are not the partner in the present couple **(Zegers et al., 2009)**. In general, it is being noticed that half of infertility related cases is caused by male factor **(Miyamoto et al., 2012)**. A male factor is corely responsible for approximately 20% infertility related cases **(Carlsen et al., 1992)**. There are so many conditions such as genetic abnormalities, immunological problems systemic or neurological infections diseases, systemic diseases, trauma, cancer, and stress which may be the responsible reason for male infertility **(Nieschlag, 2000)**. Idiopathic male infertility is a condition in which deterioration in the fertility occurs without break or may be due to unknown aetiology (**Moghissi, 1983; Dohle et al., 2010)**. The 'unexplained male infertility' (UMI) is a pattern of infertility where the cause is totally unknown **(Sigman et al., 2009**; **Moghissi, 1983**).

Routine semen analysis of these subjects showed decreased number of spermatozoa (oligozoospermia), an increased proportion of abnormal forms (teratozoospermia) and decreased motility (asthenozoospermia). This type of abnormality usually occurs and it is described as the oligoasthenoteratozoospermia syndrome **(Dohle et al., 2010)**. It includes apprantly 31% of infertile men **(Dohle et al., 2010).**

2.Prevalence

Reproductive system or genital system is system of sex organ which depends on the environment for its religious,cultural, and socioeconomic factors. The particular cause of infertility differ from community to community, one region to another and even population to population. Involuntary infertility is related to conditions such as parasitic diseases, sexually transmitted diseases, infections and toxic exposure in the environment or in diet in a major part.

2.1 International Scenario

Infertility is becoming a major global problem which affecting twelve percent couple (50-80 million) at the phase of their reproductive lives **(WHO, 1991)**. In case of Sub-Saharan Africa, the prevalence rate of the infertility ranges from less than ten percent in ogo and twenty five percentin Rwanda to about in Central African Republic and Cameroon a of women having age 20-44 years **(Larsen, 2000)**. However, it is being noticed that the one of the major causes of infertility is gonorrhea through cervical occlusion among women and tubal infection **(Frank, 1983)**. A high level of infertility is directly associated with sexual mobility, extramarital sex, divorce and prostitution. According to the Demographic and Health Surveys report (1994-2000), it was observed that 1.3 percent in Kenya and 3.3 percent in Mozambique of recently married women had no history of fertile pregnancies between 25-49 years **(Rutstein and Shah, 2004)**.

2.2 National Scenario

As per the Census on 1981 estimated around 4-6 percent cases of infertility in India and according to National Family Health Survey-1 (NFHS-1) childlessness is around 2.4 percent of recently married women having age more than 40 years in India **(Jejeebhoy, 1998)**. It is being reported that around 2.5 percent case history of childlessness in India. Among them about 5.5 percent belong to the age group of 30-49 years **(Shivaraya and Halemani, 2007)**.

According to World Health Organization (WHO), prevalence rate of primary infertility ranges between 3.9% to 16.8% **(Calverton, 2004).** Rate of infertility vary widely among different states of Indian. It is around 3.7% in Himachal Pradesh, Uttar

Pradesh, and Maharashtra **(Talwar et al., 1986)**, about 5% in Andhra Pradesh **(Unisa, 1999)** and around 15% in Kashmir **(Zargar et al., 1997)**. However, the prevalence rate of primary infertility has also been noted to vary among other tribes and castes in the same region of Indian states **(Talwar et al., 1986; Unisa, 1999)**.

It was observed thatmen are related to 40% of infertility cases whereas 40% of women and 20% cases for both sexes **(Sadockand Sadock, 2003)**.

A recent report has been published in India mentioning the status of infertility which actually stated that about 50% of infertility is associated to the reproductive abnormalities among male **(Kumar, 2004)**. About 25% of infertility related history have been reported without any cause which may be found after regular examination **(Kumar, 2004)**.

3.Risk factors of male infertility

3.1 Age factor

Level of testosterone in blood decline long with the advancement of ageing in most men, even in those who belongs to healthy subject, this particular type of decreased levelmay be found around the age of thirty **(Pasqualotto et al., 2004)**. After the age of 30 years, the testosterone level was decline of around 1% per year which is termedasandropause**(Feldman et al., 2004; Bhasin et al., 2006)**. The symptoms which are linked with the hypogonadism during aging include decreased muscle mass, libido, diminution in bone mineral, fat mass elevation, insulin resistance, emotional irritability central obesity, and erectile dysfunction, dysphoria **(Pasqualotto et al., 2008)**. According to Mahmoud et al. in Belgium focused testicular volume of elderly male was 31% less compared with the young having age group 18 to 40 years old **(Mahmoud et al., 2003)**.

3.2 Obesity

There are so many studies which have been indicated that fertility decreased in case of obese women **(Bolúma et al., 2000; Law et al., 2007)**. However, obesity plays a vital role for the onset of male infertility **(Sallmén et al., 2006)**. There is a significant diminution in the number of motile sperm which was found among men having BMI more than 25 and also observed in men with excessive fat deposion in suprapubic area **(Kort et al., 2006)**. A cohort study was conducted and also found that the risk of infertility is co-related with low BMI as well as high body mass index **(Nguyen et al., 2007)**.

3.3 Smoking

Smoking can affects percentage of motile sperm and normal sperm and finally diminish male fertility **(Nadeem et al., 2012)**. Cigarette smoking is also associated with generation of high level of free radicals. This oxidative stress influence the antioxidant content in the seminal plasma and causing oxidative damage to sperm**(Nadeem et al., 2012)**. A study publicized that smoking decreasesthe semen parameters such as viability, motility and morphology, sperm concentration **(Kunzle et al., 2003)**.

3.4 Occupational exposure

According to a study perfomed in Lebanon showed that occupational exposure to a harmful agent (physical or chemical) which is associated with high risk of male infertility **(Inhorn et al., 2008; Multigner et al., 2007)**. The workers who are

exposed with different hydrocarbon like toluene, benzene and xylene showed the alterations in different sperm parameters like viscosity, liquefaction capacity, sperm count, sperm motility whencomparison was made with unexposed males **(Ten et al., 2008)**. Ethylene Di-Bromide exposure to men low sperm count with the elevation of number of abnormal sperm **(Jensen et al., 2006)**. Workers in industrial belt and construction sitehave increase infertility rates due to greater exposure to stress **(Queiroz and Waissmann, 2006)**. Exposure to heavy metals reduces the semen quality **(Queiroz and Waissmann, 2006)**.

3.5 Alcohol and caffeinated beverages

Excessive consumption of alcohol affects the reproductive pathway **(Emanuele and Emanuele, 1998)**. Fertility decreases among the men who consume more than three cups of tea a day **(Curtis et al., 1997)**.

3.6 Electronic devices

Continues sitting posture and prolonged heat exposure to pelvic zone trigger the the risk of infertility (**Sheynkin et al., 2005)**. Moreover, using of cell phones in a continuous manner has an adverse effect on the status of male fertility that leads to poor semen quality **(Agarwal et al., 2008)**. Another study revealed that excessive use of use of cell phones diminished the actual percentage of live sperms **(Wdowiak et al., 2007)**.

3.7 Stress

There are so many studies which have rejected that stress as the only one factor for the onset of infertility. Evidence showed that stress is acts as an important and vital risk factor for infertility development. Andrological and seminal parameters areremarkably diminished in men suffer form chronic stress **(Collodel et al., 2008)**.

There are many types of stress such as which affect the male fertility **(Mishra et al., 2012)**.

3.8 Reactive oxygen species (ROS)

ROS has negative impact on spermiological sensors. The membrane of spermatazoa is very much susceptible to ROS as it contains polyunsaturated fatty acids (PUFA). High level of ROS results an visible imbalance between antioxidant defense system and amount of free radical production. Excessive free radical may damage spermatozoal membrane and ultimately cell death. Increased levels of free radicals exposure to men in a prolonged manner showed abnormal sperm and sperm counts **(Olayemi, 2010)**.

4.Causes of Male Infertility

4.1 Varicocele

Varicoceles are considered as the prime correctable cause associated with male infertility (**Cozzolino and Lipshultz, 2001)**. According to Cozzolino and Lipshultz (2001), a study showed was observed in 15% population in generalincluding teenagers and adult **(Cozzolino and Lipshultz, 2001).** Prevalence of varicocele among men ranges between 30-40% who attended the infertility clinics**(Jarow, 2001)**.Left side of the body is very prone toVaricocele. The etiology of varicocele is not clear and it has been stated that multifactor are responsible for the initiation of Varicocele **(Naughton et al., 2001)**.

4.2 Endocrinal disorder induced infertility

Male infertility is developed due to dysfunction of endocrine glands which are often referred as pretesticular causes **(Lalitha et al., 2013)**. Fifteen percent (15%) of married couples are suffering from infertility due to hormonal disturbances **(Al-**

Faisal, 2010). Abnormalities during the production of hormone may be responsible factor the development of male infertility. It is the malfunction of pituitary gland for producingadequate amounts of FSH and LH which leads to reduce sperm count and develop infertility **(Al-Daghistani and Abdel-Dayem, 2006)**. It is around 11% of oligospermic males which produces infertility caused by hyperprolactinemia. It inhibits the pulsatile secretion of the GnRH, which is responsible for decreasing pulsatile release of FSH, LH and testosterone that leads the infertility **(Singh et al., 2011)**. The thyroid hormones exerts significant regulatory role for producing spermatozoa since spermatogenesis. Therefore dysfunction in the thyroid gland may hamper spermatogenesis along with male fertility **(Singh et al., 2011)**.

