Chapter 2: Review Of Literature

Even though extensive development has been made toward understanding the semen biology and the sperm physiology, molecular and cellular stage are all essential to reveal pathology and extend the pinpointing and treatment methods, predominantly in infertile cases, (Ercan et al., 2010). Seminal plasma is a composite muddle that contains secretions of epididymis, testis prostrate, and other sex glands. It is thought that, the seminal plasma hold certain aspect that can alter the fertilizing knack of the sperm (Henalut et al., 1995). Seminal fluid is secretions from several glands, consist 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$^+$. 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, (Farkhunda et al., 2012).

2.1. CRYOPRESERVATION AND SEMEN EXTENDERS

The cryo-preservation medium compounds involved in the cryoprotective media has been divided into different groups, which thought to have incompatible in their mode of action. Many substances like DMSO, ethylene glycol, acetamide, propanediol, and methanol were added alongside with the glycerol, which all belongs to one group, will permeate into the cytoplasm of the sperm cells, (Holt, 2000). Much amalgam is being experienced for their efficiency as semen cryoprotectants, but so far much semen preservation protocols still support glycerol in the cryoprotective media, (Holt, 2000). In many recipes of the semen extender, other cryoprotectants were used, like dimethyl sulphoxide (DMSO) was if at all possible used for elephant semen preservation, (Jones, 1973). The protection and the preservative effects for the glycerol were arbitrated by its own colligative possessions; dejection in freezing endpoint and their subsequent lowering the concentrations of electrolyte, which will be obligatory during the process of freezing (Lovelock and Polge, 1954).

Many investigations were proved this particular role of glycerol (Mazur, 1984), but many researchers were arguing that this is not the only role of glycerol which protects cells during freezing, and in many research has been proved that
glycerol found to have some negative effects on the spermatozoa especially cytotoxicity. Some authors were arguing that glycerol was metabolized by many species includes human, boar and even bull spermatozoa, (Jones et al., 1979). Glycerol with its capacity while substrate, it altering the bioenergetic eminence of spermatozoa, conceivably snooping with the stability flanked by synthesis of ATP and its utilization (Hammerstedt et al., 1990), at this stage, during cooling process, the metabolic functions may be altered, which escort to the unfortunate activation of proteases and many enzymes includes phospholipases to the complete set of cell damage.

2.1.1. GLYCEROL TOLERANCE

Glycerol tolerance with its interspecific variation will be a noticeable study while preserving the human semen, some results suggests that glycerol tolerance is high in case many species during semen preservation. Some authors influenced that, when the concentration increases to 20 -25 % of glycerol in semen extender, there subsist the recovery of sperm motility, even though the fertility of the spermatozoa and their related questions were not answered (Johnston et al., 1993; Taggart et al., 1996). Also some researchers in conflict with others, studied and reported that above 2.3 % of glycerol concentration leads to the cell and acrosomal damage while semen preservation (Penfold and Moore, 1993).

2.1.2. pH MAINTENANCE

Maintaining the pH while preparing semen extender and preserving is most important in preservation of semen. Human semen pH while ejaculation was found to be between 7.2-7.8, this pH was due to the prostatic secretions which contribute to form seminal fluid. In order to maintain the same pH, that exist while ejaculation, the extender should be prepared in the pH of 7-8, but many authors were prepared the semen extenders with the slightly acidic pH of 6.8 and even 6.4 (Rota et al., 1995; Rota, 1998). Researchers were studied about the preparation of semen extenders with various pH, proved that maximum motility after post thaw were got with the pH 7-8.5 used (England, 1992).
2.1.3. BUFFERING AGENTS

Many comparative studies and progressive research has been carried out in the early 1960, the combination of 20% egg yolk, 2% sodium citrate, 1% glucose and glycine 0.8% with pH 6.6 was found to be optimized and successful semen extender for many species (Foote, 1964a). This particular recipe was used and was very successful in restoring the motility of the preserved sperm; also the post thaw motility for this particular extender was excellent. Further, the author has prepared 0.5M Tris-egg yolk-glucose extender with 10% of glycerol, and it was even flourishing than the first one without Tris buffer. The use of Tris buffered extender was more successful (Olar et al., 1989; Battista et al., 1989) for their achievement in the post thaw parameter analysis especially motility. Researchers found that extender with more than 55% of PIPES buffer in place of Tris buffer, the post thaw parameters was much more recovered and influenced, (Smith and Graham, 1984), which was argued as not victorious recipe for some researchers (Dobrinski et al., 1993). Researchers also transformed from tris buffer to citrate buffer, which was also yielding a reasonable result for post thaw motility. Semen extender containing skim milk along with standard Tris buffered-egg yolk-glucose-glycerol extender, which yield outstanding results for the post thaw semen parameters (Rota et al., 2000).

