## ***SECTION- I***

# *REVIEW OF LITERATURE*

## Introduction

Reproduction is one of basic instinct of human beings. For the fertility process to proceed smoothly, both the man and the woman should be healthy and normal. Infertility is defined as the condition in which a couple seeking for a child cannot conceive even after 12 months of unprotected intercourse (Mueller and Daling 1989; Thonneau *et al.*, 1991). Sterility means that one can never conceive and carry a child. It is almost an irreversible condition under ordinary circumstances. Infertility and sterility do not change one's ability or desire to procreate. Sterility is the permanent inability of either a male or female to produce offspring; in a woman it is an inability to conceive; in a man it is an inability to impregnate (Habbema *et al*., 2004). Similarly sub-fertility generally describes as any form of reduced fertility with prolonged time for conception (Gnoth et al., 2005). Itis a milder version of infertility which is reversible to fertility with or without medical help. Both infertility and sub-fertility are defined as the inability to conceive after a certain period of time (the length of which vary), so often the two terms overlap (Gnoth*et al*., 2005).

## Incidence and prevalence of Infertility

The incidence of male infertility varies greatly. In Western countries one in four men consulting fertility clinics has specific condition like low sperm count, motility or/ and abnormal morphology, causing infertility (Bhasin *et al.*, 1994). According to a study conducted by World health organization (WHO) regarding the diagnosis and management of male infertility (WHO, 1987), it was reported that in 20% of couple with infertility, the problem could be attributed predominantly to the male. Seshagiri (2001) has reported the incidence of infertility globally to be 13 to 18% and the male factor to be

responsible in one half of the cases. Speroff (1999) has reported 40 to 50% of infertility to be due to male factor. According to Johnson *et al.* (1994) about 15% of all married couples are burdened with infertility and the male contributing in 40 to 50 % of the cases.

However, the incidence of male sub-fertility or infertility is not yet clear, though it is estimated that approximately 13 to 19 million couples are infertile (Sharma *et al.*, 2005). An estimated 15% to 20% of couples meet this criteria and are considered infertile, with approximately 40% due to female factors alone, 50% due to male factors alone, 10% due to a combination of female and male factors, and unexplained factors. Globally, the incidence of infertility is estimated to be about 13–18% (Hull *et al.*, 1985; Mueller and Daling 1989; Thonneau *et al.*,1991; Jones and Toner, 1993; Irvine, 1996) in the human population, regardless of race, ethnic group, etc. Aetiologies of male infertility are still generally underestimated, ignored under diagnosed and under treated. Nearly 7.5 to 10% of all men in the reproductive age group are infertile and are incapable of fathering children. According to a report conducted by the International Institute of Population Sciences, infertility is growing at an alarming pace, especially in the urban area. Out of around 250 million individuals estimated to be attempting parenthood at any given time, 13 to 19 million couples are likely to be infertile (Forest, 2004).

**Incidence of infertility in India**

The recent growth of the Indian population has been unprecedented. It stands currently at over one billion and is expected to touch 2 billion by 2035 assuming an average growth rate of 2% (Seshagiri2001). Even though curtailing population growth is a major national concern, a substantial number of infertile couples in the Indian population have an equally great concern, that of having a child. This is an equally

important national problem concerning reproductive health and the infertile couples have to be treated by Medically Assisted Reproductive Technology (MART) for procreation (Seshagiri2001). A report showed that in India, 13% of married women aged 15-49 years were childless in 1981 (rural 13.4% and urban 11.3%) which increased to 16 percent in 2001(rural 15.6% and urban 16.1%) (Palatty *et al.,* 2012). Therefore over half of married women aged 15-19 years were childless in 1981, which increased to 70% in 2001. Nearly 30 million couples in the country suffer from infertility, making the incidence rate 10% (Palatty *et al.,* 2012).

**Types of Infertility:**

Infertility can be classified as primary and secondary infertility. Primary infertilityis when a couple have never had children, or unable to achieve pregnancy even after one year despite having unprotected sexual intercourse(WHO, 1983), whereas secondary infertilityis when a couple have had children or achieved pregnancy previously, but are unable to conceive at second time, even after having unprotected sexual intercourse for one year (WHO, 1983). Secondary infertility occurs more commonly than primary infertility, especially in developing countries where sexually transmitted infections are common. About 67–71% and 29–33% of patients have primary and secondary infertility, respectively (Mueller and Daling, 1989; Thonneau *et al.*,1991; Irvine, 1996). In many countries, induced abortion contributes much too secondary infertility, which accounts for 60% of the total number of infertile cases (WHO, 1983). Idiopathic infertility is a condition of couples unable to conceive for more than two years, with no abnormalities seen on repeated investigations of tubes or as regards ovulation,

luteal phase, cervical mucus, semen, sperm–oocyte interaction or intercourse (WHO, 1983).

**Male infertility**

Compared to other species, human males have relatively poor sperm producing capacity and human testicular function is very sensitive to a wide variety of environmental insults. This may be related to the human (upright) posture and hydrostatic pressure on venous testicular outflow, or other unknown factors, but it is necessary for clinicians to be aware of the high incidence of sub fertility in men. Perhaps it is a reflection of the incredible ability of humans to adapt the environment to promote their own survival or the expectation that fertility should be nearly spontaneous, but many human couples seek evaluation for infertility (Glover and Barratt, 1999).

The human male reproductive system includes the hypothalamo -pituitary-testicular Hormonal axis as well as the reproductive organs such as Testis, epididymis, vas deferens, seminal vesicles, prostate and urethra.Production of spermatozoa requires approximately 3 months from the initial mitotic divisions through the myriad changes readying sperm for ejaculation and fertilization. Highlights of this transformation include (1) the unique environment created within the testis for spermatogenesis to occur; (2) preservation of a set of stem cells relatively resistant to external injury and able to produce rapidly proliferating germ cells destined to become spermatozoa; (3) meiosis, that results in formation of the haploid gamete; and (4) the dramatic differentiation of the prospective gamete in a form that is specialized to transport chromosomal material in a structure ideally suited for transit of the female reproductive tract. The spermatozoa resulting from this complex process assumes its final shape and size in the testis. In the

normal state, it also acquires the ability to fertilize as well as a capacity for motility in the epididymis. Unfortunately, the mechanisms by which the epididymis exerts these changes on the traveling spermatozoon and the actions of the human reproductive tract after relief of chronic obstruction remain largely unknown (Glover and Barratt, 1999).

**Etiology of male infertility**

The etiology of the male infertility is multifactorial and still little is known about the causative factors dealing with impaired spermatogenesis. Male infertility has been associated with several genetic and non-genetic conditions (Poongothai *et al.,* 2009). Among the major causes of infertility, chromosomal abnormalities, microdeletions, cystic fibrosis transmembrane conductance regulator (CFTR) mutations and other genetic factors [follicle stimulation hormone (FSH) receptor mutation] are important (Irvine, 1996; Phillip *et al.*, 1998; Diemer and Desjardins, 1999; Egozcue *et al.,* 2000; Hargreave, 2000). Anatomical abnormalities such as varicocele, vesicular damage due to torsion and obstruction of testicular sperm passage can all lead to male infertility. The known causes of male infertility are quite numerous but can be grouped into a number of major categories. Non-obstructive azoospermia has a strong genetic basis where there is an excess existence of autosomal abnormalities (Hargreave, 2000). Besides, congenital bilateral absence of the vas deferens (CBAVD) associated with the phenotype of CFTR gene mutations cause obstructive azoospermia (Donat *et al.,* 1997). It is unclear that up to what extent genetic contributes. It has been reported that in a certain ethnic group, men with a particular haplotype (II) have a lower sperm concentration compared with men with haplotypes (III) and (IV) and, the frequency of haplotype (II) is more common in azoospermic men compared with normal men (Kuroki *et al.,* 1999). Based on this, it

appears that the genetic contribution towards male fertility on account of a decreased sperm concentration might be significant in some ethnic groups. There are a number of nongenetic risk factors such as Sexual Transmitted Diseases (STD s) involving N. *gonorrhoeae* and C. *trachomatis*. These cause changes semen quality and chronic infection may lead to a block of the vas deferens or seminal vesicles (Megory *et al.*, 1987). Mumps, though rare in adults, can result in azoospermia.

Besides, immunological factors operate at almost every step in the human reproductive process, antibodies induced damage to gametes and developing embryos is a major cause of immunological infertility (Carlsen *et al.*, 1992). There appears to be a world-wide concern over decreasing human sperm concentration but this has been highly controversial. Decreasing sperm counts are attributed to the deleterious effects of environmental contamination by heavy metals and estrogenic chemicals (Mehta and Anandkumar, 1997; Benoff *et al.*, 2000; Sharpe, 2000). Life style, environmental factors (Benoff *et al.*, 2000; Sharpe, 2000), including smoking (Zenzes 2000), can also affect gamete and embryo development, leading to subfertility or infertility. A combined cause of infertility is found in about 10–30% of couples (Hull *et al*., 1985; Thonneau *et al.*, 1991; Jones and Toner 1993). In addition to these causes, another important category is unexplained male infertility (UMI) or idiopathic male infertility that is reserved for infertile males with unknown origin factors with normal semen parameters in which female partner infertility factors have been ruled out. It ranges from 6 to 27% (Moghisis and Wallach, 1983; Sigman *et al*., 2009).

**Anatomy and pathology of Human male reproductive system:**

The human male reproductive system consists of a number of sex organs that form a part of the human reproductive process.The male sex organs can be classified as External genital organs and Internal Genital organs. The main external genital organs are the penis, testes and epididymis. Testis produce semen and sperm, which, as part of sexual intercourse, fertilize an ovum in the female's body; the fertilized ovum (zygote) develops into a fetus, which is later born as a child. In this type of reproductive system, these sex organs are located outside the body, around the pelvic region. The main male internal genital organs are vas deferens, seminal vesicles and prostate.

**External genital organs**

**Penis:** The penis is the male copulatory organ. It has a long shaft and an enlarged bulbous-shaped tip called the glans penis, which supports and is protected by the foreskin. When the male becomes sexually aroused, the penis becomes erect and ready for sexual activity. Erection occurs because sinuses within the erectile tissue of the penis become filled with blood. The arteries of the penis are dilated while the veins are passively compressed so that blood flows into the erectile cartilage under pressure (Figure 1). The human penis differs from those of most other mammals, as it has no baculum, or erectile bone, and instead relies entirely on engorgement with blood to reach its erect state. It cannot be withdrawn into the groin, and it is larger than average in the animal kingdom in proportion to body mass (Poncheietti *et al.*, 2001).

**Disorders of penis:** Many disorders are associated with penis such as Penile hypoplasia, Hypospadiasis, Phimosis, Paraphimosis, Peyronie's disease**,**Pudendal nerve entrapment, Penile fracture, Erectile dysfunction, etc.Thrombosis can also occur during periods of frequent and prolonged sexual activity, especially fellatio. Infection with the

herpes virus can occur after sexual contact with an infected carrier; this may lead to the development of herpes sores. In diabetes, peripheral neuropathy can cause tingling in the penile skin and possibly reduced or completely absent sensation. Priapismis a painful and potentially harmful medical condition in which the erect penis does not return to its flaccid state. Potential complications include ischaemia, thrombosis, and impotence. In serious cases the condition may result in gangrene, which may necessitate amputation (Goldenberg, 1998). Carcinoma of the penis is rare with a reported rate of 1 person in 100,000 in developed countries. Circumcision is said to protect against this disease but this notion remains controversial (Boczko and Freed, 1979).Hypospadias, micropenis ,Diphallia, or penile duplication are developmental disorders of penis considered rare condition do exist sometimes (Andrews *et al.*, 1998).