4.3 Reproductive tract infection of male

An infection in the male reproductive tract is a very common disease that can easily hamper the total process. Infection in the reproductive tract of male remarkably lower the levels of sperm quality, semen volume focusing the abnormality in the secretary purpose of the epididymis, seminalvesicles and prostate **(Marconi et al., 2009)**.

4.4 Disorders in ejaculatory ducts

The entire process of ejaculation is controlled by central as well as peripheral nervous system. Dysfunctions in ejaculation may be caused by anatomic, neurologic, psychological factors. Incomplete closure of the bladder neck shows retrograde ejaculation. Lesion in central nervous system (CNS) such as myelodysplasia and spinal cord injury ultimately leads to ejaculatorydysfunction. Ejaculation can be affected by some of medicines like anti-depressants, α- blockers, antipsychotics and antihypertensive **(Brugh and Lipshultz, 2004)**.

4.5 Immunological reason

It is being noticed that, antisperm antibodies are developed in about 9-33% of infertile couple. 8-19% among them of are male partner where this antisperm antibodies are present. There are few risk factors associated for the development of antisperm antibodies among man like vasectomy and epididymitis. Accurate mechanism behind the formation of antisperm antibodies is in gray area. Sperm having with antisperm antibodies responsible for poor sperm egg interaction and zona pellucid binding capacity that in turn may diminish the entire fertility potentiality **(Brugh and Lipshultz, 2004)**.

4.6 Chromosomal and genetic factors

About 10-15% of severe male infertility occurs due to genetic causes including chromosomal aberrations and single gene mutations **(Ferlin et al., 2006)**. Spermatogenesis, normaldevelopment of the genital tract, sperm motility, sperm viability and fertilization capacity may alter due to genetic disorder which may lead to varying degrees of malesubfertility or infertility **(Brugh and Lipshultz, 2004)**.

4.7 Psychological stress

Many forms of stress may be harmful to male reproductive potential. Stress activates the sympathetic nervous system and on the other side it involves the hypothalamus–pituitary–adrenal (HPA) axis **(McGrady, 1984)**. Psychological stress on spermatogenesis impairs testosterone secretion **(Nargund, 2015)**. A study revealed that high level of corticosterone in stressed rats showed suppresseslevel of both testosterone and inhibin**(Tohei et al., 1997)**. The psychological stress level was measured by the questionnaire **(Bhongade et al., 2015)**.

5.Testicular androgenesis-Hormonal regulation

Testicular Androgenesis and gametogensis are two major functions of testes. There are two major steroids hormones, testosterone and androstenedione, collectively known as androgens are mainly synthesized intestes. The process of steroidogenesis mainly takes place in the interstitial cell of leydig. Testosterone is a major steroid hormone and it is biosynthesized in several steps and secreted from testes. A small amount ofintermediate product of testosterone biosynthesis likes 17α hydroxyprogesterone, androstenedione are also secreted **(Winters et al., 1999)**. Testosterone is also synthesized from cholesterol, 27 carbon compounds. Cholesterol which is also synthesized in the Leydig cell and acting as major precursor for testosterone biosynthesis **(Baker and Oshaughnessy, 2001; Syed, 2007; Salman andEve, 2007)**.

5.1 Pathway of androgenesis or testicular steroidogenesis

There are two pathways known as Δ4 and Δ5 path ways of androgenesis **(Stanczik, 1997)**, the Δ4 pathway made up of the intermediates having Δ4 and 3 ketone structure but in case of the pathway of Δ5the intermediates having Δ5 as well as 3β hydroxy structure **(Stanczik, 1997)**. Testosterone, which is the end product of Δ4 pathway and different intermediates of Δ5, pathway are shifted to Δ4 pathway at four specific levels**(Stanczik, 1997)**. At C-17 position,hydroxylation of pregnalonone is involved in the pathway of Δ5 whereas Δ4 pathway beings with the oxidation of the 3β hydroxyl group of pregnanolone**(Stanczik, 1997)**. The synthesis of androgen is oxido-reduction and hydroxylation processes **(Hennebert et al., 2007)**. Dehydrogenase enzymes e.g. Δ5, 3β hydroxysteroid dehydrogenase (Δ5, 3β, HSD) again catalyzes the oxido-reduction reactions **(Vidal et al., 2000)** and 17β-hydroxysteroid dehydrogenase (17βHSD) which are acting as the rate limiting enzymes of androgenesis**(Simard et al., 2005)**. Reactions are involved systematically for the biosynthesis of androgenesis.

It occured at a specific location and needed the process of specific enzyme with co-factor.When cholesterol, the precursor of testosterone is converted into pregnanolone followed by hydroxylation at C20 and C21 of the cholesterol which produce 20-22 dihydroxy cholesterol **(Toren et al., 1962)**. Thisdihydroxycholesterol is subsequently cleaved at the bond of C20 and C22, which again catalized by C20-22 desmolase in the presence of reduced co-factor, i.e., nicotinamide adenine dinucleotide phosphate and molecular oxygen **(Stanczik, 1997)**. This result is reduction of cholesterol by 6 carbons which in turn forms 21 carbons containing biomolecules known as pregnanolone**(Baker and O'Shanghnessy, 2001)**. Now this pregnanolone is converted into testosterone involving an ordered series of enzymatic reactions **(Griffin and Wilson, 1998)**. In rodents, we find two major pathways of testosterone formation from pregnanolone, one is through the formation of progesterone, 17β hydroxysteroid progesterone and androstenedione which is known as Δ4 path way. The other is through the formation of pregnanolone, dihydroepiandrosteron and androstenedione which is known as Δ5 pathway **(Stanczik, 1997)**. For hydroxylation of pregnanolone or progesterone at 17 position17β hydroxylase is required where as 17β hydroxypregnanolone or 17β hydroxy progesterone requires C17-20 lyase or C17-20 desmolase. This reaction required NADPH and molecular oxygen **(Stanczik , 1997)**. This reaction is catalized by a single P450 enzyme which is bound to smooth endoplasmic reticulum **(Stanczik , 1997)**. This P450 enzyme accepts electron from flavoprotein called P450 reductase. The inter convertible oxidation to testosterone and dihydroepiandrosterone (DHEA) to androstenediol occurs in the cytosol by the action of 17βhydroxysteroid dehydrogenase (17βHSD), a non P450 enzyme **(Stanczik, 1997)**. Recent studies proved that there are four isoenzymes of 17β HSD **(Pautanen et al., 1995)**. Type-I isoenzyme is found to have its location in the cytosolic fraction

of placenta, ovary, breast and endometrium. It is specific for estrogens and favours the reduction of estrogen to estradiol. Again type-II isoenzyme is found in the microsomal fractions of the placenta and prostate. It catalyzes estrogens and androgens. Type III isoenzyme is found in the microsomal fractions of the testis and preferentially utilizes androstenedione and dihydroepiandrostero as substrate. Type-IV isoenzyme is scantly characterized**(Pautanen et al., 1995)**. The biosynthetic pathway with enzyme system for testicular androgenesis can be explained with the help of a diagram as shown in figure 1

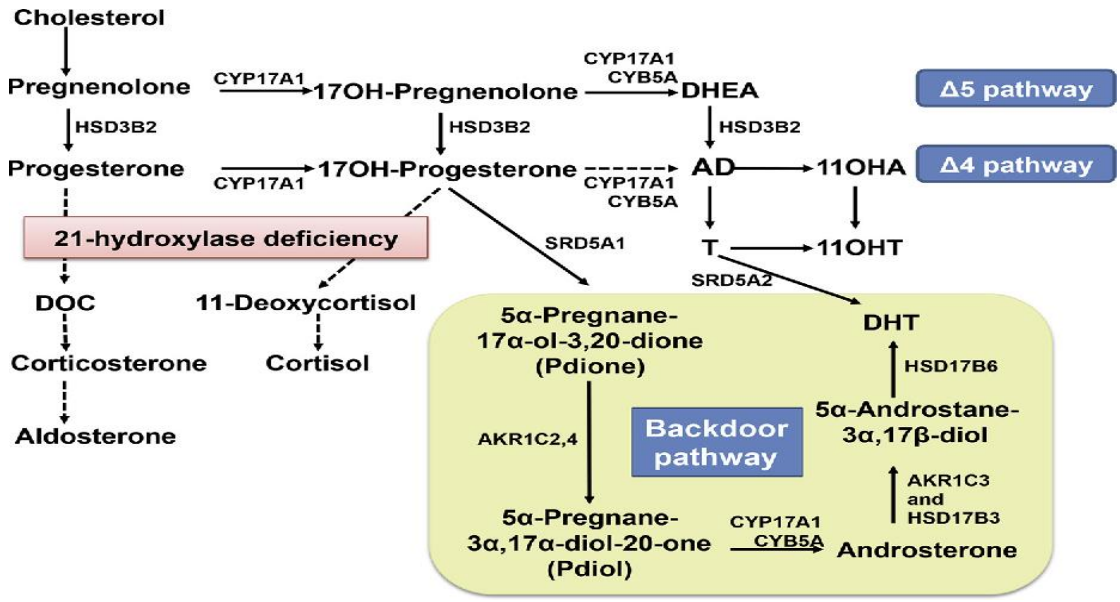


Figure 1.1: Pictorial diagram of testicular androgenesis

Androgenesis process is controlled byPituitary LH or interstitial cell stimulating hormone (ICSH) **(Udoh et al., 2009)**. This hormone is responsible for stimulating synthesis of testosterone in *in-vivo***(Hedger and Kretser, 2000)** and *in vitro***(Hall and Eik-Nes, 1962)**.Acute effect of this hormone, LH on testicular androgenesis is controlledby the activation of different mitochondrial enzyme concern with