2.1.4. ENERGY SUPPLY

Lactose or glucose was the major source of sugar found in all commonly available semen extenders, major for human and bull semen extenders (Ponglowhapan et al., 2004). Many researchers had interest with fructose as a best source for sugar supplementation for human and ram, and bull semen extenders (Akeel et al., 2011). In contrast, many researches arguing that fructose was secreted by many accessory sex organs in male reproductive system, which in turn mixed with semen and ejaculated into female reproductive tract while getting together for various reasons; though it already exists in semen. Additionally, fructose plays a major role in the activation of sperm after ejaculation (Ponglowhapan et al., 2004). Many authors have prepared the recipe with either fructose or glucose for many species related semen extenders, (Niasari et al., 2006; Michael et al., 2009; Vanmathy et al., 2012; Khalifa et al., 2013).
2.1.5. ADDITION OF GLYCEROL AND EQUILIBRATION

Many researchers found that glycerol addition, cooling studies certainly not provide the tangible pre-freeze rate for cooling, unusually studies were available with only the time depleted for equilibration and cooling. This particular idea suggests that, actual cooling rate will have more influence on the post thaw semen parameters than the time for equilibration (Zindl et al., 2006). On the whole, the most favourable concentration of glycerol which added to prepare semen extenders is conciliation flanked by protective role and adverse effects.

2.1.6. ADDITION OF DETERGENT

The addition of sodium and amine lauryl sulphate to the semen extender, author found that the post thaw parameter results (Rota et al., 1995). The plasma membrane integrity and long-lasting motility was maintained perfectly by adding the detergent, during cryopreservation the addition of detergent to egg yolk extender, probably increases the protective effects (Pursel et al., 1978).

2.1.7. FREEZE-THAW MECHANISMS

Revelation of sperm cells to hyperosmotic, nonetheless unfrozen, departure of intracellular H₂O, possible influx of ions may occur subsequent to cell contraction, (Mazur, 1984). Thawing process engrosses a reversal of these properties, and the resultant inward water flux produces disruption of cell membrane. It has been recommended that an appeasement freezing rate exist where the destructive special effects of these two dissimilar sources of cryoinjury can be curtailed, (Watson, 1990).

Available literature reveals that all the protocols designed to choose the favour cryoprotective media is depends upon the trial and error method irrespective of the species and investigations. This is due to lack of clear investigations on the action of the cryoprotectants on individual species, many protocols has been created for designing the choice of cryoprotective media as well preparing the recipe for the semen extension.
2.2. PROTEINS IN VARIOUS FRACTIONS OF HUMAN SEMEN

Various accessory male sex glands were helpful in secreting the seminal fluid, among these the main role goes to the prostate gland, seminal vesicles and testes. As we know, human semen constitutes spermatozoa matured, composed of the seminal plasma secreted by various glands (Mann and Mann, 1981). There exists as many as proteins in human semen principally in seminal plasma, for which most of the protein with its structural and functional units were not studied and understood. Even the major proteins present in human seminal plasma were not characterized (Arienti et al., 1999).

A special type of proteins called prostasomes, because originating from the prostate gland, found to be membranous in nature with vesicles, identified in human seminal plasma. The major functions of these types of protein function were not yet studied and investigated in the current research of reproductive and fertility medicine (Stegmayr and Ronquist, 1982). The major function of the human seminal plasma lay with the presence of proteins, lipids and carbohydrates. The major function of the protein present in the human seminal plasma is to neutralize the acidic nature of the vagina during ejaculation, so far found to be the most wanted and necessary function for the fertilization (Prins, 1998; Lentner, 1981).