**Scrotum:** The scrotum is a pouch-like structure that hangs behind the penis. It holds and protects the testes. It also contains numerous nerves and blood vessels. At lower temperatures, the Cremaster muscle contracts and pulls the scrotum closer to the body, while the Dartos muscle gives it a wrinkled appearance; when the temperature increases, the Cremaster and Dartos muscles relaxes to bring down the scrotum away from the body and remove the wrinkles respectively. The scrotum remains connected with the abdomen or pelvic cavity by the inguinal canal (Poncheietti *et al.,* 2001).

**Testicular Anatomy:** The human testis is an ovoid mass that lies within the scrotum. The average testicular volume is 20 cc in healthy young men and decreases in elderly men (Crane and Scott, 2002). In Asian men, testes tend to be smaller. Normal longitudinal length of the testis is approximately 4.5 to 5.1 cm (Khan *et al.*, 2010). The testicular parenchyma is surrounded by a capsule containing blood vessels, smooth

muscle fibers and nerve fibers sensitive to pressure. The functional role of the testicular capsule is unknown, but may relate to movement of fluid out through the rete testis or control of blood flow to the testis (Crane and Scott, 2002). The testis contains seminiferous tubules and interstitial cells. The tubules are segregated into regions by connective tissue septa. The seminiferous tubules are long V-shaped tubules, both ends of which usually terminate in the rete testis (Figure 2). Measurement of testicular size is critical in the evaluation of the infertile man, since seminiferous tubules (the spermatogenetic region of the testis) occupy approximately 80% of testicular volume. So, a rough estimate of spermatogenic cell capacity is provided by assessment of testicular size. Testicular consistency is also of value in determining fertility capacity. A soft testis is likely to reflect degenerating or shrunken spermatogenic components within the seminiferous tubules. The seminiferous tubules drain toward the central superior and posterior regions of the testis, the rete testis, which has a flat cuboidal epithelium. The rete coalesces in the superior portion of the testis, just anterior to the testicular vessels, to form 5-10 efferent ductules. These efferent ducts leave the testis and travel a short distance to enter the head, or caput region of the epididymis. The efferent ducts coalesce in a somewhat variable pattern within the caput epididymis to form a single epididymal tubule (Crane and Scott, 2002).

The artery to the testis is specialized in that it is highly coiled and intimately associated with a network of anastomotic veins that form the pampiniform plexus. The counter flowing vessels are separated only by the thickness of their vascular wall in some areas (Figure 2). This vascular arrangement facilitates the exchange of heat and small molecules, including testosterone (Wampler and Lianes, 2010). The transport of

testosterone is a concentration-limited, passive diffusion process in men (Wampler and Lianes, 2010). The counter-current exchange of heat in the spermatic cord provides blood to the testis that is 2 to 4 °C lower than rectal temperature in the normal individual. A loss of the temperature differential is associated with testicular dysfunction in humans with idiopathic infertility, as well as men with varicocele or cryptorchidism.

**Seminiferous Tubules**

The seminiferous tubules provide a unique environment for the production of germ cells. The structures involved in this process include germinal elements and supporting cells (Mendez and Emery, 1979). The supporting cells include the peri-tubular cells of the basement membrane and the Sertoli cells. The germinal elements comprise a population of epithelial cells, including a slowly dividing primitive stem cell population, the rapidly proliferating spermatogonia, spermatocytes undergoing meiosis, and the metamorphosing spermatids (Mendez and Emery, 1979). The seminiferous tubule also produces an environment known as "the blood-testis barrier". The testis is unique in that the differentiating germ cells are potentially antigenic, and recognizable as foreign; however, little immunological reaction is usually detectable within the testis (Mendez and Emery, 1979).

Developmentally, the testis develops from the undifferentiated gonad. These primitive germ cells are referred to as gonocytes after the gonad differentiates into a testis by forming seminiferous cords. At this time, the gonocytes are located in a central position within the seminiferous cords. They are subsequently classified as spermatogonia after the gonocytes have migrated to the periphery of the tubule. From birth to approximately 7 years of life, there appears to be very little morphological

change within the human testis. From 7 to 9 years of life, mitotic activity of gonocytes is detectable, with spermatogonia populating the base of the seminiferous tubule in numbers equal to those of the Sertoli cells (Mendez and Emery, 1979). There appears to be little further morphological change in spermatogonia until spermatogenesis begins at the time of puberty (Mendez and Emery, 1979).

**Epididymis:** The epididymis is a whitish mass of tightly coiled tubes cupped against the testicles, acts as a maturation and storage for sperm before they pass into the vas deferens, that carry sperm to the ampullary gland and prostatic ducts (Figure 3).The epididymis can be divided into three main regions: The head (Caput), the body (Corpus) and the tail (Cauda) (Jones, 1999) (Figure 4). However, these anatomical divisions have been defined based on findings in animals, not in humans. The human epididymal epithelium is relatively homogeneous as viewed under the microscope, and grossly, the epididymis does not have the same distinct gross anatomical subdivisions that are easily seen in the rat, rabbit and other animals. Unfortunately, there is little information available regarding the functional diversity of these three regions of the human epididymis (Jones, 1999). In reptiles, there is an additional canal between the testis and the head of the epididymis and which receives the various efferent ducts. This is, however, absent in all birds and mammals (Romer and Parsons, 1977).

Spermatozoa in the unobstructed testis are not motile and are incapable of fertilizing ova. Spermatozoa become functional gametes only after they migrate through the epididymis and undergo an additional maturation process, thereby acquiring the capacities for both progressive motility and fertility (Jones, 1999). Biochemical changes observed in human spermatozoa during epididymal transit involve the formation of

disulfide bonds within the sperm nucleus and tail and the oxidation of sperm membrane sulfhydryl groups. These changes are thought to provide improved structural integrity to the sperm membrane. The changes in structural integrity of sperm may be necessary for the development of progressive motility and successful penetration of eggs. Pathology of epididymis includes an inflammation of the epididymis is called epididymitis. It is much more common than testicular pain, called orchitis (Ross *et al.*, 2011).

**Internal reproductive organs**

**Vas deferens:** The vas deferens, also known as the sperm duct, is a thin tube approximately 43.2 centimetres long that starts from the epididymis to the pelvic cavity. There are two ducts, connecting the left and right epididymis to the ejaculatory ducts in order to move sperm. Each tube is about 30 centimeters long (in humans) and is muscular (surrounded by smooth muscle) (Figure 5).

During ejaculation the smooth muscle in the walls of the vas deferens contracts reflexively, thus propelling the sperm forward. This is also known as peristalsis. The sperm is transferred from the vas deferens into the urethra, collecting secretions from the male accessory sex glands such as the seminal vesicles, prostate gland and the bulbourethral glands, which form the bulk of semen. The rate of transport of fluid through the vas deferens is not known in the human. Just prior to ejaculation, the testes are brought up close to the abdomen and fluid is rapidly transported through the vas deferens toward the region of the ejaculatory ducts and subsequently into the prostatic urethra. After ejaculation, intravasal fluid is transported back toward the epididymis and occasionally into the seminal vesicles as well (Kim *et al.*, 2010). The retrograde transport

of sperm to the seminal vesicles has been documented by videoradiography during ejaculation after vasography. The return of sperm to the seminal vesicles after ejaculation may help explain the prolonged presence of sperm in the ejaculate for some men after vasectomy. The vas deferens may be obstructed, or may be completely absent (the latter a potential feature of cystic fibrosis), causing male infertility. It can be overcome by Testicular Sperm Extraction (TESE), collecting sperm cells directly from the testicles (Romer *et al*., 1977).

## Accessory glands

Accessory glands are internal reproductive organswhich provide fluids that lubricate the duct system and nourish the sperm cells. They are the seminal vesicles, the prostate gland, and the bulbourethral glands (Cowper glands) (Valerie, 2010)

### Seminal vesicles

Seminal vesicles are sac-like structures attached to the vas deferens at one side of the bladder (Figure 6). They produce a sticky, yellowish fluid that contains fructose. This fluid provides sperm cells energy and aids in their motility.

About 50-70% of the seminal fluid in humans originates from the seminal vesicles, but is not expelled in the first ejaculate fractions which are dominated by spermatozoa and zinc-rich prostatic fluid (Kierszenbaum and Abraham 2002). The excretory duct of each seminal gland opens into the corresponding vas deferens as it enters the prostate gland. Seminal vesicle fluid is alkaline, resulting in human semen having a mildly alkaline pH. The alkalinity of semen helps to neutralize the acidity of the vaginal tract hence, prolonging the lifespan of the sperm. Acidic ejaculate (pH <7.2) may

be associated with ejaculatory duct obstruction. The vesicle produces a substance that causes the semen to become sticky/jelly-like after ejaculation, which is thought to be useful in keeping the semen near the womb (Huggins *et al.,* 1942).

**Prostate gland:** The prostate gland is responsible for the proof semen, a liquid mixture of sperm cells, prostate fluid and seminal fluid(Valerie, 2010). This gland is also responsible for making the semen milky in appearance by mixing calcium to the semen coming from seminal vesicle (semen coming from the seminal vesicle is yellowish in colour); the semen remains cloudy and clumpy until the prostatic profibrinolysin is formed into fibrinolysin and lysis of the fibrinogen from the seminal vesicle fluids occurs (Myers and Robert 2000).

A healthy human prostate is classically said to be slightly larger than a walnut. The mean weight of the "normal" prostate in adult males is about 11 grams, usually ranging between 7 and 16 grams (Leissner and Tisell, 1979). It surrounds the urethra just below the urinary bladder and can be felt during a rectal exam. It is the only exocrine organ located in the midline in humans and similar animals (Figure 6). The secretory epithelium is mainly pseudostratified, comprising tall columnar cells and basal cells which are supported by a fibroelastic stroma containing randomly orientated smooth muscle bundles. The epithelium is highly variable and areas of low cuboidal or squamous epithelium are also present, with transitional epithelium in the distal regions of the longer ducts (Leissner and Tisell, 1979). Within the prostate, the urethra coming from the bladder is called the prostatic urethra and merges with the two ejaculatory ducts. The prostate can be divided in two ways: by zone, or by lobe. It does not have a capsule,

rather an integral fibromuscular band surrounds it. It is sheathed in the muscles of the pelvic floor, which contract during the ejaculatory process (Raychaudhuri and Cahill, 2008).

**Bulbourethral glands**

The bulbourethral glands, also called Cowper glands, are two small glands located on the sides of the urethra just below the prostate gland. These glands produce a clear, slippery fluid that empties directly the urethra.They are homologous to Bartholin's glands in females (McEntee, 2012). The bulbourethral glands are compound tubulo-alveolar glands, each approximately the size of a pea in humans (McEntee, 2012). They are composed of several lobules held together by a fibrous covering. Each lobule consists of a number of acini, lined by columnar epithelial cells, opening into a duct that joins with the ducts of other lobules to form a single excretory duct. This duct is approximately 2.5 cm long and opens into the urethra at the base of the penis. The glands gradually diminish in size with advancing age (Schwartz, 1988).

During sexual arousal each gland produces a clear, salty, viscous secretion known as pre-ejaculate. This fluid helps to lubricate the urethra for spermatozoa to pass through, neutralizing traces of acidicurine in the urethra (Chughtai *et al.*, 2005), and helps flush out any residual urine or foreign matter. Though the pre-ejaculate does not contain sperm it is possible for this fluid to pick up sperm, remaining in the urethral bulb from previous ejaculations, and carry them out prior to the next ejaculation. The Cowper's gland also produces some amount of prostate-specific antigen (PSA), and Cowper's tumors may increase PSA to a level that makes prostate cancer suspected (Chughtai *et al.*, 2005).