cholesterol side chain cleavage as well as activation of other enzymes involved in thepathway ofsteroidogensis**(McLachlan et al., 2002).**On the other hand the chronic effect of LH is regulated through synthesis several enzymes involved in the conversion of pregnanolone to testosterone **(McLachlan et al., 2002).**Furthermore,LH activates cholesterol esterase and this LH stimulates the release of free cholesterol from steroid lipid **(Tsang and Kinson, 1980).** Now, this LH possesses several functions from the conversion of cholesterol topregnanolone from mitochondria. These functions are as follows

1. up take of cholesterol by mitochondrial **(Jamin et al., 2005)**
2. Requirement of the NADH formation required in hydroxylation reaction **(Jamin et al., 2005)**
3. Expulsion ofpregnanolone from mitochondria**(Jamin et al., 2005)**.

LH regulates testicular androgenesis. LH receptors are fixed model receptors and membrane boundalso **(Kahn et al., 1998)**. LH receptor regulates the process of androgenesis by altering the adenylatecyclase activity and it leads to the formation of cAMP**(Dufau et al., 1973)**. Protein kinase which is activated by cAMP mainlystimulates cholesterol side chain cleavage by increasingthe testosterone production (**Hedger and Kretser, 2000)**.

5.3 Role of Follicle Stimulating Hormone (FSH) on androgenesis

FSH influences the production as well as secretion of testicular steroids **(Baker and O'Shaughnessy, 2001).**At immature state,FSH regulates the development of testicular response to interstitial cell–stimulating hormone(ICSH)**(Baker and O'Shaughnessy, 2001).**An in vitro study indicated that perfused rabbit testis cans

secret more testosterone in presence of both LH and FSHcombindly than LH alone **(Johnson and Ewing, 1971; Mahat et al.,2016).** FSH can act with LHsynergisticallyfor the production of testosterone by increasing both number and sensitivity LH receptors in the testis **(Plant and Marshall, 2001)**. FSH again enhances the activities of Δ^5, 3β-HSD and 17β-HSD in hypophysectomised immature rat **(Murono and Payne, 1979)**. FSH mediated by specific receptor which is situated in plasma membrane of Leydig cell **(Dufau and Catt, 1978; Mahat et al.,2016)** and sertoli cell (**Chemes et al., 1979; Mahat et al.,2016).** The FSH receptors are protein in nature and it has been proved bylosing their binding properties after treatment with proteolytic enzyme **(Dufau et al., 1973; Mahat et al.,2016)**.

5.4 Role of prolactin (PRL) on testicular androgenesis

Androgenesis is mediated by prolactin also, which acts on Leydig cell of rat testis **(Yadav et al., 2008)**.It increases the effect of LH for the synthesis of testosterone **(Barkey et al., 1994; Mahat et al.,2016)**. A study revealed that testicular Δ5, 3β-HSD and 17β- HSD activities have been increased in hereditary dwarf mice after the treatment with PRL **(Gunasekar et al., 2008)**. From an investigation, it has also been noticed that PRL acts with FSH and LHsynergistically to increase the quantity of LH receptors in Leydig cells **(Karthikeyan et al., 2009)**.

5.5 Role of testosterone on testicular androgenesis

Testosterone, a steroid hormone controls the activities of cytochrome P450 enzyme in microsome of the leydig cells of cultured mouse and thus regulates its own synthesis **(Quinn and Payne, 1985; Mahat et al.,2016)**. Testosterone which is produced by binding ofhCG to active site of cytochrome P450 and forms cytochrome- oxygen complex,a pseudo-substratein the presence of oxygen **(Griffin and Wilson, 1998;**

Mahat et al.,2016).After the treatment of cultured leydig cell with hCG,testosterone inhibits cAMPmediated synthesis of cytochrome P450 by activating androgen receptor mediated mechanism **(Mahat et al.,2016).**

5.6 Role of insulin on testicular androgenesis

Insulin plays some positive role in regulating the testicular androgenesis**(Adashi et al., 1985; Mallick et al., 2010)**. There are several contradictory information regarding the role of insulin and testicular activities. In case of chronic diabetes there is a significant decrease in LH and FSH **(Ballester et al., 2004; Mallick et al., 2010)**. It also impairs the process of spermatogenesis which may be due to disturbance in the functions of sertoli cells or due to disturbance in the morphology of blood testes barrier **(Okanlawon et al., 2001).**Low level of insulin in diabetes can change in the level of sex hormone binding globulin (SHBG). That Pituitary–gonadal tuning system is regulated by insulin-glucose axis. Pituitary–gonadal tuning system in chronic diabetes become blunt that diminished the sensitivity of gonadotrophins on steroidogenic cell of testes **(Adashi et al., 1985)**.

6. General review of spermatogenesis

Spermatogenesis is one of the most important functions of the testes. The seminiferous tubules which are present in testis responsible for spermatogenesis process.Spermatogoniaultimately convert into spermatozoa in the orderly process of spermatogenesis **(Paul et al., 2010).**There are two vital processes known as multiplication of mother germ cell and maturation or metamorphosis of immature germ cells which responsible for production of mature spermatozoa are formed by sequential events. This process of multiplication of mother germ cells up to spermatogonia is known as spermatocytogenesis where both the mitotic and meotic

cell division takes place**(Griffin and Wilson, 1998; Toshmori, 2003)**. At the process of maturation the round spermatid is transformed into motile tadpole like mature spermatozoa and that particular process is known as spermatogenesis **(Griffin and Wilson, 1998; Toshmori, 2003)**. The maturation and development of germ cell proceed from the peripheral basement membrane towards the lumen through which mature spermatozoa pass into excretory duct. Through the process of mitotic cell division spermatogoniawhich converts into primary spermatocytes then converts into secondary spermatocytes by first mitotic cell division. Secondary spermatocyte again develops the round spermatid by the process of second mitotic cell division **(Griffin and Wilson, 1998)**.Through a complicated process of metamorphosis or spermeogenesis, the round spermatids are converted into motile mature spermatozoa **(Griffin and Wilson, 1998)**. There are three types of spermatogonia such as dark type A-spermatogonia, which generally formed at the onset of puberty from primary primitive germ cells by the mitotic cell division. Pale type A is formed from dark type A-spermatogonia by mitotic division. This pale type A again enters into mitotic cell division and form type B spermatogonia**(Griffin and Wilson, 1998)**. Each spermatogonium releases sixty-four spermatogonia**(Toshmori, 2003).**

Spermatogenesis process is involved with the following characteristic morphological changes-

1. Formation of the acrosome and nuclear changes **(Clermont, 1986; Eddy, 1988)**.
2. Development of the flagellum **(Clermont, 1986; Eddy, 1988)**.
3. Re-organization of the cytoplasm and cell organelles **(Clermont, 1986; Eddy, 1988)**.
4. Spermiation**(Clermont, 1986; Saito et al., 2000)**.

6.1 Spermatokinetics

In seminiferous tubules the characteristics of cellular association in the cross section was established**(Johnston et al., 2008; He et al., 2009)**. According to Rossen-Range and Gisel, definition and classification of the various types of cells in the seminiferous epithelium and the detail of the kinetics of spermatogenic process has been framed (**Rossen-Runge and Gisel, 1950**). On 1952, **Leblond and Clermont** described the seminiferous epithelial cell cycle in given area of the seminiferous epithelium of the same cellular association. According to them, spermatogenesis has been divided in rat into fourteen (I-XIV) stages and nineteen steps (Step1- Step19). Particular and specific stages have been identified as per the specific arrangement of the different generation of germ cells from basal to the adluminal direction of seminiferous tubule. Different steps have been marked and identified according to the characteristics of spermatids. The number of each cell type present in each cross section of the seminiferous tubule has also been reported. The different generations of germ cell associations at different stages of the seminiferous epithelial cell are shown in figure 1.2 and 1.3.