The other major function of the seminal plasma with its proteins is liquefaction within 30 min after ejaculation while masturbation as well as during intercourse into the female reproductive tract (Mann and Mann, 1981). With the persuade of androgens in males and unrevealed testicle issues, the major accessory sex organ for male reproduction so called epididymis secretes the section of proteins that play a vital role and in functions interacts with spermatozoa (Arienti et al., 1999, Cuasnicu et al., 2002). The golden part of the epididymis is distal cauda, which the masterpiece in secreting these types of protein. These epididymal intraluminal proteins play a critical role transport of sperm and its storage for maturation. A succession of modifications was done to the sperm here in epididymis by these proteins, finally accompanied with the forward motility (Cooper and Yeung, 2006).
The route of interactions between the sperm transportation, maturation and intraluminal epididymal proteins has been raised as an emerging research in the past and present decade (Dacheux and Dacheux, 2002). The attainment of the specific intraluminal protein from the epididymis is not an easy target, as there was no clear pathway, whereas it is useful in identifying the new proteins at subcellular level (Cooper et al., 1990a; Sullivan et al., 2007).

Epididymosomes, a group of protein similar to that of prostasomes, found in the epididymal fluid collected during the vasectomy reversal process for the infertile or accidental patients have similar functions like prostasomes which have prostatic origin (Frenette et al., 2005). Thimon et al., (2008) have studied the similarity of the prostasomes and epididymosomes at proteomic and quantification approach. Frenette et al., (2002) has prepared a model by using bovine, showed the multifaceted complex nature of the epididymosomes. These author further demonstrated that the phenomenon of co-incubation of the cauda epididymal spermatozoa and epididymosomes, showed some of the proteins present in epididymosomes transferred to the sperm cells. The properties of the protein transferred from the epididymosomes to the sperm cells were more saturable, more sensitive to the pH and temperature reliant, and the properties were very much efficient with the presence of the ions especially zinc.

It was very well conjectured that epididymosomes are concerned with the recovery of novel proteins, and thus proving it has a major role in the motility and in sperm maturation process (Sullivan et al., 2007). Additionally, epididymosomes and prostasomes have been characterized with the antibacterial and immunomodulatory activities which play a critical role in protecting the spermatozoa from free radicals and oxidative stress (Carlsson et al., 2000). In disparity, the many functions of the prostasomes and epididymosomes were not deliberated in any of the available literature.

Human semen can be fractionated by centrifugation at different speed of centrifugation (RPM) with different low temperatures (Arienti et al., 1999; Arienti et al., 1997). The quantification and the analysis of the different fractions collected from the human semen have its own importance and on the whole, have diagnostic importance. The major fractions were found to be spermatozoa, cell debris devoid of
cells and the fluid, prostatomes, epididymosomes and seminal plasma (Arienti et al., 1997). In many studies the concentration of the protein in various fractions of the human semen were done and correlated with the semen quality includes, sperm count, sperm motility, sperm normal morphology and pH (Vickram et al., 2012). The recovery of the purified prostatomes were high than the fraction without percol gradient (Arienti et al., 1999).

2.3. SEMINAL FRUCTOSE, GLUCOSIDASE AND MICROELEMENTS

In epididymis, spermatozoa are stored during the epididymal transit period followed by the spermatogenesis and maturation; during these process spermatozoa remnants immotile until the instance of ejaculation (Eddy and O’Brien, 1994). The volume of fluid, count of spermatozoa, concerto of seminal plasma were found to be the important parameters for normal sperm functions (Weiske, 1994). Seminal plasma, after all the cellular components present, various chemicals, enzymes and microelements were present, that have its own properties and assigned for a particular function in the male genital system. Spermatozoa are assorted with secretions of a variety of male accessory sex organs, and at that time motility was induced (Lindholmer, 1974). Accessory sex gland and their functions play a decisive role in proper functioning of sperm (Mann, 1964; Robert and Gagnon, 1996). It has been studied and reported that there is a correlation between the accessory sex organ secretions and the time of sexual abstinence (Cooper et al., 1993).