**Role of Hormones in male reproduction**

Endocrine system is the second key regulator of organ system functions after nervous system in human body. Hormones are actual messengers in endocrine signaling. A man's sperm production is controlled via a complex interplay of hormones in the brain and the testicles. The control starts in the brain with the hypothalamus (under the brain) which releases Gonadotropin-releasing hormone (GnRH), a substance that promotes the pituitary gland to release two important hormones, Follicle stimulating hormone (FSH) and leutinizing hormone (LH). Follicle stimulating hormone travels in the blood to the testicles where it signals certain cells such as sertoli cells to produce sperm. When enough sperm is produced, the testicular cells produce inhibin, a hormone that travels in the blood and gives signal to the pituitary gland to stop production and secretion of FSH. Leutinizing hormone also comes from the pituitary and travels to the testicles, where it signals to different cells, the Leydig cells to secrete testosterone (Heaton and Jeremy 2003). Testosterone is the male hormone but is not active reproductively until it is converted to dihydrotestosterone via an enzyme, 5-alpha-reductase. Dihydrotestosterone stimulates male reproductive tract growth and function. Testosterone and dihydrotestosterone inhibit pituitary production and secretion of LH when enough testosterone is synthesized (Mooradian *et al.,* 1987). Prolactin is a pituitary hormone that in men is not directly involved with reproduction. However, certain medical conditions can cause prolactin to increase, such as pituitary tumors, which in turn causes decreased production of FSH and LH by a variety of mechanisms. Estrogen, usually thought of as a female hormone, is present in men as well and is called estradiol. Estradiol levels are typically low, but may be elevated in certain conditions, such as obesity (Mooradian *et*

*al.,* 1987). Testosterone is converted to estradiol by an enzyme called aromatase. Aromataseis present in fat cells (Bassil*etal*.,2009).

By observing the levels of the various reproductive hormones, especially when analyzed in relation to a comprehensive history and physical examination, one can get a very good sense of a male's reproductive status as well as his prognosis for natural, biological fatherhood. In addition, there are hormonal conditions that can be treated with medical hormonal manipulation, often resulting in the patience's return to natural fertility. In addition, a comprehensive endocrine assessment will also detect potentially life-threatening medical conditions that are present in 2% of male fertility patients.

**Diagnosis of male reproductive system through Ultrasonography**

**Medical imaging in infertile males**

Technological advancement in the field of urology and imaging has led to enhanced diagnostic evaluation of the infertile male. Urological imaging and urology has coevolved replacing several invasive techniques like vasography, venography etc., that was previously employed (Anton and Mark, 2011). Several imaging techniques are available to evaluate men with obstructive infertility such as scrotal ultrasonography, transrectal ultrasonography (TRUS), vasography, Magnetic Resonance Imaging (MRI), TRUS guided seminal vesiculography etc. These advanced, highly specific imaging studies have enabled to identify specific abnormalities of physiological or anatomical significance which were previously ill defined, leading to selection of therapies designed to treat previously unmanageable post testicular abnormalities (Zahalsky and Nagler, 2001).

**Obstruction of seminal tract**

Post-testicular causes include obstruction of the sperm delivery route, anti-sperm antibodies and retrograde ejaculation. Obstruction can occur at any region of the seminal tract, either proximal, affecting the epididymis and scrotal portions of the vas deferens and distal which includes inguinal, pelvic and ampullary portions of the vas deferens, and ejaculatory ducts (Goluboff *et al.*,1995; Brugh and Lipshultz, 2007). Seminal tract obstruction may be congenital or acquired. Congenital causes include atresia (failure of normal opening) or stenosis (abnormal narrowing) as well as midline prostatic cystic lesions, e.g. utricular, Mullerian and ejaculatory duct cysts. Acquired causes may be of inflammatory or traumatic origin of the prostate, seminal vesicle or ejaculatory duct (Goluboff *et al.*,1995). Obstruction of the seminal tract is observed to be the underlying cause among 6 % of men complaining of fertility disorders (Schlegel, 2009).

**I. Ultrasound Scanning**

Ultrasound is cyclic sound pressure with a frequency greater than the upper limit of human hearing. The production of ultrasound is used in several fields including medicine, where the sound waves are directed to penetrate a tissue and measure the reflected signals called as echoes.This can reveal details about the structure of the tissue enabling to study any associated abnormalities.

**Principle**

The principle involves directing sound waves by means of a transducer (probe of appropriate length) to the area of interest. The waves interact with tissues of different density, absorptive and reflective characteristics (Honig, 1994). The piezoelectric crystals located in the probe transmit equal and opposite electric voltages that are transformed

into ultrasound waves (Zhalsky and Nagler, 2001). Some of the transmitted sound waves are reflected back to the transducer which is transformed in to electric potentials which are then converted to computer generated images (Honig, 1994).

**Types**

Ultrasound imaging techniques comprise real-time ultrasound, Doppler ultrasound, and Color Flow Doppler (CFD) imaging. Normal scrotal Ultrasound imaging employs a high resolution, ultrasound probe which is placed externally at the scrotum and movement in transverse as well as longitudinal plane a few millimeters provides real time images of the testis (Kim and Lipshultz, 1996). High frequency probe results in lower tissue penetration, high attenuation and better resolution. Real-time ultrasound images are generated when high-speed ultrasonic beams create independent images at high rates, providing a dynamic image. This series of dynamic images may then be recorded in the form of a video or may be viewed as individual frames. For superficial examination of the scrotum and penis, a high-frequency probe (10 MHz) is optimal, providing greater resolution of the superficial areas of interest. A slightly lower frequency probe (6.5 - 7.5 MHz) permits deeper penetration, and is used for TRUS, where the ultrasound probe is placed within the rectum to view seminal vesicles, ejaculatory duct and prostate in real time (Honig, 1993).

Doppler ultrasonography enables to deduce the direction of blood flow. It is based on the pulsatile emission of ultrasound waves towards the moving target The reflected tissue interface i.e. blood, is moving, a frequency difference is created. This difference is converted into a visual signal which is then recorded electronically as a graph of

frequency against time. A pulsed Doppler system measures the velocity of the moving tissue by the transmission at regular intervals of short bursts of ultrasound waves that are reflected from a moving tissue. Duplex Ultrasound combines pulsed Doppler with real time imaging and is extremely useful in imaging small blood vessels. Color flow Doppler ultrasonography also combines real time ultrasonography with pulsed Doppler and employs color identification of blood flow. Any alteration in the direction of blood flow is represented by a color reversal (Honig, 1993).

**1. Scrotal Ultrasonography**

Ultrasonography (US) is the primary imaging modality for assessing scrotal abnormalities (Kim and Lipshultz, 1996). Testicular abnormalities, which can be identified by means of ultrasonography include testicular tumors (benign and malignant) testicular cysts and testicular microlithiasis. The imaging is also used to calculate testicular volume and texture (Lenz *et al*.,1994). Testicular torsion can also be accurately diagnosed when no blood flow to the testicle is demonstrated by CFD ultrasonography (Paltiel *et al*.,1998). Adnexal abnormalities, such as spermatoceles and hydroceles, are well visualized with scrotal ultrasonography, ruling out the need for further diagnostic exploration (Zahalsky and Nagler, 2001).

Other abnormalities that can be identified using US are varicoceles (pathological dilation of network of small veins draining the scrotum) and epididymal cysts. Scrotal US is of immense help in evaluation of azoospermic (semen devoid of sperms) subject as to whether the condition is due to obstruction or non obstruction as the imaging can detect abnormalities in testis, epididymis and the proximal vas deferens (Donkol, 2010).

## 1.1 Scrotal Ultrasonography in non obstructive Azoospermia and other infertile conditions

### a ) Size and texture

Scrotal US are employed to examine the size and echotexture in transverse as well as longitudinal plane. Testicular volume is positively correlated with the level of spermatogenic activity). The size has got profound influence on the sperm count as well as motility (WHO 2010). Lenz *et al.,* (1994) employed a low frequency 7.5 millihertz transducer to measure the testicular volume (volume of ellipsoid). They demonstrated the mean volume of both the right and left testicles to be smaller when compared to the volume of normal men. In their study they were also able to identify a positive correlation between the volume and sperm count. The study also involved evaluation of the texture on a scale of 1 to 5 for increasing degree of irregularities. The asmedian score for the infertile group with regard to texture was observed to be 3 when compared to the median score of 2 observed in the normal men. This is indicative of increased damage of seminiferous tubules in the testis of the infertile individuals.

### b) Testicular tumor

Testicular tumors are observed to be more among infertile males when compared to normal individuals. The frequency of testicular tumors in infertile men was found to be 1 in 200 when compared to 1 in 20,000 among normal individuals (Zahalsky and Nagler, 2001). Testicular tumors cannot be appropriately detected by means of physical examination but can be made evident through color Doppler ultrasonography. In a review of ultrasonographic reports by Pierik *et al*., (1999) it was identified that 60% of

sonographic findings of abnormalities were not evident on palpation with only one in seven cases of tumors was suspected in physical examination.

**c) Testicular torsion**

Testicular torsion is a condition in which twisting of the spermatic cord results in progressive impairment of testicular venous drainage which ultimately leads to arterial ischemia and testicular infarction (Cuckow *et al*.,2000). Testicular torsion is one of the leading causes of male infertility accompanied by acute scrotal pain. Torsion can lead to unilateral or bilateral anorchia, testicular atropy, low sperm count, diminished motility and in severe cases may lead to azoospermia (Singh *et al.,* 2012). Persistent torsion can lead to ischemia and reperfusion injury which is associated with excessive production of Reactive Oxygen Species (ROS) leading to germ cell apoptosis, DNA damage, testicular atropy and impaired spermatogenesis (Ichikawa *et al.*,1993; Turner *et al*.,1997). Testicular torsion can be identified by CFD ultrasonography the observation being lack of blood flow to the testicle (Paltiel *et al.*,1998).

**d) Hydrocele**

Another abnormality that can be detected using scrotal ultrasonography is hydrocele which is an abnormal collection of fluid between the parietal and visceral layers of tunica vaginalis. Hydrocele is the most common cause of painless scrotal swelling (Rubeistein *et al.,* 2004). Primary hydrocele is usually of idiopathic etiology whereas secondary hydrocele can be caused by testicular torsion, infection, truma, tumor etc (Singh *et al*., 2012). Mihmanli *et al.,* (2004) observed that testicular size was larger in men with hydrocele which they propose is due to stasis in the venous and lymphatic

outflow owing to pressure induced obstruction in the vessels of testis. They also observed that the testis returned to normal size after hydrocele excision.

The hydrostatic pressure of a hydrocele is more than the pressure in blood vessels within the scrotum. This affects the normal arterial blood flow which might result in an ischemic effect on the testis (Rados *et al.*,1996). Mihmanli *et al.*, (2004) employed color Doppler ultrasonography to evaluate the blood flow before and after surgical removal of hydrocele and observed that the high-resistance flow in the intratesticular arteries prior to excision was substituted by a low-resistance flow after hydrocele excision which also resulted in the elimination of the high pressure. This altered blood pressure indicates that hydrocele has an ischemic effect on the testicular tissue ultimately leading to infertility.