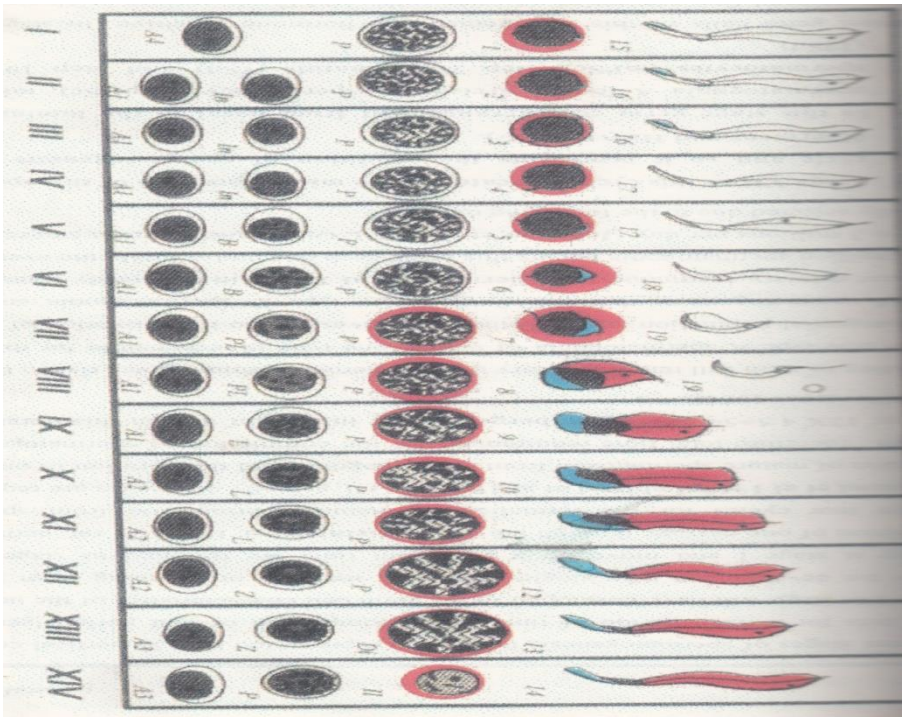


Figure 1.2: Arrangement of different generation of germ cells at different stages of spermatogenic cycle at rat

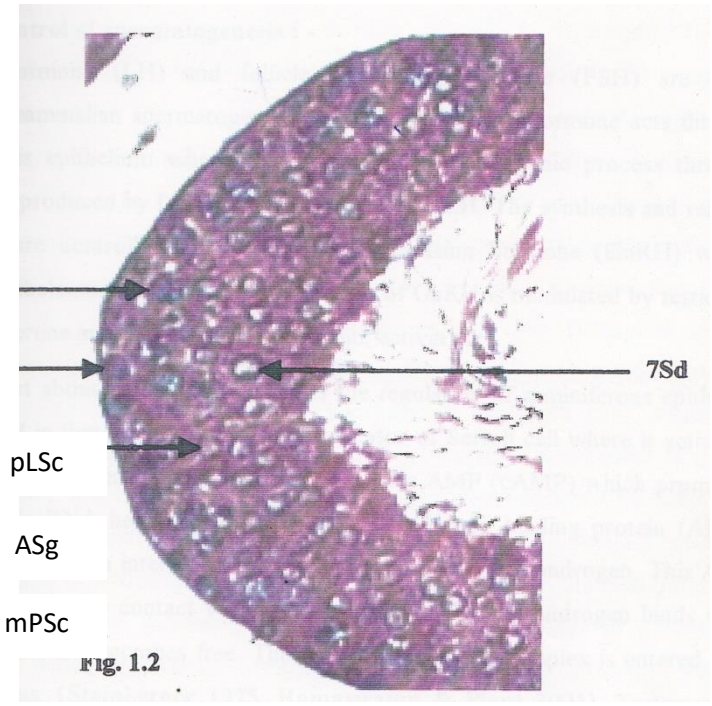


Fig. 2

Figure 1.3: Testicular cross section at stage VII of semineferous epithelial cell cycle shows different generation of germ cells (Fig. 2 X 1000)

column and each stage lasts for a particular and fixed period of time.Germ cell in its specific stage at the expiry of the fixed duration, it is converted into characteristic

germ cells of the next stage. At the end approximately of 34.8 hours, Step-I spermatid in rat (stage I) would be converted to Step–II spermatidshaving all the characteristics of stage II. When germ cells ultimately reached to the final stage i.e. Stage XIV, it progresses to secondary spermatocytes at the third layer of germ cell.

Entire duration of the cycle is equals to the duration of the different stages. The total duration of spermatogenesis is much as each germ cell passes through four cycles in rat before its ultimate release as spermatozoa at stage VII **(Leblond et al., 1963)**. Spermatokineticshelps for quantitative evaluation of changes in seminiferous epithelium following hypo-physectomy**(Clermont and Morgantaler, 1959)**. The knowledge aboutspermatokinetics also helps to evaluate the damage caused by noxious agent (**Steinberger, 1962; Chaudhury and Steinberger, 1975**). Spermatogenesis process is completed by 4.5 cycles which was started from primary spermatocytes to 19 steps.The total duration of each cycle is approximately 12 days in rat and so the entire duration covered by 4.5 cycles would be 12 days × 4.5 = 54 days. But in the case of human being total duration will be 72 days **(Clermont, 1986)**.

The stem cell renewal theory is also vitalfor the process ofspermatokinetics. Type Aspermatogonium starts proliferation at stage IX of seminiferous epithelium cell cycles. The daughter cell further divides successfully at stage XII and stage I of the semineferous cell cycle **(Clermont et al., 1959**). This type Aspermatogonium produces two new types A spermatogonia. One of these spermatogonia is responsible for the renewal of spermatogonial population. The other type Aspermatogoniumproduces intermediate spermatogonium. This said intermediate spermatogonium again produces type B spermatogonium and ultimately produces primary spermatocytes. The condition of spermatogenesis is presented by the quantification of spermatogenesis, the count of different generation of hormone

sensitive germ cells at stage VII of seminiferous epithelium, cell cycle (**Clermont and Harvey, 1967**). In this stage, there are different generations of germ cells such asspermatogonia A (ASg), Pre leptotene spermatocytes (pLSc), Midpachytine spermatocytes (mPSc), Stage VII spermatid (7 Sd) and step 19 spermatids (19 Sd) or spermatozoa. Step 19 Sd are not encountered here they are present in clump state.

6.2 Hormonal control of spermatogenesis

Spermatogenesis is mainly controlled by the leutinizing hormone (LH) and follicle stimulating hormone (FSH). LH regulates the processof spermatogenesis by testosterone which produced from Leydig cells. Beside this FSH directly acts on the seminiferous epithelium. Hypothalamus secrets gonadotrophin releasing hormone (GnRH) which synthesis and releases of LH and FSH, .GnRH secretion is modulated by testicular steroids like testosterone and peptides such as inhibin and activin.

The modern concept revealed that FSH is first bound with its specific receptor of sertoli cells where it activates the adenylatecyclase which results in the formation of cyclic AMP (cAMP) and this promotes the RNA synthesis. The formation of androgen binding protein (ABP) is helped by the synthesis of RNA. ABP is then transported into intercellular space and binds with androgen. Androgen binds with specific receptor where then ABP will become free. Receptor – androgen complex penetrates into the germ cell nucleus **(Ramaswamy and Plant, 2001)**. The process of spermatogenesis is then controlled by the testosterone but the actual mechanism on germ cell after this particular stage is not clear.

For the development of germ cells three steps have been followed which are known as known as initiation, maintenance and re-initiation. This said process is regulated by specific hormones **(Baker and O'Shanghnessy, 2001)**. The first stage of

spermatogenesis is initiation i.e testicular spermatozoa are formed at the stage of puberty.

Initiations of spermatogenesis in rodents are mainly regulated by FSH and testosterone –

6.2.1 Role of FSH on spermatogenesis

Administration of FSH proves that FSH has an important role in initiation of germ-cell division (**Plant and Marshall, 2001, Udoh et al., 2009**). It is being reorted that FSH maintains the meiotic cell division since the ratio of 4C cells to 1C cells (spermatids) is reduced to 10 % of control by following anti-FSH treatment (**Plant and Marshall, 2001**). The production of spermatozoa cannot be reach with FSH alone despite the importance of FSH for germ cells development in immature testis. Therefore, the questionmay arises about the ability of FSH only to start the normal spermatogenesis, but it is also reported that FSH can regulate the multiplication and differentiation of type A spermatogonia and increase the number of pachytene spermatocytes along with presence of testosterone **(Plant and Marshall, 2001, Ramaswamy and Plant, 2001)**. On other hand FSH again increased the mitotic rate and inhibited the degeneration of spermatogonia in immature rat (**Ramaswamy and Plant, 2001)**. FSH plays a prime role in the maturation of testis by maintaining the size of sertoli cell population. After hemi castration,FSH also helps to proliferate thesertoli cells **(Plant and Marshall, 2001)**.

6.2.2 Role of testosterone on sperm production

Testosterone has an important role for the initiation of spermatogenesis (**Hedger and Kretser, 2000; McLachlan et al., 2002**). At pre-pubertal period of a rat (within third to eighth day during), the plasma level of testosterone attains its pick (**Novak et al.,**

2007). GnRH regulates the level of testosterone which is also proved by immunization **(Pitteloud et al., 2009**).

