Chapter 1 : General Introduction

1.1. HUMAN MALE INFERTILITY

Male Infertility can be distinct as the inability to fulfill pregnancy subsequent to a reasonable time of sexual intercourse without contraceptive measures taken, (Farkhunda et al., 2012). Male infertility is accountable for more than 40 % of infertility in the world, (Cuneyt et al., 1998). No precise reason can be found in about 10%-15% of infertile couples, (Murad and Devrim, 2009). Human male infertility is found to be the stressful issue to couple as well as their families. Under these circumstances, World Health Organization (WHO, 2010) has announced human male infertility as the global problem. WHO defines infertility as biological inability of a person to contribute to conception. Approximately 10–15 of every 100 couples reported to be incapable to produce offspring. Among these, it is estimated that in about 30–40% of these cases, defects were identified among the males. Almost 30 to 50% of infertility in the couples is due to male factor and it is due to the deficiency in the sperm quality and quantity. Sperm quality represents the total number of sperm present, total motile sperms, total rapid progressive motile sperms and total number of morphologically normal sperms present in an ejaculate. The quantity represents the total volume of the ejaculate. (Adamopoulos and Deliyiannis, 1983).

1.2. HUMAN SEMEN

Male contributes semen for the conception. Human semen consists of seminal plasma and spermatozoa and is secreted by various glands like prostate, seminal vesicles, testes and many accessory glands. Reproductive fluids (semen in humans) of different mammals exposed that the existence of multilamellar vesicles and thought to contain much protein, ions and high cholesterol to phospholipid ratio. (Mann and Mann, 1981).

Seminal fluid is secretions from several glands, comprises of several organic and inorganic compounds includes free amino acids, proteins, lipids, fructose, glucosidase and its derivatives, zinc and other microelements that includes Mg$^{2+}$, Ca$^{2+}$, K$^+$, Na$^+$. (Elzanaty et al., 2004).
1.3. HUMAN SEMINAL PLASMA (HSP) PROTEINS

Some proteins present in the seminal plasma were bound to spermatozoa, to quantify the overall concentration of the protein present in an ejaculate is to measure the concentration of protein in each fraction of the ejaculate. Human seminal plasma was found to be the most protein rich fluid in the body compared to any other fluid or compartmental portions of the body. The different fractions separated from human semen were spermatozoa, cellular debris, prostasomes, epididymosomes, and seminal plasma. (Arientia et al., 1999). The maximum concentration was found to be in the seminal plasma which was mentioned by number of researchers who were working with biochemistry of human semen. The quantity of protein in different fractions was varying in different categories of male infertility, when compared to the normospermia and control samples. In recent years, many proteins present in seminal plasma were recognized and characterized, and particularly epididymal proteins were categorized which has identified as fertility associated proteins. Prostasomes were identified in the year 1978, which was membranous in nature, isolated at very higher RPM, with low temperature. Prostasomes were secreted by prostate gland into the seminal fluid, finally isolated at 80000 rpm.

It has been demonstrated in human sperm surface that property of the ejaculated spermatozoa surface is coated with a number of seminal plasma proteins. These surface proteins have various biochemical activities such as haemagglutination, heparin-binding or zona pellucida-binding. Majority of these proteins are 12–16 kDa. Glycoproteins that bind to the sperm surface which helps in acrosome reaction. Human seminal plasma (HSP) contains a family of major proteins designated HSP-A1/A2 and HSP-A3 with molecular masses ranging from 15 to 16.5 kDa and HSP 30 kDa with an approximate molecular mass of 28–30 kDa. These proteins, collectively called human seminal plasma proteins (HSP proteins), bind to the sperm surface and modulate sperm functions, (Asadpour et al., 2007). These proteins are important in proper management of sperm-oocyte binding and motility. In recent years, various proteins from the seminal plasma have been identified, isolated and characterized. The protein composition of the seminal plasma is found to be different in different species and is associated with the fertility and thought to increase the semen quality.
There are number of glycoprotein responsible for the sperm-egg interaction. The cascade of reactions starts with the cell-specific binding site at the surface of the zona pellucida (ZP) and the complementary receptor on the sperm plasma membrane should recognise. This results in the recognition of the sperm and egg which ultimately result in the acrosomal reaction following the penetration of the sperm into the oozyte.