Various substances were identified in human semen, but fructose was found to be the most significant and correlated to many semen parameters. In the initial study conducted by many researchers (Ivanov, 1931; Huggins and Johnson, 1933). The biochemistry of the human semen, and concluded that there is a reducing substance called sugar, constantly believed that it was glucose (Ivanov, 1931; Huggins and Johnson, 1933).

Finally Mann (1946a) was proved that the reducing sugar present in the semen is fructose and he proved with a lot of chemical methods. With this demonstrated result, many research from this period started to work on the biochemistry of the fructose and its correlation to many semen parameters (Kimmig,
1956; Schirren, 1961). After this, researchers not only focused on the fructose concentration in different species, they almost focused on the relationship of fructose and semen parameters and the nature of definite endocrine glitches. Schoenfeld et al., (1979) reported that, fructose is mainly secreted by the seminal vesicles, and also a small quantity from the deferens ducts, which is helpful in metabolism of spermatozoa, maintaining the normal morphology especially the midpiece, motility inducing acting as energy source. The male infertile men with low ejaculated volume, there is an absence of fructose concentration, which indicates the seminal vesicle dysfunction (Aumuller and Riva, 1992). Low level of testosterone hormone in male infertile patients will have dysfunction in the seminal vesicles, will result in the low concentration of fructose in the ejaculated semen (Moon et al., 1970).

This initiative result helps in identifying the relationship between seminal plasma fructose level and androgen functions. Some studies correlated the fructose concentration with the sperm count, positively correlated, while for the sperm normal morphology it was negatively correlated (Phadke et al., 1973). Epididymis plays a major role in the spermatozoa maturation and functions in fertilizing capacity, many markers were available for the prediction of the epididymis functions, includes glycercyolphosphocholine, carnitine and glucosidase. When compared to all the markers available for the epididymal function, glucosidase play vital role and act as a perfect marker to elucidate the function of epididymis (Garcia et al., 1992).

Measuring the concentration of glucosidase will be helpful in localizing the site of male genital tract obstruction; majorly it will focus in identifying the partial obstruction in the epididymis (Guerin et al., 1986). Low level of glucosidase in the semen is allied defectiveness of sperm maturation and erectile dysfunction (Von der Kammer et al., 1991). The binding capacity between spermatozoa and zona pellucida, the relationship with glucosidase activity was studied, reported high level of glucosidase has strong relationship with this (Ben Ali et al., 1994). addition of glucosidase to the prepared sample for intrauterine insemination (IUI) samples yield good success rate for getting pregnancy (Milingos et al., 1996).

Zinc concentration in human seminal plasma acting as a major role in the physiology of the sperm like sperm maturation, encouraging the sperm motility, and sperm capacitating nature. Ionic environment and the nature of sperm as its own
influence on the sperm function (Hamameh and Gatti, 1998). Several trace elements were present in human seminal plasma and each and every element present have its own functions. All the trace elements present in human seminal plasma plays an important role in normal functioning of the sperm.

Intracellular calcium present in human semen in important for the sperm motility, metabolism of the sperm, marinating the normal morphology of the head of each sperm present in the ejaculated semen (Morton et al., 1974; Peterson and Freund, 1976; Warren et al., 1987). Semen has to be matured by various enzymes present in the semen, that all reactions were controlled and induced in the presence of the magnesium present in the seminal plasma which was initially secreted into the seminal plasma by the epididymis and other accessory male sex glands.

Magnesium acting a most important role in ejaculation during intercourse, magnesium supply energy and pressure to the semen to ejaculate into female reproductive tract (Omu et al., 2001). Initially magnesium was secreted by prostate gland and finally secreted into the seminal fluid, mixed with spermatozoa. The drastic change in the concentration of magnesium leads to the erectile and ejaculatory dysfunction (Edorh et al., 2003).

2.4. ANTIOXIDANTS AND MALE INFERTILITY

Oxidative stress at the cellular level turns out to be apparent, when the oxidants present in the semen or spermatozoa devastate the overall antioxidant defence arrangement in spermatozoa. The remaining oxidants play a major role in unspecific chemical reactions with in close proximity of cells in particular with DNA, protein and some lipids especially unsaturated. Oxidative stress to the spermatozoa may occur due to the squat level of enzymatic and non-specific enzymatic antioxidant defence molecules.