The actual function of testosterone depends on the different doses because in low testosterone produces atrophy in seminiferous epithelium whereas high testosterone level does not show any adverse effect. This type of finding is due to the suppression of gonadotrophinsfrom pituitary followed by the low level of testosterone while the high level of testosterone can maintain spermatogenesis process in spite of low level of LH (**Karthikeyan et al., 2009; Paul et al., 2010)**. Testosterone hormone cannot control alone the first initial wave of spermatogenesis. It has also been revealed the conversion of primary spermatocytes to prophase and metaphase based on testosterone (**Hedger and Kretser, 2000**). Testosterone also regulates the formation of primitive spermatogonia A from gonocytes and the reduction division of primary spermatocytes (**Hedger and Kretser, 2000**). LH influences the spermatogenic development when hypophysectomized immature rat was treated with androgen or animal exposed to estrogen followed by androgen treatment. It was found by Collins and others that testosterone treatment in hypophysectomized rats resulted in the progression of spermatid development up to 8-12 steps (**Collins et al., 1981**).

7.Oxidative stress and male infertility

Producing offspring is a part of living. Unfortunately about 15% to 25% of the couples are struggling for bearing child and they need medical advice for improving their chances of successful pregnancy **(Trussell, 2013)**. As per World Health Organization guidelines, the male factor is one of the causes of infertility directly associated with poor sperm concentration, motility **(WHO, 2010)**. This problem is further increased due unidentifiable causes. Currently, oxidative stress (OS) is believed to be an immerging factor and one of the probable causes of idiopathic male

infertility **(Saalu, 2010)**. Matured spermatozoa are known to be equipped with antioxidant systems and they can able to neutralize ROS and protect the sperm from oxidative injury **(Henkel, 2011; Henkel, 2011; Trussell, 2013)**.

Statistics of United States indicated that oxidative stress is a responsible factor for male infertility **(Lanzafame et al., 2009)**. Spermatozoa are reported to be susceptible with freer radical. Sometime the damage may be restored by antioxidant supplementation **(Bansal and Bilashpuri, 2011)**.

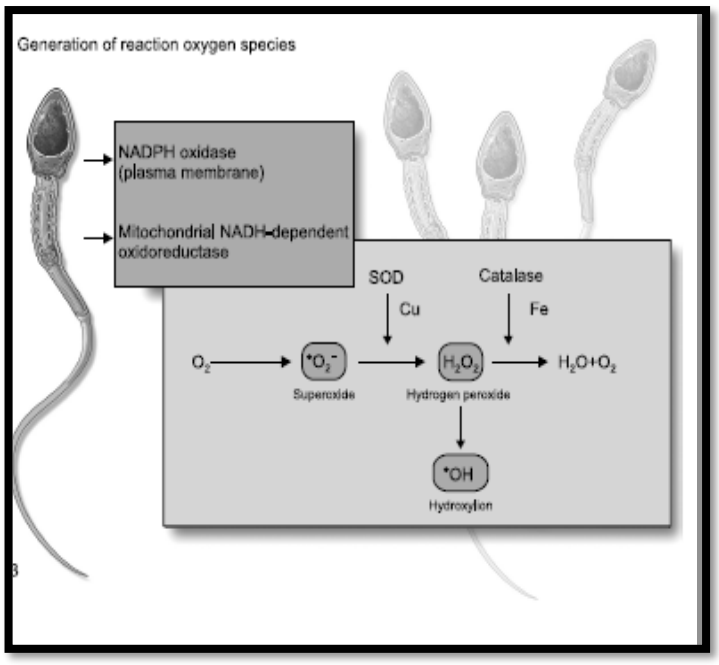
Subsequently, Due to LPO, a remarkable loss of intracellular adenosine tri-phosphate (ATP) has been marked which is associated with diminution in sperm viability, and decreased sperm motility **(Bansal and Bilashpuri, 2011; Gharagozloo and Aitken, 2011)**. Therefore, Now a days, OS has become a great concern for doctors and research personnel that directly affect the rate of pregnancy, neural tube defects etc **(Tremellen, 2008)**.

7.1 Reactive oxygen species and free radicals

ROS, also designated as free radicals which have one unpaired electron. They are the agents generated as byproducts from the metabolism of oxygen. They form highly reactive molecules due to the unpaired electron in the outer orbit **(Miranda-Vilela et al., 2010)**. There are broad range of radicals (e.g., hydroxyl ion [OH-], superoxide ion[O2 -], nitric oxide [NO], peroxyl [RO2], lipid peroxyl [LOO], and Thiyl [RS-]) and non-radical molecules (singlet oxygen [-1O2], hydrogen peroxide [H2O2], hypochloric acid [HOCL], lipid peroxide [LOOH], and ozone [O3]) which represents ROS (**Bansal and Bilashpuri, 2011)**.

7.1.1 Generation of reactive oxygen species

Current research has indicated that ROS causes leakageof the electron from activespermatozoa which were mediated by intracellularredox activities. There are twomethods which are responsible for the generation of ROS in spermatozoa(1) the nicotinamideadenine dinucleotide phosphate oxidase system at the levelof spermatozoal membrane and/or (2) mitochondrial nicotinamideadenine dinucleotide-dependent oxido-reductasereaction. Spermatozoaare generally rich in mitochondria as constant supply of energyis needed for the motility of spermatozoa **(Henkel, 2011)**. Therefore, the presenceof non functionalspermatozoa in the semen remarkablyelevates the production of ROS, which in turn affectsmitochondrial function ultimatelysperm motility.The major ROS which is generated in human spermatozoa is$O_2^{-\cdot}$ This O_2^-, electron-reduced product reacts with itselfvia dismutation to generate H_2O_2. This H_2O_2 and O_2^{-} undergo the Haber-Weiss reaction to generate extremely reactive as well as destructive OH^- in presence of transitionmetals such as iron and copper, (Fig. 4).Normal sperm function can be lost in case of the generation of OH^- radicals which are exceptionallypotent initiators of the LPO cascade**(Sikka, 2001; Chen et al., 2013)**.According to a recent



report, the production of O_2^- in spermatozoa explored that the presence of a calcium-dependentNADPH oxidase called NOX5 (encoded by the NOX5gene) has been estabilshed within the particular region specially acrosome and midpiece of human spermatozoa **(Sabeur and Ball, 2007)**.NOX5 gene was initially detected in the testis of human and is activated during Ca_2 binds with cytosolic N-terminal EF-handdomain. This binding generally brings conformational changes tothe cellular integrity by inducing OS. This type of finding provides furthersupport that NOX5 gene is a major source of ROS human spermatozoa**(Chen et al., 2013)**.

Figure 1.4: Generation of reactive oxygen species in human spermatozoa. NADPH: nicotinamide adenine dinucleotide phosphate, NADH: nicotinamideadenine dinucleotide, SOD: superoxidedismutase, Cu: copper,Fe: Iron.

7.1. 2 Sources of reactive oxygen species in seminal plasma

There are various endogenous and exogenous sources which are responsible for the generation of ROS in spermatozoa. The human ejaculatessemen which consists of different types of cells including mature, immature cells, round cells from different stages of spermatogenesis, leukocytes and epithelial cells. Immature spermatozoa and leukocytic cell mainly neutrophils and macrophagesare considered as the main endogenous sources of ROS, whereas several factors such as smoking, alcohol consumption, pollution and different environmental factors such as radiation and toxins can contribute to exogenous sources of ROS **(Esteves, 2002**;**Choudhary et al., 2010)**.

7.1.2.1 Endogenous sources of reactive oxygen species

❖ Production of immature spermatozoa

At the time spermatogenesis, developing spermatozoa extrude their cytoplasm in order to ready for fertilization. But in case of damaged spermatozoa which retain excess cytoplasm around the midpiece due to cessation spermiogenesis. This condition is designated as excess residual cytoplasm (ERC). NADPH system was activated byERC that is the hexose-monophosphate shunt, which spermatozoa use as a potent source of electrons for producing ROS and ultimately leads to OS**(Rengan et al., 2012)**. Moreover, ERC affects different spermiological parameters such as motility, morphology, and fertilization potential, which may lead to male infertility **(Hampl et al., 2012)**.

❖ Leukocytes

Peroxidase-positive leukocytes include about 50% to 60 % of polymorphonuclearleukocytes and 20%-30%macrophages **(Saleh et al., 2003)**. These huge proportions of these peroxidase-derived leukocytes originate from theseminal vesiclesand prostate also. When the ROS are activated and generated by various different intracellular or extracellular stimuli, such as infection or inflammation, they can discharge up to 100 times more ROS than normal and NADPH production via the hexose monophosphate shunt is being increased **(Lavranos et al., 2012)**. An increase level of pro-inflammatory cytokines, interleukin (IL)-8, and a decrease in the activity antioxidant, superoxide dismutase (SOD) can result in a respiratory burst which produces of high levels of ROS, and ultimately leads to oxidative injury. This oxidative injury causes sperm damage if seminal leukocyte concentrations are abnormally high in the case ofleukocytospermia**(Lu et al., 2010)**As per the report generated by World Health Organization that per millilitre of semencontains one million peroxidase-positive**(WHO, 2010)**. Now a day's extensive research has been

carried out to establish a link between the presence having leukocytes in the semen and a male factor as the cause of infertility.Various studies focus a correlation between decreasedsperm function and seminal plasma with the abnormal elevation in the level of ROS, IL-6, IL-8, and tumor necrosis factor (TNF),which result LPO**(Lavranos et al., 2012)**.