1.4. SEMINAL FRUCTOSE, GLUCOSIDASE AND MICROELEMENTS

Other than protein, the major components of the seminal fluid were fructose, enzyme glucosidase especially neutral α-glucosidase, and microelement in particular Zn. Fructose was secreted by seminal vesicles, glucosidase was secreted by epididymis and Zn with other microelements was secreted by prostate gland. The presence of sugar in human semen was identified in early 1930, finally after a decade, researches were started working with the entire biochemistry of human semen, finally identified that the major sugar present in the human semen is fructose and not the glucose (Schoenfeld et al., 1979). Still many researchers arguing the concentration of the fructose varies from the person to person, and even to the same person at different time of ejaculation.

One can easily find the change in the biochemistry of his own semen sample at different time, even may be within a week. The major function of the fructose in human semen is to give energy acting as a motility factor; the forward progression of sperm inside the female reproductive tract is abundantly due to the presence of fructose. The perfect biomarker to check the function of the epididymis is to elucidate the concentration of glucosidase present in the seminal plasma, because the enzyme neutral α-glucosidase is purely secreted by only epididymis. (Garcia et al., 1992).

By elucidating the concentration of fructose, glucosidase and Zn in human seminal plasma, the biochemists can easily identify and diagnosis for the male infertility. These three parameters were used as the biochemical markers for male fertility. Fructose, glucosidase and Zn were the markers for the functions of the seminal vesicles, epididymis and prostate respectively, (Hamameh and Gatti, 1998).
There has been increasing interest in the evaluation of essential trace elements present in different concentrations in the human body fluids and their correlation to the human health. Trace elements play important roles in a number of body functions. All the essential trace elements have their own range of adequacy. Smaller levels result in various abnormalities because of their specific biochemical changes. The analysis of essential trace elements will serve two purposes: first, to determine the concentrations and profiles of the various trace elements, and, second, to determine and detect the presence of potential toxic metals. This raises serious concern about male fertility.

Zinc is an important micronutrient for human health. It is associated with many physiological functions, including its role in metalloenzymes that relate to intermediary metabolism. Zinc plays a major role in semen ejaculation as well as being a cofactor for the DNA-binding proteins with Zn fingers. Zinc is thought to be the part in superoxide dismutase for the repair of damaged DNA. It plays a major role in the transcription and translational process. Zinc in the seminal plasma stabilizes the cell membrane and nuclear chromatin of the sperm. The total content of zinc in human semen is very high and is found to have a critical role in spermatogenesis. However, there is controversy about zinc content and sperm quality. Zinc is thought to be one of the major factors that affect spermatozoa motility and morphology. It controls the effects by modulating the activity of the Ca\(^{2+}\) ATPase enzyme. Zn plays an important role in the development of testes and secondary sexual characteristics, and in a few sperm physiologic functions. Zinc acts as a growth factor, an immune-regulator, and a cryoprotectant with anti-inflammatory effects. Decrease in the concentration of Zn in the seminal plasma causes hypogonadism, decrease in the size of the testes, inadequate development of secondary sexual characteristics, and atrophy in somniferous tubules. Consequently, these result in the spermatogenesis failure.

Calcium is an important element for determining the sperm metabolism, sperm motility, sperm vitality, and acrosome reaction. Calcium plays a vital role in the regulation of the motility, chemotaxis, capacitation, and hyperpolarization. Magnesium is thought to be in high concentrations in the prostate gland and is released into the semen during ejaculation. Drastic reduction in the magnesium concentration in the seminal plasma will therefore result in male reproductive disorders (Kumosani et al., 2008).
Sodium is present in the seminal plasma of humans at higher concentrations. The development of apposite and steadfast analytical techniques such as atomic absorption spectroscopy enabled determination of multivariate elements in a much faster way. The objective of the current study is to analyze the effect of different trace elements in the human seminal plasma (Zn, Mg, Na, and Ca) and their correlation with different semen parameters, and to compare their correlations with different categories of infertile (oligoasthenozoospermia, asthenozoospermia, oligozoospermia, azoospermia, and normozoospermia) and fertile semen samples.

1.5. ANTIAOXIDANTS AND MALE INFERTILITY

Oxidative attack to spermatozoa in the ejaculated semen was the common problem for the motility associated issues during capacitating process. Seminal vesicles and prostate gland secretes the maximum quantity of the seminal fluid which contains antioxidants. Epididymis provides the most wanted antioxidant system that prevents the oxidative damage of spermatozoa in the ejaculated semen.