Oxidative stress leads to many major diseases, which remains untreated by any research, particularly cancer, diabetes, heart diseases, and finally male and female infertility. According to the report of Scopus database, (2013 and 2014), 377 articles were reported with the oxidative stress related male and female infertility. Fortuitously, it has been reported first time that spermatozoa were vulnerable to oxidative stress. Authors established the spermatozoa oxidative damage and loss of
motility when the semen sample was preserved with the oxygen rich atmosphere. When the same sample was preserved with the antioxidant catalase, he was able to recover the motility of spermatozoa, this was the first hypothesis proved, later many researches has been carried out to establish further in the antioxidant capacity of spermatozoa in both preservation and insemination steps (MacLeod, 1943).

There exists a patho-physiological consequence in the nature of various types of male infertility, when the reactive oxygen species present in semen has been recognised as significant (Aitken, 1994; Iwasaki and Gagnon, 1992; Zalata et al., 1995a). Per-oxidation of many unsaturated fatty acids occurs due to the oxidants i.e. free radicals interference in the seminal plasma and it plays negative role in normal functioning of the sperm especially in the sperm plasma membrane (Aitken and Clarkson, 1987). This unsaturated fatty acids having the capacity to engender the ROS into the seminal plasma and crating the oxidative stress, which cannot be solved by the sperm on its own, if antioxidant defensive system not working well (Aitken and Clarkson, 1987). The chemical and structural modifications in the sperm nuclear DNA, and the modifications in the lipids, protein structure and functions were mainly due to the increase in the content of the free radicals in the seminal plasma (Jones et al., 1979). Many antioxidant defensive mechanism were available for the spermatozoa, which is due to presence of catalase, glutathione, superoxide dismutase, and the lipid peroxidise in the human seminal plasma, helps in protecting the spermatozoa from the cellular oxidative damage and stress related damage (Parviz and John, 2011).

Prominent ROS level were detected in 20-45%, and in case of spinal cord injured male patients 98% of samples showed elevated levels of ROS (De Lamirande et al., 1995). In the ejaculated volume of semen, there exists some 50000 leukocytes on an average and leukocytes are having the capacity to induce and activate the free radicals the so called ROS (Sharma et al., 2001). Even, some researchers argued that the trace amount of free radicals generated initially by the respiratory mechanism is always necessary for the normal functioning of the sperm includes capacitation, acrosomal reaction, and ejaculation (Chi et al., 2008; Griveau and Le Lannou, 1997; Kim and Parthasarathy, 1998, Aziz et al., 2004).
Epididymis plays a major role in the generation of antioxidant defence mechanism as because it secretes a trace amount of antioxidants like extracellular SOD and catalase (Potts et al., 1999; Williams et al., 1998). GPX epididymal mRNA were also detected in the epididymis in a trace amount, which acts as an antioxidative protective mechanism (Zini and Schlegel 1997a). Epididymis provides a best possible atmosphere for the sperm storage and later on the maturation. During this process the role of the epididymis in protecting the spermatozoa from the oxidative stress were not clearly studied so far (Potts et al., 1999). Even spermatozoa itself is able to produce and protect themselves from the free radicals, by its own thiol groups, uric acid and α-tocopherol (Potts et al., 1999; Lewis-Jones et al., 1996; Ochsendorf et al., 1998). Epididymis posses many site specific antioxidants that helps in protecting the spermatozoa from the oxidative stress (Potts et al., 1999).

2.5. STUDIES ON FERTILITY ASSOCIATED PROTEINS IN SEMINAL PLASMA

Investigations reveals that the seminal plasma proteins, their nature and properties were characterized in the recent past years by several researchers (Maja and Miroslava, 2010; Pang and Cheung, 2007; Thimon et al., 2008; Kopers et al., 2011; Fariello et al., 2012; Jun Wang et al., 2009; Peonim et al., 2013; Asadpour et al., 2007). These authors stated that most of the major proteins present in human seminal plasma were adsorbed to the facade of the sperm which was ejaculated during sexual intercourse, and so the functions of the major proteins present in plasma is tough to investigate and characterize (Asadpour et al., 2007; Desnoyers and Manjunath, 1992).