❖ **Varicocele**

Varicocele is a condition which defines as an abnormal dilation of veins inthe pampiniform plexus around the spermatic cord. It is detected in about 40% of male partners of all infertile couples that is considered the leading and responsible factor for the development of maleinfertility**(Will et al., 2011)**. It has been exposed that the level ofseminal ROS depends on the grade of varicocele;that is, the higher the grade of varicocele is, the greater isthe level of ROS detected **(Shiraishi et al., 2012)**.

7.1.2.2 Generation of reactive oxygen species: Exogenous sources

➢ **Radiation**

Radiation is a natural source of energy. It has significant clinical and detrimental effects on human body. Several studies have yet been implicated about the effect of radiation on human body.Mobile phone generates radiationwhich helps to increasethe production of ROS in human semen with impaired semen quality **(Aitken et al., 2005**;**Agarwal et al., 2008)**. A report has been published on *in-vitro* studies which demonstrated that generation of electromagnetic radiation induces ROS production which causes damage in human spermatozoalDNA.Radiation induces ROS further decreases the vitality and motility of sperm cells as their concentration depends on the duration of radiation exposure**(De Iuliis et al., 2009)**. This type of radiofrequency

generation can negatively affect the electron flow in the internal membranes of the cell which result generation of numerous charged molecules within the cytosol, thus hampersthe normal cellular and organelle function **(Lavranos et al., 2012)**.

- **Smoking**

At present tobacco is one of the major preventablecauses of death. Cigarettes generally contain more than4,000 chemical compounds. Itincludes nitrosamines, alkaloidsand some inorganic molecules. Among them some of the chemicalsshowedan imbalance between ROS and antioxidants defencein the semen of smokers **(Lavranos et al., 2012)**. This ROS and antioxidantdisparity affects the overall semen quality.A recent study indicated that smoking has been shown to result about 48% increase inthe level of seminal leukocyte and about 107% increase inseminal ROS levels **(Saleh et al., 2003)**. Moreover, it is being noticed that smokers have decreasedactivities of the antioxidants such as vitamin E and vitamin C in seminal plasma. This said finding has been confirmed by a significantincrease in the levels of 8-OHdGin the seminal plasma of smokers which is the another biomarkerfor the assessment of oxidative stress **(Esteves, 2002)**. A comparative study has been carried out on the semen profiles of smokers versusnon-smokers which showed that spermatozoa from smokers weresignificantly more sensitive to acid-induced denaturation of nuclic acid than those of non-smokers and also resulted in higher levelsof DNA strand breakage **(Jarow, 2003)**. There is an another study which performed onsmokers estabilished that the increasedconcentrations of heavy metal such as cadmium and lead in their blood and semen led to enhance the production of ROSwith an accompanying decrease in sperm motility**(Kiziler et al., 2007)**. Furthermore, it was again proved that prolonged exposure oftobacco smoke is closely

linked to increase production in sperm which leads to DNA damagethrough apoptosis and ultimately increase the chances of male infertility.

- **Alcohol consumption**

Alcohol can be actesas promoter of ROS production and hampersthe body's antioxidant defencesystem, particularly in the liver. There are many factors involved in causing alcohol-induced OS. Acetaldehyde which is the by-products of ethanol metabolism interacts with lipids and protein and ultimately ROS is formed. This causes damage to proteins, lipids, and DNA. Therefore, excessive alcohol consumption showed decreased percentage of normal spermatozoa in asthenozoospermic patients **(Agarwal and Prabhakaran, 2005)**. A study indicatedthat 46 alcoholic men with reproductive age showed significant increase in serum LPO by-products and a decrease in antioxidants which is providing the further evidence of alcohol-induced OS in testes **(Saalu, 2010)**.

- **Toxins**

Toxins generate from industrialproducts and structural materials accumulate in the human body which increases ROS production in the testes. It creates negative impacton thestructural integrity of sperm and function**(Esfandiari et al., 2002)**. Plastic materials which are largely used in domestic or industrial purpose as well produces ROS which leads to oxidative damage**(Pant et al., 2008)**. From another study it has been found that plastic materials impair spermatogenesis and causeDNA damage in spermatozoa **(Kasahara et al., 2002)**. Moreover, it was being noticedthat the workers who were regularly dealing with toxinsin the form of metals such as

chromium,cadmium, lead,manganese, and mercury were more likely to have decreasedsperm quality such as count, volume, and density **(Jurasović et al., 2004)**.

7.1.3 Pathological role of ROS

7.1.3.1 Lipid peroxidation

Lipids are very much essential for the fluidity of membrane layers and the changes which occur at the time of capacitation in the female reproductive tract **(Sanocka and Kurpisz, 2004)**. Plasma membrane of spermatozoa in mammals is significantlydiffering from mammalian somatic cells in terms of the composition of lipid. The plasma membrane of spermatozoa contains high level of poly unsaturated fatty acid (PUFAs) which contain unconjugated double bonds separated by methylene groups. The placement of a double bond that is adjacent to a methylene group weakens the methyl carbon-hydrogen bond, consequently making hydrogen extremely susceptible to abstraction and oxidative injury. The levels of ROS within the cell are high which will attack PUFAthat produces a cascade of chemical reactions called LPO **(Makker et al., 2009)**. Spermatozoa which contain 50% of the fatty acids in human are composed of DHA having22-carbon chains and six *cis*double bonds. DHA is playing a major role in connection of the regulation of spermatogenesis and membrane fluidity **(Aitken et al., 2010)**. If LPO cascade occurs in the sperm, 60% of the fatty acid is lost from the sperm membrane. It affects its function by decreasing fluidity, and increasing non-specific permeability to the ions and inactive membrane-guided receptors and enzymes. Since LPO is an autocatalytic self-propagating reaction which may be developed with abnormal fertilization, it is very critical to understand the mechanism behind the entire process, which can be easily separated into three main steps, generally called initiation, propagation, and termination **(Tremellen et al., 2008)**.Initiation defines the abstraction of hydrogen atoms which is

associatedwith carbon-carbon double bonds that resultin the free radical production. This newly form free radicals react with fattyacid chains and produce lipid radicals. This lipid radical reactswith oxygen to form peroxyl radicals. These peroxylradicals can abstract hydrogen atom from lipid molecules in presence of metals such as copperand iron. This ion causes an autocatalytic chain reaction. The radicalseventually react with hydrogen to form lipid peroxides**(Saalu, 2010)**. These radicals act on lipids which form cytotoxicaldehydes caused byhydroperoxide degradation.In the propagation stage, peroxyl and alkyl radicals are regenerated in a cyclicalstep until they react with each otherto form a stable end product called malondialdehyde(MDA). Thus,MDA is used in biochemical arrays to monitor the degreeof peroxidative damage to spermatozoa **(Sanocka and Kurpisz, 2004)**. There is anotherbyproduct of LPO namely 4-hydroxynonenal. It is producedfrom low-density lipoproteins. Hydroxynonenals are hydrophilicand can cause severe cell dysfunction at both genomicand proteomic levels **(Hampl et al., 2012)**.

7.1.3.2 ROS induced DNA damage

Spermilogical parameters such as concentration, motility, viability and morphology are used to determine the potentiality of sperm in fertilization from ejaculate. Though it provides an overview of the quality of sperm but it does not provide any information regarding the genetic materials of sperm **(Zribi et al., 2011)**. From a study, it was reported that chromatin in the sperm nucleus is susceptible to oxidative damage. It leads to DNA fragmentation and base modifications **(Zribi et al., 2011).** During the process of spermiogenesis, sperm chromatin undergoes a series of

modifications in which histones are replaced with transition proteins and subsequently.

This DNA compaction and organization helps to protect the sperm chromatin from oxidative injury when making them particularly resistant to DNA damage **(Hampl et al., 2012)**. DNA is more susceptible and vulnerable to OS which actually produces base-free sites, deletions, frame-shift mutations, chromosomal rearrangements and DNA which is observedobserved in testicular, epididymal, seminal vesicle and ejaculated human spermatozoa **(Kemal et al., 2000)**. Terminal uridine nick end labelling or comet assay are used to detect single- and double-stranded DNA breaks.. Single-strand breaks of DNA results oxidative damage on sperm DNA, whereas double-strand breaks may arise from exposure to 4-hydroxyl-2-nonenala, a major product of LPO **(Badouard et al., 2008)**. In human spermatozoal DNA there are two DNA adducts such as 8-hydroxy-2-deoxyguanosine and ethenonucleosides (1, N6-ethenoadenosine and 1, N6-ethenoguanosine), the two major found in human sperm DNA considered as key biomarkers ofDNA damage due to OS **(González et al., 2012)**. Despite of these findings,DNA damage is not a cause for concern during intrauterine insemination and in vitro fertilization (IVF), because the coexisting LPO damage by ROS eliminates the possibility of fertilization. However, if normal natural selection is bypassed during intracytoplasmic sperm injection (ICSI), sperm with significant amounts of DNA damage have the opportunity to fertilize the oocyte **(Makker et al., 2009)**. When DNA damage occurs minimally, spermatozoa can undergo self-repair and potentially regain the ability to fertilize the oocyte and proceed with the further development **(Aitken and Koppers, 2011)**. Moreover, the oocyte which is also capable torepaire damaged sperm DNA. In other cases, before the initiation of the first cleavage division,the oocyte can successfully repair sperm

DNA-strand breaks and therefore producing normal offspring. From a report Ithas been revealed that 80% of the structural chromosomal aberrations are of paternal origin in humans **(González et al., 2012)**. DNA damage is a contributory factor to apoptosis, poor fertilizationrate, high frequency of miscarriage, and morbidity in offspring **(Chen et al., 2013)**.