**e) Epididymal cyst**

Epididymal cyst or spermatocele are usually an associated finding but not contributing factor to infertility and are best detected by scrotal ultrasonography. However, in certain conditions an epididymal cyst may become obstructive resulting in oligoasthenospermia or azoospermia condition. Chronic epididymal inflammation may lead to an enlarged, thickened epididymis with mixed echogenicity and calcification as a result of inflammatory response. Acute epididymitis may be confirmed from the findings of an enlarged epididymis with decreased echogenicity during an ultrasound scan (Kim *et al.*, 1996).

**f) Cryptorchidism**

Cryptorchidism is the absence of one or both testicles from the scrotum due to failure of descend through the normal anatomical pathway (Lee *et al*.,1995). Cryptorchidism, whether congenital or acquired can lead to infertility by affecting the sperm concentration and sperm count. The extent of damage depends on several factors like testis location, temperature, hormone titre and associated structural anomalies. Semen analysis from men with untreated bilateral cryptorchidism reveals that the subjects were more inclined to be azoospermic and have higher rates of germ cell apoptosis than individuals with untreated unilateral cryptorchidism (Hadzisellimovic and Herzong., 2001; Chung and Brock, 2011).Moreover, 44–100% of men with treated bilateral cryptorchidism have been reported to have a low sperm count, low motility and abnormal morphology. Also more than 50% of the treated individuals were found to be azoospermic (Lee *et al*., 1995).

**g) Varicocele**

Varicocele is the pathological dilation of the pampiniform plexus of veins (network of many small veins draining the scrotum) of the spermatic cord (Agarwal *et al*., 2009). Several mechanisms have been demonstrated to be the pathophysiology of varicocele induced male infertility. These include hypoxia, testicular venous hypertension, elevated testicular temperature, stasis etc. These effects the normal testicular function causing a decline in semen parameters like count, motility morphology etc. (Marmar *et al*., 2001). Clinical varicoceles are diagnosed by physical examination and confirmed by Ultrasound Color Doppler scanning which has better diagnostic accuracy than physical examination.

Scrotal US is highly reliable to diagnose varicocele and has been reported to have 97% sensitivity and 94% specificity (Trum *et al.*, 1996). Real-time ultrasonography has also been used to diagnose the type of varicocele (grade 1, II and III). Using high-frequency ultrasound (7 to 10 millihertz) probe, a varicocele is identified if there is dilation of the venous structures with the Valsalva maneuver (Mclure *et al*., 1991). Color flow Doppler ultrasonography has been demonstrated to have superior sensitivity and more noninvasive when compared to venography in diagnosing varicocele (Petros *et al.*, 1991). Employing CFD the characteristic alteration in the direction of blood flow in varicocele condition can be identified by the color reversal (red to blue or blue to red) (Zahalsky and Nagler, 2001).

### 1.2 Scrotal Ultrasonography in obstructive Azoospermia and other infertile conditions

Evaluation of the epididymis and testicular volume with scrotal US are vital for distinguishing obstructive azoospermia from non-obstructive azoospermia among infertile men. Testicular volume measured by scrotal US is higher for obstructive azoospermia than for nonobstructive azoospermia. A study conducted by Moon *et al.*, (2006) revealed that the median testicular volume in obstructive azoospermia was 11.6 mL (range, 7.7-25.8 mL) and that in nonobstructive azoospermia was 8.3 mL (range, 1.2-16.4 mL). Scrotal US are employed to detect abnormalities in the proximal portion of the seminal tract by demonstrating dialation in the proximal seminal duct (mediastinum testis, epididymis, and intrascrotal portion of the vas deferens) and can also provide insight into secondary changes of the proximal seminal duct caused by obstruction in the distal part of the seminal duct (terminal vas deferens, ampulla of the vas deferens,

seminal vesicle, and ejaculatory duct) (Beddy *et al.,* 2005). The epididymal abnormalities depicted with scrotal US are significantly associated with obstructive azoospermia. Sensitivity, specificity, and accuracy of scrotal US for differentiation of obstructive from nonobstructive azoospermia were found to be 82.1%, 100% and 87.5% respectively (Moon *et al.,* 2006).

**II Transrectal Ultrasonography**

Transrectal Ultrasonography is most commonly performed if the diagnosis of distal seminal tract obstruction associated with vassal ampullae, seminal vesicles and ejaculatory ducts and is widely used in the diagnosis of distal genital ductal system abnormalities (Galuboff *et al.,* 1995; Kim *et al.,*1996). Prior to the advent of TRUS, the seminal vesicles and ejaculatory ducts were imaged by vasography which is associated with risk of vassal scarring and subsequent obstruction (Honig, 1993). Recent improvements in TRUS with the use of higher frequency multiplanar transducers have enabled the ejaculatory ducts to be visualized and imaged more frequently than in the past (Clements *et al.,*1991). TRUS findings that affect infertility consistent with Ejaculatory duct obstruction (EDO) include ejaculatory duct calcifications, ejaculatory duct cysts, and dilated ejaculatory ducts or seminal vesicles. The primary use of TRUS is to assess obstructions and to determine the absence or hypoplasia of the seminal vesicle and ejaculatory ducts. TRUS has been combined with seminal vesiculography to search for distal ejaculatory duct obstruction, thereby greatly reducing the need for the more invasive open vasography. Currently, the most important indication for TRUS to assess for obstruction, the absence or hypoplasia of the seminal vesicles and the ejaculatory

ducts, is low ejaculate volume, azoospermia, severe oligospermia and asthenospermia (Zahalsky and Nagler, 2001).

The advantage of TRUS is that it is non-invasive, low cost, wider availability and assists in visualizing the normal and abnormal seminal vesicles, the vasa deferentia, ejaculatory ducts and the prostate. Absolute indications for performing TRUS include low volume azoospermia in the absence of testicular atrophy and low volume severe oligoasthenospermia, when (for both) retrograde ejaculation is not present. When an ejaculatory duct obstruction is suspected, TRUS is now considered the initial diagnostic modality (Kim and Lipshultz, 1996).

**a) Ejaculatory duct obstruction**

Ejaculatory duct obstruction is a correctable cause of male infertility and is now considered to affecting 1–5% of infertile men and can be due to either acquired or congenital causes (Engin *et al.*, 2000; Purohit *et al.*,2004). The acquired causes include trauma, inflammation, calculus formation and infection while congenital abnormalities include atresia, stenosis, cysts (Mullerian, utricular and wolffian) and genetic abnormalities as well as Müllerian, utricular and Wolffian cysts (Singh *et al.*, 2012). Ejaculatory obstruction primarily leads to infertile condition while other symptoms include decreased ejaculate force, pain during ejaculation, hematospermia, perineal or testicular pain, prostatitis-like symptoms etc (Galuboff *et al.*,1995). Physical examination of individuals with EDO reveals normal testis, vas deferens and secondary sexual development (Mclntyre and Fish, 2010), while semen analysis records low-volume, azoospermia or oligozoospermia, negative or very low fructose content, and low or immotile sperms (Philip *et al.*, 2007). When the diagnosis is complete EDO, TRUS

guided aspiration of dilated or cystic ejaculatory ducts or seminal vesicles is undertaken to look for presence of sperm. If sperms are observed, then surgical endoscopic relief of the obstruction by transurethral resection of ejaculatory ducts (TURED) is performed which restores communication between ejaculatory duct and urethra (Heshmath and Lo, 2006). When the aspirate is devoid of sperms than vasotomy or vasography are performed to visualize the anatomy of the seminal vesicles, ejaculatory ducts and distal vasa deferentia to exactly demarcate the site of obstruction as well as to identify any associated atresia or stenosis in the distal vas deferens. This is followed by microsurgical epididymal sperm aspiration without EDO repair. If sperm are identified in the vasal aspirate, endoscopic relief of EDO is generally performed. In the absence of vasal sperm, (Shabsigh *et al.*, 1989; Kim and Lipshultz, 1996).

**b) Congenital absence of Vas deferens**

The vas deferens is a paired tubular structure that extends from the caudal region of the epididymis into base of the bladder through the inguinal canal. At the internal ring, the vasa deferentia curve laterally and then pass medially downward into the pelvis where they join the seminal vesicles to form ejaculatory ducts. The human vasa deferentia have a total capacity of 0.45 ml, which accounts for roughly 10% of the volume of normal ejaculate (Singh *et al.,* 2012). Congenital absence of the vas deferens (CBVAD) is diagnosed in 1.3% of men who are subjected for fertility evaluation of which 4.4–17.0% of men record azoospermic condition and 25% of men record obstructive azoospermia (Jequier *et al.,* 1985; Goldstein and Schlossberg, 1988; Futterre *et al.,*2008).

Vasal agenesis can be partial or complete, unilateral or bilateral, and in few cases found to be associated with epididymal hypoplasia. Infertile individuals with CBAVD

display a spectrum of abnormalities including preserved caput epididymis, and absence or abnormal seminal vesicles (Oates and Amos, 1993). The testis is usually found to be normal in both size and function among these individuals. They are observed to be azoospermic with low semen volume (<1 ml), low levels of fructose (seminal vesicular origin), α-glucosidase (epididymal origin) and more acidic (Oates and Amos, 1993).

Among men complaining of fertility disorder an approximate 1% of men have unilateral vasal agenesis and these men are usually fertile owing to single vas deferens. However they are more prone to become infertile than the general population as they have a single functioning testis. It is observed that 20% of affected men have aplasia of the contralateral seminal vesicle and atresia of the ampullary portion of the contralateral vas deferens.Hence a subset of men with unilateral vasal agenesis has azoospermia or other abnormal semen parameters (Hall and Oates, 1993). TRUS is employed to evaluate the ampullary portion of the contralateral vas deferens and the seminal vesicles.Obstructive azoospermia in these individuals is treated by testicular or epididymal sperm retrieval techniques followed by Invitro Fertilization (IVF) or IntraCytoplasmic Sperm Injection (ICSI).

**c) Abnormalities associated with Seminal Vesicles**

The seminal vesicles are paired, symmetric, saccular, elongated organs which lie cephalad to the prostate and posterior to the bladder. They are best visualised through TRUS by means of transverse imaging. The vasal ampullae are best visualized in transverse section just medial to the seminal vesicles (Kim and Liphultz, 1996). The

seminal vesicles serve as reservoirs of seminal fluid but no significant change in volume has been demonstrated after ejaculation. Ejaculatory duct obstruction is often associated with seminal vesicle dilation but is not found in every individual. However asymmetry in the pair is an indication for ejaculatory duct obstruction. Almost 90% of infertile individuals with unilateral CAVD might have aplasia of the ipsilateral seminal vesicle, and close to 20% of these individuals might have aplasia of the contralateral seminal vesicle. All these abnormalities can be identified and characterised by means of TRUS. In those men with CBAVD, 16% had bilateral aplasia of the seminal vesicles, whereas 21% had unilateral seminal vesicle aplasia and contralateral seminal vesicle hypoplasia. Hypoplasia has been defined as a decrease in normal size of 30% (Kim and Liphultz, 1996).

TRUS-guided seminal vesiculography is a technique that combines Ultrasonography with radiography to evaluate seminal vesicle abnormality. Seminal vesiculography is performed by means of fine needle puncture of the seminal vesicle to inject contrast material for radiography. Seminal vesiculography has helped imaging of the distal male reproductive tract (vas deferens, seminal vesicles, and ejaculatory ducts). Real-time TRUS visualization has also been used to guide aspiration of seminal vesicles to diagnose EDO. The presence of sperm in the aspirated fluid confirms the presence of obstruction (anatomical or functional) as well as rules out more proximal obstruction. It also confirms the presence of normal spermatogenesis. The presence of more than three motile sperm per high-power field in the seminal vesicle aspirate is considered as an indication of obstruction (Jarow, 2001).