The total antioxidant system in the seminal plasma was due to the presence of many antioxidants like catalase, SOD, and glutathione, which presents in the seminal plasma, secreted by various accessory sex organs (Griveau and Le Lannou, 1997). This study will be useful in comparing the antioxidants and the secretory capacity of the epididymis between the low range and high range of sperm count. Always, there will be a positive correlation between the antioxidants in the seminal plasma and the total number of spermatozoa present in the ejaculated semen. Epididymis has the capacity to self synthesize the extracellular SOD and even catalase in a minimum amount which will be helpful in preventing the oxidative damage for sperm.

The discrepancy between the production of the reactive oxygen species and the total antioxidant capacity (TAC) in the seminal plasma leads to the male infertility. The amalgamated score for ROS and TAC will have a strong impact on male infertility rather than the single score of ROS and TAC. Spermatozoa and seminal plasma have the capacity to produce the antioxidants which will be helpful in counterattacking the harmful effects of the reactive oxygen species and free radicals. The balance between the production of ROS and antioxidants in the seminal plasma will be positively correlated with the sperm count, total motility and many seminal
parameters. The abnormal production of ROS and free radicals in the seminal plasma will lead to the impaired metabolism, low motility, morphologically abnormal sperms. The males whose spermatozoa produces excess amount of ROS was likely impregnate their wives during the sexual intercourse.

1.6. CRYOPRESERVATION OF HUMAN SEMEN

Cryopreservation is a technique by using that, semen can be preserved to sub zero temperatures, usually at \(-196\) °C. During this process the biological and chemical bustle of the semen in particular sperm, is paused until thaw process. The freezing of semen desires vitrification mediators that diminish wreck to the cells (spermatozoan) during the freeze and thaw process. Cryopreservation of semen is rife, because is flourishing for most species, including human semen samples, but for some species, nuisance of cryoinjury made it unsuitable and challenging. Challenge to perk up cryopreservation protocols have incorporated in changing freezing charge, thawing rates, varying the panorama and category of cryoprotective mediators, altering the temperatures and rates at which cryoprotective mediators are supplemented to and separated from the cells and by means of vitrification in the nonexistence of cryoprotectants, (Leibo et al., 2002). Using cryopreservation, the quality of the semen has been increased in the latest years, by which the achievement rate for the assisted reproductive techniques has increased in an agreed way. The freezing of semen was begun over 55 years and still the research and the focus was there on to increase the quality of the preservation techniques. All semen extenders were used for freezing at cold temperatures should have the following features (in spite of species):

1) endow with nutrients as energy resource;
2) defend from cold shock throughout cooling;
3) will have cryoprotectants to diminish the quantity of freezing damage to the cells present in the semen;
4) prevents the intensification of bacteria;
5) contains buffer aligned with destructive alter in pH; and
6) Being an osmotic pressure and concentrating the electrolytes.

A standard recipe is full of egg yolk, a sugar which will be metabolized and glycerol, and buffer to adjust the pH. Recently, the formula for preparing the standard
and modern extender is the basic of the first successful extender called BSA extender technique. Now by means of all the technical development, the recipes were modified from the standard BSA extender and varied from each species. The current focus of the research work is to prepare the best recipe for the human semen extension which will be suitable / apt to preserve the human semen samples for the assisted reproductive techniques. This will be very much useful to improve the success rate for human assisted reproductive techniques.

Therefore, in view of the development of novel approaches to male infertility, overall understanding of the biochemical and molecular composition and its role in regulation of sperm quality and enable to be potential human spermatozoa is highly desirable. With this view the following objectives designed and executed in this research work

In spite of availability of elucidated details of semen components, infertility in male is an ever increasing problem. Much more understanding of semen components, micro elements, seminal plasma proteins, proteins/factors associated with the development, maturation of spermatozoa, fertility efficiency and to prediction of lacking factors are all essential to alleviate the problem of infertility. In our current effort to design a medium which must preserve the efficiency / retainment of semen capacity of fertility, to identify fertility associated microelements and proteins, and to predict the causes of infertility using basic and common parameters. This study will enable us to understand and to predict the causes of infertility. Which intern required to alleviate the problem of human male infertility.
1.7. OBJECTIVES OF THE STUDY

To compare the semen parameters before and after freezing using different extenders

To evaluate the protein concentration of different fractions of semen among male infertile semen categories

To perform biochemical assays of semen to assess the function of the accessory sex organ and their malfunction leads to male infertile

To measure the level of antioxidants that determines semen quality (using standard antioxidant markers)

Efforts to identify the fertility associated protein(s) from human seminal fluid

To design, construct and standardize a suitable model(s) to identify causes of infertile semen using basic semen parameters by Artificial Neural Network (ANN) models