7.1.3.3 ROS induced apoptosis in germ cell

Apoptosis, programmed cell death, in biology, a physiological mechanism that allows cells to self-destruct that cause cells to die in a controlled manner **(Makker et al., 2009)**. There is anestablished theory regarding DNA breakage of sperm and impaired fertilization due to unsuccessful apoptosis. At the time of early development, apoptosis is very essential and vital also in the ontogeny of the germ line cellsthat means of regulating the ratio of germ cell to Sertoli cell. In maturity, apoptosis plays a crucial role in destroying the premeioticspermatogoniaselectively during the first phase of spermatogenesis by resisting the overproduction of germ cells from seminiferous tubules in respect of the response to ROS **(Tremellen, 2008)**. During the process of initiation, the human individual ejaculate expresses different apoptotic markers which are responsible for the initiating apoptosis. These markers are some of which include Fas, phosphatidylserine (PS), Bcl-Xl, and p53. Fas is designated as a type I membrane protein which belongs to the family of tumor necrosis factor-nerve growth factor receptor. It is secreted by the Sertoli cells located specially on surface of germ cell **(Agarwal et al., 2008)**. To further support this theory in this aspect, the similar study reported that the percentage of Fas-positive spermatozoa showedabnormal sperm parameters about the50% in men **(Agarwal et al., 2008)**. Additionally, this pathway of apoptotisiactivates the mitochondrial (inner and outer) membranes to release of the signaling molecule cytochrome C through permeability

transition pore.It triggers different caspasesvizcaspases 3 and 9, and annexin- V binding (Annexins are calcium-mediated phospholipid- binding proteins that binds with PS). This pathway mainly leads to apoptosis of thesperm**(Aitken and Baker, 2013)**. For the detection of early apoptosis it was reported that in a study that annexin-V staining was used here to study the externalization of PS−a marker. From a study, it was revealed that spermatozoa from infertile patients with increased ROS levels showed significantly higher levels of apoptosis in respect of mature spermatozoa from the control individual**(Agarwal and Said, 2003)**.

✓ **Mechanism of oxidative stress induced germ cell apoptosis**

Spermatogenesis is a dynamic andsynchronized process of maturation ofspermatogonia into mature spermatozoa which takes place in the seminiferous tubules.This process generally involves mitotic development of spermatogonia and its differentiationinto spermatocytes followed by the development of spermatids and maturespermatozoa. According to the Russell et al (1999) the number of cells present in the seminiferous tubules which is determinedby aactive balance between apoptotic cell death and cell proliferation **(Russell etal., 1999).**

➢ **Germ cell apoptosis via intrinsic or mitochondrial pathway**

In the mitochondrial pathway, proapoptotic members of the Bcl2 family such as Bax,Bak, or Bid that locates to the mitochondrial membrane and are involved in themitochondrial permeability transition pore (mtPTP) formation **(Gulbins et al., 2003;Green and Kroemer, 2004)**. Opening of the mtPTP damages the mitochondrialmembrane potential, causing mitochondrial swelling, ion equilibration and release ofproteins, including cytochrome c, from the intermembrane space into the cytosol**(Wallace, 2005).** Cytochrome c leads to form an apoptosome, which

activatescaspase-3 and ultimately results cell apoptosis. There are a number of othermechanisms, aside from activation of the death receptors, through which the caspasecascade can be activated. Antiapoptotic members of the Bcl-2 family (Bcl2 and Bcl-XL) seizes Bax, Bak, and/or Bid in the cytosol, by this way, translocation of theseproapoptoticsignaling molecules to the mtPTP is prevented and inhibits apoptosis.

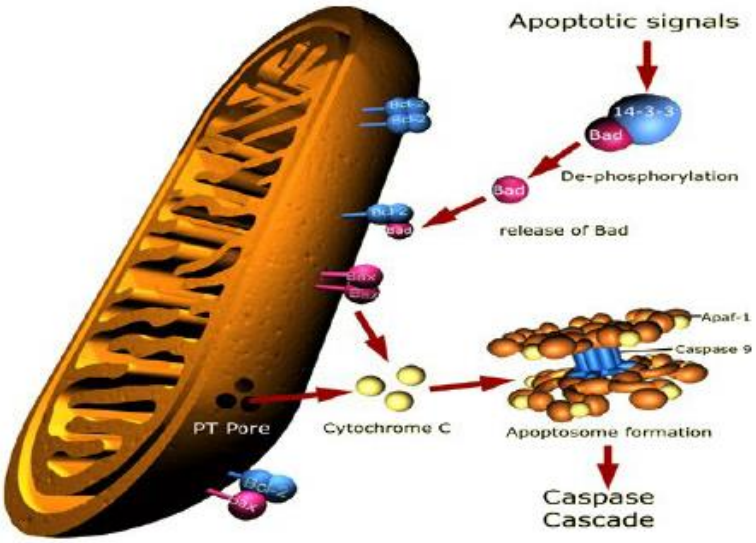


Figure 1.6: Apoptotic mitochondrial pathway (signalling pathway) for the activation of caspase cascade system

In death receptor pathway, the death receptor (e.g., Fas) binds with a ligand (e.g., FasL), which results activation of a caspasesignaling cascade to induce cell death **(Timmer et al., 2002; Wallace, 2005)**. The extrinsic pathway is activated upon ligation of the cell surface death receptor(s), that activates downstream effector mechanisms orchestrated by the caspase family of cysteine proteases **(Green, 2004).**The extrinsic signaling pathway leading to apoptosis involves transmembrane death receptors that are members of the tumor necrosis factor (TNF) receptor gene

superfamily. Members of this receptor family bind to extrinsic ligands and transducerintracellular signals that ultimately result in the destruction of the cell **(Elmore, 2007)**. The most well characterized ligands of these receptorsto date are FasL, TNF-alpha, Apo3L, and Apo2L. Corresponding receptors are FasR,TNFR1, DR3, and DR4/DR5, respectively **(Roy and Nicholson, 2000; Ashkenazi etal., 2008).** Molecules that stimulate the activity of these pro-apoptotic proteins oractivate these receptors are currently under evaluation for their therapeutic potential inthe treatment of cancer, including hematologic malignancies. The signal transductionof the extrinsic pathway involves several caspases which are proteases with specificcellular targets. Once activated, the caspases affect several cellular functions as part ofa process that results in the death of the cells **(Ashkenazi et al., 2008).**

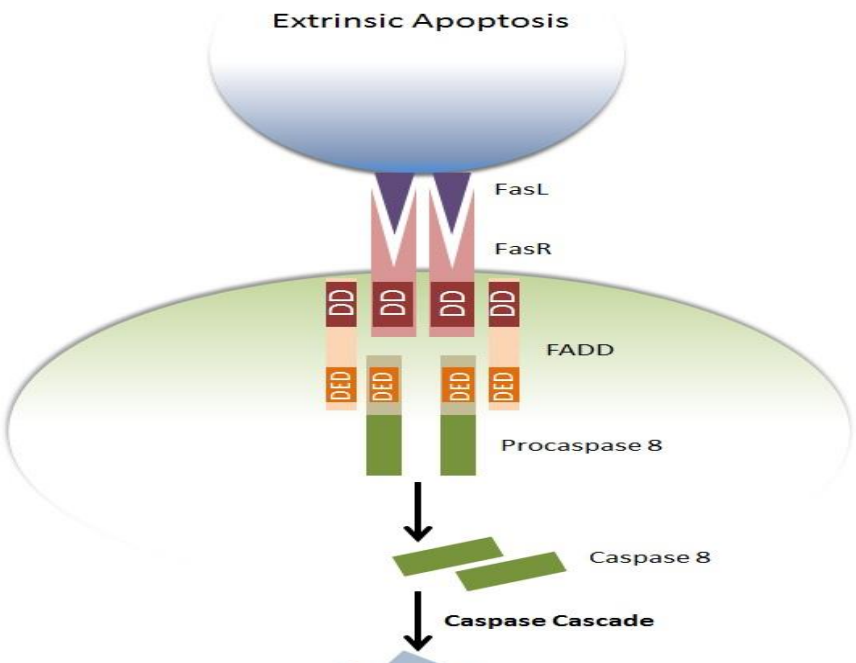


Figure 1.7: Extrinsic pathway for maintaining programme cell death

8.Management of oxidative stress induced male infertility

8.1 Prevention of oxidative stress

Two main mechanisms protectDNA of spermatozoa from OS. First, the genetic materials DNA is coiled tightly and packaged into chromatin so that the genetic material is not fully or minimally exposed to ROS **(Lampiao, 2012)**. On other hand, seminal plasma contains natural antioxidants and so spermatozoa assistto minimize the production ofROS towards normal levels. There are some natural antioxidants such ascatalase, peroxidise and SOD and some non-enzymatic compounds like vitamins C, E and carotenoids. These antioxidants help to scavenge ROS production and prevent the onset ofOS and keep intact thefunction ofspermatozoa **(Sharma, 1996)**.Mature spermatozoa contain lactoferrin, the antioxidants and coenzyme Q10 **(Lanzafame et al., 2009)**. Aadequate amount of antioxidants have to be consumed in diet to prevent OS from onset **(Lanzafame et al., 2009)**. Moreover in case of some patients who actually suffer from male infertility,there might be either an

overproduction of ROS or less production of antioxidants, which hampers as well as disrupts the balance

8.2 Management of oxidative stress

In the management of OS, the first step to take is to ascertain the underlying cause of the imbalance and treat it **(Agarwal et al., 2004)**. For instance, chlamydia infections can be treated with antibiotics and anti-inflammatory medication, while varicocele can be corrected by surgery **(Tremellen, 2008)**. Thereafter, antioxidant treatment may be given to supplement the natural antioxidants and increase the ability of the seminal plasma to combat OS **(Agarwal et al., 2004)**. The following section explores the different methods, including lifestyle changes and antioxidant supplements, which can be employed to help reduce OS.