Fine needle aspiration of abnormally dilated cysts, seminal vesicles, or ejaculatory ducts is performed through guidance by means of TRUS. The aspiration of cystic structures can also be performed and the fluid can be evaluated for the presence of sperm. A contrast material is then injected into the punctured structure and then plain radiographs are taken. The "vesiculogram" films then are reviewed for the presence of dilated seminal vesicles, EDO, or other correctable anatomic abnormalities that may be causing infertility (Zahalsky and Nagler, 2001).

**d) Cystic lesions of the Prostate**

Imaging techniques such as TRUS and endorectal MRI has improved the detection of cystic lesions of the prostate (congenital or acquired), which affects 0.5–7.9% of men. Two types of cyst, namely midline prostatic cysts and ejaculatory duct cysts can obstruct the ejaculatory ducts and lead to infertile condition (Hamper *et al.*, 1990). Midline prostatic cysts can be divided into three types: prostatic utricle cysts (previously called Mullerian duct cysts), cystic dilatation of the prostatic utricle and enlarged prostatic utricles (Galosi *et al*., 2009). A prostatic utricle cyst results from failure of the Mullerian ducts to regress and affects 5% of men with obstructive azoospermia. Prostatic utricle cysts do not communicate with the urethra and hence aspirations from these cysts do not contain spermatozoa (Singh *et al*., 2012; Kato *et al.*, 2002).The condition is usually asymptomatic, but patients in the third or fourth decade of lifemay develop irritative and obstructive urinary symptoms as well as hematuria, hematospermia, bloody urethral discharge, ejaculatory pain, urinary tract infection, epididymitis, infertility and constipation.

Cystic dilation of the prostatic utricle (cystic utricle) arises due to obstruction of the junction between the utricle and the urethra hence communicates with the posterior urethra. These cysts are smaller than prostatic utricle cysts and are localized to the midline. Both prostatic utricle cysts and cystic utricles can enlarge and compress both ejaculatory ducts resulting in abnormal semen parameters and might also cause azoospermia (Kato *et al.*, 2002; Kato *et al.*, 2005).

The third type of midline prostatic cyst is an enlarged or hypertrophied prostatic utricle that communicates with the prostatic urethra and is frequently found in children with urogenital malformations, such as proximal hypospadias or virilization defects. TRUS and cystourethrography usually reveal an enlarged prostatic utricle that is midline and posterior. This type of cyst does not typically obstruct the ejaculatory ducts.

**e) Ejaculatory duct cysts**

The ejaculatory duct is formed by the confluence of the seminal vesicle and the terminal ampullary portion of the vas deferens. The ampulla of the vas deferens can be imaged in both the transverse and sagittal planes. They appear as a pair of oval, convoluted, tubular structures medial to the seminal vesicles and cephalad to the prostate (Kim and Liphultz, 1996). Ejaculatory duct cysts (congenital or acquired) originate from the Wolffian ducts and occupy a paramedian or median position in the prostatic gland above the level of the verumontanum. They can be unilateral or bilateral and etiologies include partial distal obstruction caused by chronic infection, transurethral manipulation, tuberculosis or urethral foreign body andare commonly associated with obstructive azoospermia. Small cysts appear asintra-prostatic masses lateral to the midline at the base and midline at the level of the verumontanum.during TRUS. If the cysts are large

these lesions resemble cystic utricles and prostatic utricle cysts. Small cysts are usually asymptomatic while large ones can cause hematospermia, ejaculatory pain, azoospermia and male infertility.

The condition is diagnosed by TRUS and ultrasonography guided transperineal aspiration of cystic fluid is employed to detect the presence of sperm. Small and asymptomatic cysts do not require treatment but cysts that cause hematospermia, low semen volume, abnormal semen parameters, infertility and infections must be treated. Treatment modalities include simple transrectal aspiration, sclerotherapy of the cyst under TRUS guidance, open surgical removal, and TURED. These treatments usually result in appearance of sperm in the ejaculate and restores fertility.

**Significance of Transrectal Ultrasonography in management of male infertility**

- Evaluation of size, shape and position of Vas deferens, seminal vesicles prostate and their anatomical and pathological changes
- Early diagnosis of carcinoma of the prostate (CAP) based on biopsy results along with abnormal digital rectal examination findings, elevated prostate-specific antigen (PSA) levels, or both (Ultrasonographic findings alone cannot be used to establish or exclude the diagnosis of CAP.)
- Evaluation of men with azoospermia to rule out ejaculatory-duct cysts, seminal vesicular cysts, müllerian cysts, or utricular cysts

Infertile men with primary testicular failure can proceed directly to an advanced assisted reproductive technique such as intracytoplasmic sperm injection or IVF. On the other hand infertile condition arising due to obstructions of the seminal tract may be

amenable through surgical or interventional correction. This has enabled to devise systemic simpler therapies instead of prematurely restoring to Assisted Reproductive Techniques. The rapid progress in diagnostic options employing Ultrasonography and TRUS combined with improvements in the treatment modalities has allowed physicians to treat the infertile male successfully. In case of assessment of infertile individuals, the enhanced ultrasound resolution provided by high-frequency transducers has enabled better resolution with reference to the number of pathologic processes that may be observed within the testes, paratesticular structures, genital ducts, and accessory reproductive organs. Abnormalities that were rarely identified have become easily visible resulting in more precise and appropriate treatment for male infertility.

The present investigation is maiden report in south India in which an attempt has been made to study the male infertility systematically in relation to anatomical and pathological changes in the reproductive organs in Mysore.

**Objectives:**

1. *To carry out physical examination of the subjects with reference to reproductive organs including per rectal examination of the internal genital organs.*
2. *To establish the spermiogram and to carry out hematological analysis through physical and chemical parameters.*
3. *To investigate the reproductive hormones such as Testosterone, Estrogen, Luteinizing hormone (LH), Follicle stimulating hormone (FSH), and Prolactin for the subjects.*
4. *To investigate the external genital organs through Ultrasound scanning by color doppler for testis and epididymis and Trans Rectal Ultrasound Scanning (TRUS) for internal reproductive organs among infertile males.*

***SECTION- II***

# *MATERIALS AND METHODS*

**Study population**

The study subjects were classified according to the diagnosis based on their fertility condition.

**Infertile subjects:** Infertile subjects were selected when two consecutive semen samples (with minimum three to five days of sexual abstinence) were abnormal with respect to the routine parameters mentioned before.

**Control subjects:** This group was recruited with proven fertility. This group either already fathered a child naturally or semen parameters were normal.

**Inclusion criteria**

All males aged between 21-50 years, clinically diagnosed with infertility or sub-fertility including azoospermia, oligospermia, aspermia, asthenospermia, teratozoospermia or combined conditions were included as cases and males with proven fertility who passed all the criteria of the WHO (2010) guideline dealing with spermiogram were included as controls.

**Exclusion criteria**

All males below 21 and above 50 years of age were not considered for the study. Men with obesity, cardiovascular problem, HIV positive and Hepatitis (HBsAg) positive were excluded from the study.

**Source of the samples**

The subjects in the present investigation including infertile males and control group were recruited through Mediwave IVF and fertility research hospital, Mysore.

**Sample size**

A total of 274 clinically diagnosed infertile males as well as 130 healthy fertile males were recruited as controls from the Mediwave IVF and fertility research hospital, Mysore. The age of the subjects ranges between 21 to 50 years old.

**Ethical clearance**

The study was approved by the Institutional Human Ethical Committee (IHEC) of University of Mysore numbered IHEC-UOM No. 54/Ph. D/ 2011-12. Informed consent in English and regional language (Kannada) to participate in the study was obtained from the subjects or their spouse.

**Physical examination**

A general physical examination is an integral part of the evaluation of male infertility. In addition to the general physical examination, particular focus should be given to the genitalia including 1) examination of the penis; including the location of the urethral meatus; 2) palpation of the testes and measurement of their size; 3) presence and consistency of both the vasa and epididymis; 4) presence of a varicocele; 5) secondary sexual characteristics including body habitus, hair distribution and breast development;

and 6) digital rectal exam. The diagnosis of congenital bilateral absence of the vasa deferentia (CBAVD) is established by physical examination.

**Semen Collection and Analysis**

After 3-5 days of ejaculatory abstinence the semen samples were collected in a sterile plastic container by the process of masturbation from the subjects (WHO, 2010). Semen samples were collected in the laboratory room in a clean, dry, biologically inert container. In case of oligozoospermic or azoospermic patients, three semen samples were collected 3 times on different days with three days abstinence and thorough examination was carried out.The collected samples were allowed to liquefy at 37°C for 30 minutes and analyzed within one hour after collection. The semen samples were centrifuged at 3,000 rpm for 10 minutes and the seminal plasma was separated and stored under -80$^{o}$C for further analysis. Macro and microscopic assessment of the semen was carried out to measure semen volume, sperm count, concentration, sperm motility, viability, morphology and leukocyte count according to the World Health Organization (WHO, 2010) Guidelines.

**SEMEN ANALYSIS:**

**Examination of semen by physical characteristics (WHO, 2010)**

**Coagulation:** Semen will be ejaculated in a gel state which starts to liquefy after the ejaculation. Absence of coagulation is indicative of a congenital absence of vas deferens and seminal vesicles.

**Liquefaction:** The gel state of the semen will be liquefied between 20 to 30 min after ejaculation in normal men. Abnormality in the liquefaction is indicative of a problem with the prostate and / or the seminal vesicles.

**Odour:** A normal sample has a distinctive smell which is unpleasant. Occasionally a sample may smell of spices such as garlic and cloves which is quite normal. Malodorous, pungent smelling semen is indicative of an infection, while odourless semen is associated with abnormal prostate function.

**Color:** Most samples have a whitish opacity on ejaculation but after liquefaction they acquire a grayish, translucent color. Creamy, white samples are indicative of a high sperm concentration while those having low sperm concentrations will appear clear.

**Volume:** The volume of the ejaculate will be measured to the nearest 0.1ml in a graduated centrifuge tube. The volume of the sample would be categorized as normal ranges in between (1.5ml to 4.5ml), high (>4.5ml) or low (> 1.5ml). If the volume is less than 1 ml it is important to establish whether it was a complete sample.

**pH:** pH should be determined immediately after liquefaction by placing a drop of semen on to pH paper (range pH 6.4 to 8.0). Normal values range between 7.2 and 7.8 but pH will be higher if samples are left standing prolonged periods.

**Examination of semen by microscopy**

Around 10 to 15 µl of liquefied semen was placed on to a clean glass slide using a Pasteur pipette. Then covered with cover slip and examined under phase contrast microscopy using a 40x objective or under light microscopy with the condenser lowered. The following aspects studied from the microscopy were as follows:

**Motility:** A simple method for grading motility was recommended by WHO (1999). That distinguishes spermatozoa based on the motility rate such as progressive or non-progressive motility from those which are immotile. The motility of each spermatozoan was graded as follows

1. Rapid linear and progressive (**grade a**)
2. Sluggish, linear and progressive(**grade b)**
3. Non progressive (**grade c**)
4. Immotile (**grade d**)

Within a given microscopic field, all spermatozoa with **grade a** and **b** were counted first. Subsequently spermatozoa with non-progressive motility and immotile spermatozoa were counted in the same field. Motility of at least 200 different spermatozoa were observed and expressed in percentage.

**Sperm Vitality:**

Sperm vitality was estimated by using eosin and nigrosin staining to assess the membrane integrity of the cells. The percentage of viable cells normally exceeds that of motile cells.