Seminal plasma proteins have a property of protective medium for the ejaculated sperm; play a vital role in their endurance and the channel through the female reproductive tract. Several authors investigated on proteomic analysis of human seminal plasma, found to have number of extracellular proteins and erstwhile proteins which were secreted by prostate gland, testes and epididymis (Fung et al., 2004; Pilch and Mann, 2006; Duncan and Thompson, 2007; Wang et al., 2009; Hynes and Yamada, 1982; Fusi and Bronson, 1992).
Seminogelin proteins present in the seminal plasma was rarely used as the confirmatory test for detecting the semen inside the female reproductive tract in case of sexually assault females at the time of investigation. Seminogelin proteins were the group of proteins with specific characteristics, secreted by seminal vesicle (Pang and Cheung, 2007). Seminogelin proteins have a special property with non-covalent interactions and immediately making coagulum, that cannot be digested by proteases within minutes, this can be the better step to analysis the vaginal swab of sexually assaulted female for the presence of seminogelin group of proteins through one and 2D gel electrophoresis (Robert et al., 1999; Bjartell et al., 1996; Peter et al., 1998; Lundwall et al., 2002).

Two dimention gel electrophoresis and mass spectroscopy has been used to spot and identify the molecular weight of proteins present in human seminal plasma (Jun Wang et al., 2009). More than 780 spots were detected in 2D gel electrophoresis for fertile male human seminal plasma sample which was done immediately after ejaculation, whereas more than 580 spots were detected for the samples which was freeze and thawed (Starita Geribaldi et al., 2001). About 950 proteins in human seminal plasma by means of Fourier transform mass spectroscopy while nearly 150 proteins were identified as prostatic origin (Pilch and Mann, 2006; Li et al., 2008). However all these findings were not successful with predicting the functions and the role of seminal plasma proteins in fertility. A deep knowledge and understanding towards the seminal plasma proteins is in need for finding out the reasons behind the motility associated issues for male infertility. It has been identified that about 50 and 56 proteins threefold up regulated and down regulated respectively (Jun Wang et al., 2009). Regression model with seminal plasma proteins is to represent and predict the fertility of the sample (de Souza et al., 2007).

Sperm cells were entered into the epididymis from testis by the process called sperm maturation, on this time a group of cysteine rich secretory proteins present in human seminal plasma were adsorbed to the surface of the spermatozoa, which participates and enhance the motility. This type of proteins cannot be isolated from the seminal plasma as just they are adsorbed to the spermatozoa before ejaculation itself (Koppers et al., 2011). These proteins were also acting as a decapacitation factor once the sperm enters into the female reproductive tract by mixing with the fluid secreting by the female genital. So the isolation of cysteine rich secretory
proteins can be isolated and characterized by using the swab taken from the female reproductive tract immediately after sexual intercourse, but it is very tough to isolate from the sample taken by masturbation (Reddy et al., 2008; Jalkanen et al., 2005; Nolan et al., 2006).

Gelatin binding proteins present in human seminal proteins was found to be the biomarkers for the secretory functions of testes and epididymis at molecular level. The presence of fibronectic proteins in human seminal plasma between normospermia and infertile category semen samples (Maja and Miroslava, 2010). A comparative analysis proteome of human seminal plasma between normospermia and asthenozoospermia samples, found many protein spots were not detected in the infertile category which was efficiently spotted in the case of control and normospermia (Jun Wang et al., 2009).

Seminal plasma proteins in humans have not been fine categorized (Dubiel, 1974; Bruschi et al., 1979; Vickram et al., 2012). Many researchers were cross compared the protein profile between seminal plasma, spermatozoa, prostasomes and between the control and various infertile categories (Vickram et al., 2012). Identification of fertility associated proteins in human seminal plasma proteins has substantial advantage, can be more helpful in diagnosing, increase the fertility, increase the success rate for assisted reproduction in humans, and even as a contraceptive (Killian et al., 1993). Heparin was found to have a necessary role in the penetration of the sperm inside the female reproductive tract, while heparin is a carbohydrate; fibronectin proteins have the same role inside the female reproductive tract (Sinowatz et al., 1997).