▪ Lifestyle changes

Modernization, affluence, and the accompanying stressesof the society have resulted in an increase in negative behaviors,including, but not limited to, smoking, substanceabuse, obesity, and an unbalanced diet. All these have beenshown to contribute to OS, and therefore, minimizing suchdetrimental behavior is likely to aid in alleviating OS **(Tremellen, 2008)**.It is also recognized that exposure to heat, pollution, toxins,and heavy metals play a role in the development of OS.In addition, any activity that may cause the scrotum's temperatureto increase, such as hot baths, saunas, extendedperiods of driving, and long and sedentary office hoursshould be avoided. Lastly, adequate protective equipmentand aeration should be ensured at work places to limit exposureto any chemical or vapor that may cause OS **(Tremellen, 2008)**.Undertaking these lifestyle changes can contribute tothe reduction of the ROS production and help correct theimbalance causing OS.

▪ Antioxidants

Another precautionary measure that can be taken is antioxidantsupplementation. Antioxidants work by haltingthe oxidative chain reaction-eliminating, taking up, or reducingthe formation of ROS **(Saleh et al., 2009)**. They can be divided into two types on the basis of their actions: (1) preventive antioxidantsare metal chelators or binding proteins, such aslactoferrin and transferrin, which prevent the formation ofROS; and (2) scavenging antioxidants, like vitamins C andE, remove the ROS that is already present **(Lampiao, 2012)**. There havebeen various studies conducted to elucidate the effectivenessof each individual antioxidant. However, resultshave been inconclusive as most experiments have a smallsample size, differ in dosage and duration of therapy, andlack controls **(Agarwal et al., 2004)**.Moreover, antioxidants work cooperatively, and thus, itis extremely challenging to measure the effect of any singleone alone **(Sharma and Agarwal, 1996)**. This is supported in theory because asuitable combination of antioxidants with their differentprofiles will neutralize any ROS in its vicinity, hence resultingin an additive effect on the decrease in the total OSlevel of the body **(Gharagozloo and Aitken, 2011)**. Antioxidants are categorizedas enzymatic and non-enzymatic. Some enzymaticoxidants, or natural oxidants, include glutathione reductase(GSH), SOD, and catalase, while some non-enzymaticoxidants include vitamins C, E, and B; carotenoids;carnitines; cysteines, pentoxifylline, metals, taurine, hypotaurine,and albumin **(Bansal and Bilaspuri, 2011)**. The non-enzymatic oxidantsare acquired from fruits or vegetables containing the supplements**(Lampiao, 2012)**.

▪ Enzymatic antioxidants

➢ Glutathione reductase and glutathione peroxidase

GSH/glutathione peroxidases are the main reducing agentsin the body and act as scavenging antioxidants in the epididymisand testes **(Mora-Esteves and Shin, 2013)**. Their modification of the spermatozoamembrane confers protection on the lipid constituents,thus preserving sperm viability and motility **(Lanzafame, 2009)**.Previous *in vitro* studies have shown that GSH preservesthe tail-beat frequency, reduces LPO, and improves thesperm membrane characteristics **(Griveau and Le Lannou, 1994)**.

- **Superoxide dismutase and catalase**

SOD protectssperm from superoxide anions by catalyzing the conversionof superoxide into oxygen and H_2O_2, thereby preventingLPO and improving motility **(Agarwal et al., 2004)**. On the other hand, catalase aids in the decomposition of H_2O_2 into waterand oxygen **(Mora-Esteves and Shin, 2013)**. Thus, both SOD and catalase assist inremoving ROS that has the potential to damage sperm.

- **Non-enzymatic antioxidants**

  - **Vitamin E**

Vitamin E (α-tocopherol) is a chainbreakingantioxidant found in the sperm's cell membraneand acts by neutralizing H_2O_2 and quenching free radicals**(Bansal and Bilaspuri, 2011)**, hence halting chain reactions that produce lipid peroxidesand protecting the membrane from the damage induced by ROS **(Lampiao, 2012)**. Furthermore, it improves the activityof other scavenging oxidants **(Mora-Esteves and Shin, 2013)**. In these ways, vitaminE helps to preserve both sperm motility and morphology**(Agarwal and Nallella, 2004)**.

  - **Vitamin C**

Vitamin C (ascorbate) is another chain-breaking antioxidant that plays a significant role (upto 65%) in combatting OS in the seminal plasma **(Sharma and Agarwal,**

1996). It reactswith OH-, O2-, and H_2O_2 in the extracellular fluid,thus protecting sperm viability and motility **(Lampiao, 2012)**.However, vitamin C is only a weak ROS scavenger in thecell membrane and, hence, has almost no effect within thecell **(Lanzafame et al., 2009)**.

▪ Nutritional therapy

The word "Nutraceutical" was coined in the 1989 from "nutritional" and "pharmaceutical" terms, and it is today used to indicate food, or part of the food, which provides important benefits for human health, as prevention and/or treatment of disease**(Kalra, 2003)**. In recent years, especially in industrialized countries, this concept has received an important boost towards the development of new therapeutic opportunities that can improve patients' health, by the contemporary identification of unbalanced food regimens. Poor heterogeneous diets, caused by environmental factors such as air pollution, stress, chemicals and other toxic agents in foods, have been considered in some studies as possibly responsible of the decreased male semen quality observed in the last 50 years**(Carlsen et al., 1992; Salas et al., 2017)**. Recently, other studies evaluated the effects of specific nutrients along with important nutritional supplements on inferlity of men **(Giahi et al., 2016)**.

Lycopene is one of the cyclic carotenoids naturally obtained from red fruits and vegetables. It has been searched to have the maximum rate of physical quenching constant with singlet oxygen and its plasma level is higher than β-carotene **(Di et al.,1989)**. The antioxidant features of lycopene plays a pivotal role in oxidative systems as an

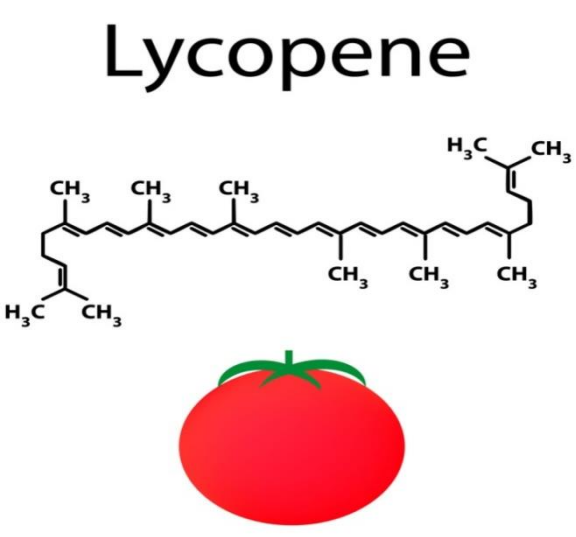


Figure 1.8: Chemical structure of lycopene

important anti radical antioxidant in the protection of lipid peroxidation **(Klebanov et al., 1998)**. Deficiency of lycopene has been associated with immune-infertility. A study demonstrated that an outstanding significant result was noted in lycopene level in the seminal plasma of immune-infertile men when compared with the fertile. The fertile men postulate a role for infertility management by inclusion of antioxidants in their diet **(Palan and Naz, 1996)**. Lycopene is naturally derived from red and orange fruits and vegetables.

It has been identified to have the maximum rate of ROS-quenching in plasma levels higher than β-carotene **(Klebanov et al., 1998)**. A high level of lycopene is found in the testes and seminal plasma but a low concentration is noticed in infertile men. Gupta and Kumar (2002) reported that a twice a day dose of 200 mg for 3 months resulted in a statistically significant improvement in sperm concentration of 66% of patients and motility of 53% **(Klebanov et al., 1998)**. Some experiments have been reported that lycopene is used for rmale inferlity management. Low semen lycopene levels with antibody –linked infertility compared to fertile controls in men.