**Vitality test using eosin–nigrosin**

This one-step staining technique uses nigrosin to increase the contrast between the ackground and the sperm heads, which makes them easier to discern. It also permits slides to be stored for re-evaluation and quality-control purposes (Bjorndahl *et al.,* 2003).

**Procedure**

A drop of 1% aqueous solution of eosin-Y and 10% aqueous solution of Nigrosin was taken in a tube. A drop of well mixed semen was added and mixed well using Pasteur pipette. A wet preparation of this mixture was observed under optical microscope. Dead sperms were stained red and live ones will remain unstained. At least 200 spermatozoa were counted and the incidence of live versus dead spermatozoa was estimated and expressed in percentage.

**Sperm and germ cell morphology by Papanicolaou staining:**

Papanicolaou staining is the widely used procedure for examination of germ cell morphology since it distinguishes clearly between basophilic and acidophilic cell components and allows a detailed examination of the nuclear chromatin pattern. This method gives a optimal results for analysis of sperm morphology and immature male germ cell.

**(Stains used: Orange-G, EA-36, Haematoxylin)**

**Procedure:** Wet smear of the semen sample was prepared in such a way that all the sperms lie in a single focal plane. Slides were air dried and fixed in ether-alcohol (1:1) mixture for 20 minutes.

**Staining Method:**

| | | |
|---|---|---|
| 95% Alcohol | - | 1x 10 dip |
| 50% Alcohol | - | 1x 10 dip |
| Running water | - | 2minutes |
| Haematoxylin | - | 2 minutes |
| Running water | - | 2minutes |
| Alcoholic ammonia | - | 1x 10 dip |
| 70% Alcohol | - | 2x 10 dip |
| 95% Alcohol | - | 2x 10 dip |
| Orange G | - | 4 minutes |
| 95% Alcohol | - | 2x 10 dip |
| EA 36 | - | 4 minutes. |
| 95% Alcohol | - | 2x 10 dip |
| 100% Alcohol | - | 3x 10 dip |
| Xylene | - | 1x 10 dip |

After 30minutes, slide was cleared in Xylene and mounted in DPX. Slides were analyzed under optical microscope using an oil immersion x100 objective and enumerated different types of sperm morphology abnormality

**Estimating Sperm concentration (sperm count) by cytometry**

**Solutions:**

Dilution media was prepared by dissolving 50 g of sodium bicarbonate in 10ml 0f 40% formalin, 5ml of a saturated aqueous solution of gentian violet was added and make up to final volume of 1000 ml with distilled water.

**Procedure:**

Around 10 - 15µl of semen sample was taken onto a cytometer and a cover slip was placed over it. The sample was allowed to settle down for about 5 minutes. High (>100 X 106. ml-1) density semen samples would require further dilution while low (<10 X 106. ml-1) density samples would require lesser dilutions. Number of spermatozoa in the central square of the Neubauer counting chamber was counted. The number of squares was examined for sperm enumeration will depend on the average number of spermatozoa present in a square. If there are <10 spermatozoa per squares should be examined, the occurrence of 10 – 40 spermatozoa per square would necessitate counting of 10 squares, 5 squares should be examined if there are >40 spermatozoa per square.

**Statistical analysis**

The obtained data were subjected to statistical analysis using statistical software SPSS version 16.0. Data were expressed as Mean± Standard deviation. Student t-test, one way analysis of variance (ANOVA) was done to compare means. Statistical significance was analyzed by Chi-square test. Pearson correlation was performed to assess the linear relationship between semen parameters.

**HORMONE ANALYSIS OF THE SUBJECTS THROUGH ELISA**

All study subjects have undergone sex hormone analysis including FSH, LH, Testosterone, Esteradiol and Prolactin using ELISA kit method (ERBA, DRG, ).

**Serum Follicle Stimulating Hormone (FSH):**

Human follicle-stimulating hormone (FSH, follitropin) is a glycoprotein produced and secreted by the basophilic cells of the anterior lobe of the pituitary gland. Secretion of FSH is stimulated by gonadotrophin-releasing hormone (GnRH). In men, determination of FSH is useful in the diagnosis infertility, hypogonadism, gynaecomastia and tumours in the reproductive organs.

**Material required:**

- A micro titre plate calibrated reader (eg., the DRG instruments micro titer plate reader).
- Calibrated variable precision micropipettes
- Absorbent paper
- Distilled water
- Semi logarithmic Figure paper or software for data reduction

**Preparation of Sample**

Dilute samples with concentrations above 135mlU/mL 1:1 with test distilled water

**Procedure:** 5 standards and 1 blank were included

- Micro wells from pouch were removed, and required micro well was taken and unused stripes were returned in the sealed pouch to refrigerator. Around 25µl of calibrators and patient samples were pipette into the wells and incubated at room temperature for 15 minutes.

- 100µl of Enzyme conjugate was added to the wells except for blank well and incubated 15minutes at room temperature.
- After 15 minutes, 300µl of distilled water was added (decant or aspirate). This step was repeated for 4 additional times.
- 100µl of Substrate solution was pipette into each micro well using the same order and timing as for the addition of the substrate solution.
- 100µl of stop solution was added into each micro well using the same order and timing as for the addition of the substrate solution.
- Absorbance of each micro well at 450 nm against blank was taken using a micro plate reader (BIOTEK ELX800-MS).

**Normal Reference Values:**

| **Sample** | **Range mlU/mL** |
|---|---|
| Male | 2.0-14.0 |

**Serum Lutenizing hormone (LH):**

The LH test is based on simultaneous binding of human LH to two monoclonal antibodies, one immobilized micro well plates, and the other conjugate with horseradish peroxidase (HRP). After incubation the bound separation is performed by a simple solid-phase washing, and then the substrate solution is added. After an appropriate time has elapsed for maximum color development, the enzyme reaction is stopped and the absorbance was determined. The color intensity is proportional to the LH concentration in the sample.

**Material required:**

- A micro titter plate calibrated reader (e.g. the DRG instruments micro titter plate reader).
- Calibrated variable precision micropipettes
- Absorbent paper
- Distilled water
- Semi logarithmic Figure paper or software for data reduction

**Preparation of samples:**

Samples with concentration over 200 mlU/ ml, dilute the sample 1:1 with standard A.

**Test procedure:** 5 samples and 1 blank was included,

- Micro wells from pouch were removed, taken required micro well were taken and returned unused stripes in the sealed pouch to the refrigerator. The micro wells were carefully placed into the extra provided holder.
- 25µl of standards and 25µl patient samples were pipette into the wells and incubated for 10 minutes at room temperature
- 100 µl Enzyme conjugate was added to the well except for blank well and incubated for 60 minutes at room temperature.
- Approximately 300µlof distilled water was added, decant (tap or blot) or aspirate. This step was repeated for 4 additional times.
- 100µl of substrate solution was pipetted into each micro well in the same order and timing as for the enzyme conjugate, including the blank well and incubated for 10 minutes at room temperature in the dark.

- 100µl of stop solution was added into each micro well using the same order and timing as for the addition of the substrate solution.
- Absorbance of each micro well at 450 nm against blank was taken using a micro plate reader (BIOTEK ELX800-MS).

**Normal reference values for men:** Range 4 .0 to 10.0 mlU/mL

**Serum Testosterone:**

Testosterone is a C19 steroid with an unsaturated bond between c-4 and c-5, a ketone group in c-3 and a hydroxyl group in the beta position at c-17.This steroid hormone has a molecular weight of 288.47. Testosterone is the most important androgen secreted into the blood. In males, testosterone is secreted primarily by the Leydig cells of the testis, Testosterone is responsible for the development of secondary male sex characteristics and its measurements are helpful in evaluating the hypogonadal states. In men, high levels of testosterone are associated to the hypothalamic pituitary unit diseases, testicular tumors, congenital adrenal hyperplasia and prostate cancer. Low levels of testosterone can be found in patients with the following diseases: Hypopituitarism, Klinefelter's syndrome, Testicular feminization, Orchidectomy and Cryptorchidism, enzymatic defects and some autoimmune diseases.

**Material required:**

- A micro titer plate calibrated reader (eg., the DRG instruments micro titre plate reader).
- Calibrated variable precision micropipettes
- Absorbent paper

- Distilled water
- Semi logarithmic Figure paper or software for data reduction

**Procedure:**

- 25 µl of each standard, control samples were dispense with new disposable tips into appropriate wells.
- 200 µl enzyme conjugate was dispensed into each well and thoroughly mixed for 10 seconds. (It is important to have completed mixing in this step) and incubated for 60 minutes at room temperature.
- After 60 minutes of incubation briskly shook out the contents of the wells and rinsed the wells for 3 times with diluted wash solution.
- 200 µl of substrate solution was added to each well and incubated for 15 minutes at room temperature.
- After incubation the enzymatic reaction was stopped by adding 100µl of stop solution to each well.
- Absorbance (OD) of each well at 450(±10) nm was determined using micro plate reader (BIOTEK ELX800-MS).
- **Normal reference values formen :** Range : 2.0 to 6.9 ng/ml

**Serum Prolactin**

Prolactin is a hormone secreated from the lactotrophs of the anterior pituitary consisting of a single polypeptide chain containing approcimately 200 amino acids. Prolactin may be involved in steroidogenesis in the gonads, acting synergistical with

luteinizig hormone. High levels of prolactin appers to inhibit steroidogenesis as well as inhibiting LH and follicuole stimulatin hormones synthesis at the pituitary gland.

**Material required:**

- A micro titter plate calibrated reader (e.g. the DRG instruments micro titter plate reader).
- Calibrated variable precision micropipettes
- Absorbent paper
- Distilled water
- Semi logarithmic Figure paper or software for data reduction

**Procedure:**

- 25 µl of each standard, control samples were dispense with new disposable tips into appropriate wells.
- 100 µl enzyme conjugate was added to each well and and incubated for 60 minutes at room temperature.
- After 60 minutes of incubation briskly shook out the contents of the wells and rinsed the wells for 3 times with diluted wash solution.
- 100 µl of TMB substrate solution was added to each well and incubated for 15 minutes at room temperature.

- After incubation the enzymatic reaction was stopped by adding 50µl of stop solution to each well. Absorbance of each well at 450(±10) nm was determined using micro plate reader (BIOTEK ELX800-MS).

**Normal reference valuesfor men:** Range 1.82 to 17.0 mlU/mL

**SerumEstrodiol**

**Material required:**

- A micro titter plate calibrated reader (e.g. the DRG instruments micro titter plate reader).
- Calibrated variable precision micropipettes
- Absorbent paper
- Distilled water
- Semi logarithmic Figure paper or software for data reduction

**Procedure:**

- 25 µl of each standard, control samples were dispense with new disposable tips into appropriate wells.
- 200 µl enzyme conjugate was added to each well and and incubated for 2hours at room temperature.
- After 2 hours of incubation briskly shook out the contents of the wells and rinsed the wells for 3 times with diluted wash solution(1 ml wash buffer + 39 ml of distilled water).

- 100 µl of substrate solution was added to each well and incubated for 15 minutes at room temperature.
- After incubation the enzymatic reaction was stopped by adding 50µl of stop solution to each well. Absorbance of each well was determined using micro plate reader (BIOTEK ELX800-MS)

Normal values in men: Range: 11 to 36 pg/ml

**COLOUR DOPPLER SCANNING AND TRANS RECTAL ULTRASOUND SCANNING (TRUS) OF THE STUDY SUBJECTS**

The principal application of transrectal ultrasonography in the infertile men is to evaluate patency of distal ductal system (Vas deferens) and internal genital organs. Although gold standard for evaluation of male ductal system is vasography, TRUS has the advantage of being non-invasive. Transrectal ultrasound allows visualization of seminal vesicles, prostate and ejaculatory ducts.