2.6. ARTIFICIAL NEURAL NETWORKS

Three different artificial neural network models (ANN) like Multilayer perceptron (MLP), Decision Tress (DT) and Support Vector Machines (SVM) has been used to predict the parameters involved in the male fertility and sterility potential (Gil et al., 2012). Furthermore, the use of artificial neural networks has also been used to predict various medical applications.

The major advantage of artificial neural networks in prediction for biological samples was
a) Potential to sustain clinical decision making,

b). Cheap and sensitive tool to predict maximum number of samples within the time limit,

c). Easy to compare the results with the existing sample database,

d. Helpful in creating a biological and biochemical parameter datasets in single software (Lisboa and Taktak, 2006).

Much number of classifiers was available within artificial intelligence (AI) for instance, artificial neural networks (ANNs), SVM (Gil et al., 2012; Polat et al., 2009b; Conforti and Guido, 2010). Many Artificial insemination researchers were used hybrid techniques like combination of ANNs and fuzzy logic and finally named as fuzzy neural networks (FNN), which found to have wide applications in predicting the various parameters in male infertility during the diagnosis period for both male and female (Kahramanli and Allahverdi, 2008).

Patients with male infertility under azoospermia category has some hope with techniques like testicular sperm extraction (TSE), percutaneous epididymal sperm aspiration (PESA), and many other surgical techniques involved in assisted reproduction for pretty enhancing the fertility potential (Devroey et al., 1994; Silber et al., 1996). In fact the success rate for assisted reproduction has been increased by these surgery techniques, many were concern about fibrosis and vascularisation, which creates lot of burden and financially not in the scope for doing assisted reproduction (Schlegal and Su 1997; Tournaye et al., 1997). Many researchers were trying to get rid of these problems by estimating many non-invasive parameters includes assessment of volume, testicular estimation, hormonal estimation and many other parameters were used to predict the sperm retrieval assay, but however the results were not fully reliable with the available database of that particular patient (Vernaeve et al., 2002; Yi et al., 2011).

An artificial neural network model that predicts the outcome of the assisted reproduction by using different biochemical parameters (Yi et al., 2011). An artificial neural network model to predict the quality of the spermatozoa before the diagnosis of male infertility and compared the results with the proper standard regression model. Authors concluded that his model was predicting with low error than any other standard models (Murat and Dogan, 2004).
Predicting the assisted reproduction is not merely easy by regression models, but the development of the perfect model can be possible by artificial neural networks followed by the Bayesian algorithm. The results of prediction techniques for the IVF outcomes and diagnosis of male infertility by ANNs is still not reliable (Corani et al., 2013). Scientists have developed three different AI to predict the semen quality assuming the semen has been influenced by the environmental and stress related issues. His results were very much useful in elucidating and validating the diagnosis of male infertility (Gil et al., 2012). The amount and the number of parameters used to predict the semen quality and fertility potential is found to be the important factor in the application of AI for improving the accuracy and to elucidate the most favouring parameter (Subashini et al., 2009; Gil et al., 2009; Gil and Johnsson, 2010a, 2010b; Gil et al., 2011). In various illustrations artificial neural networks can recognize a huge range of relations than any other statistical techniques and even with many computer based algorithms (Ripley, 1994).

Vickram et al., (2013) developed an artificial neural network model by using back propagation algorithm and finally called as back propagation neural network model (BPNN) to predict the concentration of Zn in the normospermia samples with the semen parameters as input. He has used six different input parameters and only one output (Predicting the concentration of Zn), finally he compared the prediction results with the original result obtained through atomic absorption spectroscopy (AAS). When the number of variables in the model increases, those time the easy and the accuracy of the prediction decreases with the model (Bustillo et al., 1993; Jurisica et al., 1998). Data mining is the concept and the combination of sophisticated statistical model and the artificial intelligence model that can be used in predicting the outcome of the in vitro fertilization (IVF) and its various factors influencing the success rate (Jurisica et al., 1998; Kim and Jung, 2003; Ruey-Shiang et al., 2011). The most noteworthy traits and their relationship in influencing the pregnancy success rate followed by the IVF and in diagnosing the male fertility potential were currently accepted as fascinating breakthrough in the medical field for the realm experts in the AI field (Ruey-Shiang et al., 2011).