**Preparation, positioning, and contraindications**

For Transrectal scannig fasting is not advised, but the patient is instructed to empty his bladder before the examination. It is advisable to do the scanning in empty rectal condition. For this, a purgative (Dulcolax 2 tablets) is given to the patient to be taken in the previous night. He is advised to empty the bowel before scanning.

Subjects were asked to remove their inner garments. Scanning can be done in left lateral, lithotomy, or knee-elbow positions. Lithotomy was most comfortable position for subjects in our study and this was done. Knee joints were supported using supportive strands attached to a gynecological examination table. Two leggings were used to cover

the legs for patient's and examiner's convenience. To make the US room more comfortable for the patient, the room was kept semi dark during assessment. The patients were explained the technique of the scanning. For transrectal ultrasound, the ultrasound transmissiongel was applied to the endorectal transducer and it was covered with a sterile probe cover or a sterile condom.

Although, in our study, the procedure was performed without any infiltrative anesthesia in the past it is a common practice to use lidocaine infiltration in the periprostatic area. Pareek *et al*., (2001) described a technique of periprostatic nerve blockade. Accordingly 2.5 mL of lidocaine was injected (using a 5-in 22-gauge spinal needle through the ultrasound probe) on each side at the prostate base at the junction of the prostate and the seminal vesicle. In a randomized, double-blind, placebo-controlled study, Pareek *et al*., (2001) showed significant pain control during and after biopsy. Alavi *et al.*, (2001) compared the efficacy of intrarectallidocaine gel with that of periprostatic nerve block and concluded that the nerve block was superior for pain control. Using this technique, saturation biopsies, with up to 20 cores, could be performed. However, if only diagnostic TRUS is done, without taking biopsy, as in this study, no need of injection is required as it is almost a painless procedure.

Currently, the most widely used probe is a 7-MHz transducer within an endorectal probe, which can produce images in both the sagittal and axial planes. Scanning begins in the axial plane, and the base of the prostate and seminal vesicles are visualized first. A small amount of urine in the bladder facilitates the examination. Seminal vesicles are identified bilaterally, with the ampullae of the vas on either side of the midline. The seminal vesicles are convoluted cystic structures that are darkly anechoic. Men who have

abstained from ejaculation for a long period may have dilated seminal vesicles.Measurements were taken. Length of the seminal vesicle is taken using dotted lines along the curvature of the organ in its midline. Width is taken at the midpoint. Volume is calculated using inbuilt calculator in the scanning machine. Vas deferens is visualized and the diameter of the vas deferens was measured.

Next, the base of the prostate is visualized.Volume assessment of the prostate is an important and integral part of this procedure. Several formulas have been used, the most common of which is the ellipsoid formula, which requires measurement of three prostate dimensions. The length and breadth of the prostate was taken in the longitudinal axis of the prostate. Thickness was taken in the transverse axis. The ellipsoid volume formula is then applied, as follows:Prostatic volumes were calculated by using the ellipsoid volume formula of: length × breadth × thickness × 0.52. This was available in inbuilt calculator of volume in the software of the scanning machine Prostatic scanning was done in various slices.

The central zone comprises the posterior part of the gland and is often hyperechoic. The mid gland is the widest portion of the gland. The peripheral zone forms most of the gland volume. Echoes are described as isoechoic and closely packed.

The transition zone is the central part of the gland and is hypoechoic. The junction of the peripheral zone and the transition zone is distinct posteriorly and is characterized by a hyperechoic region, which results from prostatic calculi or corpora amylacea. The transition zone is often filled with cystic spaces in patients with benign prostatic hyperplasia (BPH).

Scanning at the level of the verumontanum and observing the Eiffel tower sign (anterior shadowing) help to identify the urethra and the verumontanum. The prostate distal to the verumontanum is composed mainly of the peripheral zone. The capsule is a hyperechoic structure that can be identified all around the prostate gland. Several hypoechoic rounded structures can be identified around the prostate gland. These are the prostatic venous plexi. The position of the neurovascular bundles can often be identified by the vascular structures. Imaging in the sagittal plane allows visualization of the urethra. The median lobes of the prostate are often visualized.

**Scrotal scanning:**

**Preparation:** For scrotal ultrasound patient is instructed to shave the parts and come for scanning.An ultrasound examination of was done per cutaneous to investigate the testis. Testes and the epididymal tail were visualized in longitudinal plane scrotal wall, with the later present at the distal extremity of testes.

The scanning was done by using latest 4D color Doppler scanner manufactured by Shenzhen Mindray Biomedical Electronics Co., Ltd., Germany, with linear-array 10Mz transducer. The sector transducer was used whenever testis was larger than the size of the linear transducer. Scanning was performed with the patient in the supine position. The scrotum was supported by a towel between the thighs. Ultrasound gel was applied over the scrotum. Testicular scanning was done in both longitudinal and transverse axis. The length and breadth of the testis was taken in the longitudinal axis of the testis. Thickness was taken in the transverse axis. Testicular volumes were calculated by using the empiric formula of Lambert: length × breadth × thickness × 0.71. This was available in inbuilt calculator of volume in the software of the scanning machine. Then, the testis and

paratesticular area, mediastinum testis, epididymal head, epididymal body, and epididymal tail were examined sequentially.

Spermatic chord and vasculature were studied. Additional techniques such as Valsalva maneuver was used for venous evaluation. Enlargement of veins was assessed. Diameter of veins was measured both in resting posture as well as in Valselva maneuver. Presence of Varicose veins if any was noted. Gradations of varicosity was done.

***SECTION III***

# *OBSERVATION*

**Age:**

The age distribution of the participants in both infertile and control males is shown in **Table 1**. Of the 274 infertile subjects, 22.3% of them were between 21-30 years, 69.7% of them were between 31-40 years, 8.3% of them were between 41-50 years. Among control subjects, 32.3% of them were between 21-30 years, 56.9% of them were between 31-40 years and 10.8% of them were between 41-45 years (**Table1** and **Figure 7** ).

**Anthropometric measurement:**

Anthropometric assessment including height, weight, and BMI were performed for all participants. BMI was used as the main criteria to categorize subjects in to obese subjects and non-obese group. According to BMI, subjects were divided into 3 groups. Cases with BMI between 25 to 29.9 $kg/m^2$ were considered as overweight, BMI between 30-34.9 $kg/m^2$ as obese and subjects with BMI above 35 $kg/m^2$ include obese type II and III or morbid obesity.

Accordingly, in infertile group, 7.66% had BMI < 20 $kg/m^2$ , 45.98% had BMI between 20-24.9 $kg/m^2$, 32.84% of them were between 25-29.9 $kg/m^2$, 13.5% had BMI >30-34.9 $kg/m^2$. In control group, 11.53% had BMI < 20 $kg/m^2$, 41.53% had BMI between 20-24.9 $kg/m^2$, 37.69% of them were between 25-29.9 $kg/m^2$ and 9.23% had BMI above 30 $kg/m^2$. Data obtained from the analysis of BMI criteria showed no statistical differences between obese infertile and obese fertile groups as shown in **Table 2** and **Figure 8**.

**Seminal analysis**

Semen analysis is the most important test in the evaluation of an infertile male. In fact it remains the cornerstone for detection of male infertility. Further, male factor infertility has been linked with numerous irregularities including sperm count motility, viability and morphology.

**According to the obtained data from semen analysis infertile groups were classified into different subgroups as below (Table 3)**

**Aspermia:** In the present investigation, 5.1% of infertile males were unable to give a semen sample either due to severe erectile dysfunction or obstruction.

**Azoospermia:** Among 274 infertile males, 98 (35.7%) of them including had no sperm in their semen samples after 2 attempts. These cases were considered as azoospermic cases.

**Oligozoospermia:** In the present study, 20 (7.3%) of infertile males were found to be oligozoospermic (< $20\times10^6$/ml of semen).

**Asthenozoospermia:** Among infertile males,31 (11.3%) showed low progressive motile sperm (< 50%) than controls.

**Oligoasthenzoospermia:** Among infertile group, 41 (14.9% of them were found to be oligosthenozoospermic, a condition with low sperm (< $20\times10^6$/ml of semen) count and <50% progressive motile sperm.

**Oligoasthenoteratonecrozoospermia:** Out of 274 infertile males in the present study, 17.5% were diagnosed with oligoasthenoteratonecrozoospermia, a combination of low

sperm count, less sperm motility and high abnormal sperm morphology associated with necrosis.

**Oligoasthenonecrozoospermia:** In the present study, 6.2% were diagnosed with oligoasthenonecrozoospermia, a combination of low sperm count, less sperm motility associated with necrosis.

**Oligoasthenoteratospermia:** Out of 274 infertile males in the present study, 1.8% were diagnosed with oligoasthenoteratospermia, a combination of low sperm count, less sperm motility and high abnormal sperm morphology.

**Table 4** shows theage-wise distribution of different infertility among infertile group. Data revealed that all aspermic cases were belonging to the last age group (41-50 yrs). In azoospermic subjects, 6% were 21-30 yrs, 6% were 31-40 yrs and 7% were above 40 years old. 1% of oligospermic subjects were 21-30 yrs, 1.5% were between 31-40 yrs and 5% were above 40 years old. All 3 asthenospermic subjects were between to 31-40 yrs and 41-50 yrs and the only teratozoospermic case was belonging to 41-50 yrs age group. In OAT subgroup, 2 cases were between 31-40 yrs and 5 subjects were above 40 yrs and in OAT subgroup, most of the subjects (5.5%) were belonging to third age group followed by 3% of the subjects were between 31-40 yrs and 5 subjects (**Table 4**).

In infertile group, BMI frequency was analyzed and its distribution among infertile subgroups is shown in **Table 5**. In aspermic subgroup, 2 subjects had BMI < 20 $kg/m^2$, 6 had BMI between 20-24.9 $kg/m^2$, 3 of them were between 25-29.9 $kg/m^2$ and 3 had BMI above 30 $kg/m^2$. In azoospermic subgroup, 11 had BMI < 20 $kg/m^2$, 51 had BMI between 20-24.9 $kg/m^2$, 25 of them were between 25-29.9 $kg/m^2$ and 11 had BMI above 30 $kg/m^2$. In oligospermic subgroup, non of them had BMI < 20 $kg/m^2$, 8 had BMI

between 20-24.9 kg/$m^2$, 11 of them were between 25-29.9 kg/$m^2$ and 1 of them had BMI above 30 kg/$m^2$. In asthenozoospermic subgroup, 1 subject had BMI < 20 kg/$m^2$ , 13 had BMI between 20-24.9 kg/$m^2$, 11 of them were between 25-29.9 kg/$m^2$ and 6 had BMI above 30 kg/$m^2$. In oligoasthenozoospermic subgroup, 3 had BMI < 20 kg/$m^2$ , 17 had BMI between 20-24.9 kg/$m^2$, 16 of them were between 25-29.9 kg/$m^2$ and 5 had BMI above 30 kg/$m^2$. In oligoasthenoteratonecrozoospermic subgroup, 2 had BMI < 20 kg/$m^2$ , 22 had BMI between 20-24.9 kg/$m^2$, 19 of them were between 25-29.9 kg/$m^2$ and 5 had BMI above 30 kg/$m^2$. In oligoasthenoteratozoospermic subgroup, all 5 cases had BMI between 20-24.9 kg/$m^2$.

Assessment of semen characteristics with respect to their BMI among both groups has been depicted in **Table 6.** Data showed that in subjects with BMI <20, infertile subjects had impaired semen parameters when compared with the control males. Sperm count was the only parameter that its value was between normal levels. In subgroup with BMI between 20-25 kg/$m^2$, a considerable decrease was observed in all the semen parameters included in the study among infertile subjects. The similar data was obtained for the two subgroups with BMI of 20-25 and >30 kg/$m^2$.

**Table 7** shows mean and standard deviation of different parameters in both infertile and control groups. Data obtained by physical semen assessmentamong infertile group is as follows. Out of 274 subjects, 147 (53.6%) of them showed abnormal liquefaction time and in control subjects, 21 (7.6%) cases were found with abnormal liquefaction time and the rest of subject in both groups expressed normal values (**Table 7).**

In the present investigation, among 274 infertile subjects, 185 individuals (67.5%) had abnormal semen pH values (7.2-8.2) and rest of the infertile subjects expressed a

normal pH value. While among control group 115 subjects (88.4%) had normal and 15 subjects (11.6%) showed abnormal semen pH (**Table 7).**

Independent t-test with respect to motility as a variable revealed that the infertile males showed the lower progressive motility values (9.59±14.46) compared to controls (54.41±12.25) and this difference was significant ($p<0.0001$) at 95% confidence interval (CI) (**Table8**).

Comparison of sperm viability between study groups showed a significant difference. Paired t-test results showed that like sperm count, motility and sperm viability was significantly decreased in infertile group (27.82±28.98) compared to control group (72.05±10.55) ($p<0.0001$).

Results of independent t-test for sperm and germ cell morphology assessment showed the higher values in control group (17.55±11.03) when compared with infertile group (4.50±6.74) and the difference was significant (*$p<0.01$* ) (**Table 8**). Pearson correlation analysis showed a significant negative relationship between BMI and all the quantitative semen parameters (**Table 10**) but the relationship between age and quantitative semen characteristics was negatively significant only for sperm viability and morphology (**Table 9**).

With respect to presence or absence of fructose in semen, data obtained in the present study revealed that the fructose was present in all 130 controls. In infertile group out of 258 subjects who were subjected for the fructose test showed complete absence of fructose in 3 (1.2%) subjects and partially detected in 19 (7.4%) subjects.

**Coital frequency**

In the present study, analysis showed that coital frequency per week was 2.71±1.29 among infertile group and 2.35±1.37 in control subjects (**Table 7**). No significant relationship was observed between coital frequency, age and BMI (**Table 8**).

**Consanguinity**

With respect to type of marriage among subjects included in the present study, in infertile group, 19 subjects were unmarried, 234 subjects had non-consanguineous marriage and 35 couples were found with consanguinity. In control group, out of 130 subjects, 5 of them were unmarried, 6 of them were found with consanguinity and 119 subjects with non consanguineous marriage **(Figure 9).**

**Assessment of Hormone**

Description of the infertile and control males subjected for assessment of hormones are shown in **Table 11**. Statistical analysis using Levene's independent test for hormones in both groups was performed and the data is depicted in and **Table 12**.

Data revealed that **LH** levelwas higher in infertile males (5.59± 5.07) when compared to the controls (4.55± 3.13, p=<0.05) **(Table 11).**

**FSH** levels was found to be higher in infertile males (10.28±10.65) when compared to the controls (4.89±3.05) and the difference was significant (p=< 0.001).

Datashowed that **Prolactin** levels were lower in controls (11.85±7.17) when compared with the infertile (12.48±9.95) but the value was not significantly different between groups (p=0.62) at 95% level (**Table 11 and Table 12**).

In the present study, control group showed higher levels of **Testosterone** when compared to the controls and the difference was significant at p<0.01 (12.74±67.6) **(Table11 and Table 12**).

**Estradiol** levelswere found to be higher in infertile group (41.35±39.97) when compared the controls (33.71±16.08) but the difference was not significant (p=0.061) **(Table11 and Table 12).**

**Colour Doppler scanning and Trans Rectal Ultrasound Scanning (TRUS) of subjects for reproductive organs**

**Testicular volume**

In the present study, mean value of right testicular volume was 11.83±3.20cc in control group that was significantly higher when compared with the infertile subjects (7.62±4.05, p=0.0001).Similarly, the left testis volume was found to be significantly higher in controls (11.08±2.84) than infertile group (6.99±3.60) (**Table13).** Moreover, the mean of total testicular volume was also higher in control group (22.91±5.38) when compared with the infertile group (14.61±7.21) and the difference was significant (p=<0.0001) at 95% (**Table14).**

Significant positive correlation was seen between total testicular volume and semen volume (r=0.172, p<0.0001) that might be because the testis contributes 5 % of the semen volume. Pearson correlation test was strongly significant between testicular volume and total sperm count (r=0.508, p<0.0001). Highly positive relationship was observed between testicular volume and sperm motility (r=0.476, p<0.0001). Testicular volume was also observed to be significantly lower in men with low semen volume (2.0 ml vs. 22.91 in men with normal semen volume) **(Figure 12).** Both sperm count/ml and total

Sperm count were directly related to total testicular volume (**Figure 13).** 69.9% (267) of men had normal total sperm count per ejaculate (39 million and above) demonstrating the mean testicular volume of 20.63 ml. Mean sperm count per ejaculate was 84.86 millions **(Table 15).Figure14** shows total motility with total testicular volume excluding azoospermia and aspermia. **Figure15**shows Image of ultrasound scanning of the normal testis with respect to the testicular volume andsubject with testis hypoplasia was shown in the **figure 16** in which the testis volume is less than normal.

**Epididymis**

In our study, out of 274 infertile subjects, 22 (8%) subjects were found with cyst in right epididymis, 16 (5.8%) subjects with cyst in left epididymis and 11 (4%) subjects were detected to have a cyst in bilateral. Further, 2 (0.7%) subject were detected with epididymis atrophy and the rest of the subjects were normal with respect to epididymis.In the present study **figure 18** shows the image of ultrasound scanning of the normal epididymis andabnormal epididymis with cysts was shown in the **figure 19.** The accumulation of fluid in the epididymis was represented in the **figure 20.**

**Prostate volume**

In the present study, **table 16** shows the description of the study groups for prostate volume assessment. In the present study independent t-test revealed that the mean value of prostate volume was 12.38±3.98cc in infertile group that was significantly lower when compared with the control subjects (14.16±4.16, p=0.0001**)( Table 17).**

Significant positive correlation was seen between prostate volume and age, BMI semen volume, sperm count, total sperm count, sperm motility, normal sperm

morphology and sperm viability. Pearson correlation test values were negative between prostate volume and pH (r=-0.040, p=0.450) that shows the relation was not significant. Similarly, no significant negative relationship was observed between prostate volume and semen liquefaction time (r=-0.026, p=0.628) **(Table 18).Figure 21** shows the image of ultrasound scanning of the normal prostate whereas **Figure 22 a) and b)** represent image of ultrasound scanning of the abnormal prostate with calcification. Image of ultrasound scanning of the abnormal prominent prostatic cyst was seen the **figure 23.**

**Seminal vesicle volume**

In the present study, mean value of right seminal vesicle volume was 0.97±1.09cc in infertile group that was lower when compared with the control subjects (1.05±0.93) but the difference was not significant (p>0.05). Accordingly, the left seminal vesicle was found to be not significantly higher in controls (1.05±0.95) than infertile group (0.95±1.12, p>0.05) (**Table 19).**

Pearson correlation test revealed a significant positive relationship between left seminal vesicle volume and BMI (r=0.113, p=0.032) but not with right seminal vesicle volume and BMI. No significant relationship was observed between age and both right and left seminal vesicle volume. A significant correlation was found between semen volume and left seminal vesicle volume (r=0.124, p=0.019). Sperm viability showed a significant positive correlation with both right and left seminal vesicle volume. The highly significant positive relationship was also observed between right seminal vesicle volume and left seminal vesicle volume (r=0.829, p<0.0001) **(Table 20)**. **Figure 24** represents the image of ultrasound scanning of normal seminal vesicle and **Figure 25** with abnormal cystsin the seminal vesiclein the present study.

**Vas deferens**

In the present study in 17 of the infertile men, vas deferens could not be visualized on both the sides. Both control and rest of the infertile individuals are with normal Vas deferens. **Figure 26** shows the image of ultrasound scanning of normal Vas deferens

**Varicocele**

In our study, out of 274 infertile subjects, 8 (2.9%) of them were detected with right varicocele, 40 (14.6%) of them were found to be associated with left varicocele and 51 (18.6%) of them were detected with bilateral varicocele (**Figure 10**). In the present study **Figure 27** shows the image of ultrasound scanning of left varicocele

**Hydrocele**

In current research, out of 274 infertile subjects, 14 (5.1%) of them were detected with right hydrocele, 19 (6.9%) of them were found to be associated with left hydrocele and 35 (12.7%) of them were detected with bilateral varicocele (**Figure 11).** In the present study the image of ultrasound scanning of the abnormal testis with hydrocele was shown in the **figure 17**

**Table 1: Age-wise distribution of 274 Infertile men and 130 controls (n= number of study subjects).**

| Conditions | 21-30 Years | | 31-40 Years | | 41-50 Years | |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % |
| Infertile (n=274) | 61 | 22.3 | 191 | 69.7 | 22 | 8.3 |
| Control (n=130) | 42 | 32.3 | 74 | 56.9 | 14 | 10.8 |

**Table 2: Distribution of subjects with respect to Body Mass Index (BMI) in both study groups (n= number of study subjects).**

| BMI | <20 Kg/m$^2$ | | 20-24.9 Kg/m$^2$ | | 25-29.9 Kg/m$^2$ | | >30 Kg/m$^2$ | | Total (%) |
|---|---|---|---|---|---|---|---|---|---|
| Group | No. | % | No. | % | No. | % | No. | % | |
| Infertile (n=274) | 21 | 7.66 | 126 | 45.98 | 90 | 32.84 | 37 | 13.50 | 274 (100) |
| Control (n=130) | 15 | 11.53 | 54 | 41.53 | 49 | 37.69 | 12 | 9.23 | 130 (100) |

**Table 3: Distribution of different infertility conditions among infertile subjects. (n= number of infertile men).**

| Cases<br>Conditions | | Infertile<br>(n=274) | |
|---|---|---|---|
| | | **No. Of subjects** | **Percentage(%)** |
| **1.** | **Aspermia** | 14 | 5.1 |
| **2.** | **Azoospermia** | 98 | 35.7 |
| **3.** | **Oligozoospermia** | 20 | 7.3 |
| **4.** | **Asthenozoospermia** | 31 | 11.3 |
| **5.** | **Oligoasthenospermia** | 41 | 14.9 |
| **6.** | **Oligoasthenoteratonecrospermia** | 48 | 17.5 |
| **7.** | **Oligoashtenonecrozoospermia** | 17 | 6.2 |
| **8.** | **Oligoasthenoteratospermia** | 5 | 1.8 |
| | **Total** | **274** | **100** |

**Table 4 : Age-wise distribution of different infertility among infertile group (n=274)**

| Conditions | Age group in years | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | 21-30y | | 31-40y | | 41-50y | | |
| | No. | % | No. | % | No. | % | |
| Aspermia | 1 | 0.36 | 11 | 4 | 2 | 0.7 | 14 |
| Azoospermia | 30 | 10.9 | 58 | 21.2 | 10 | 3.6 | 98 |
| Oligozoospermia | 5 | 1.3 | 12 | 4.4 | 3 | 1.1 | 20 |
| Asthenozoospermia | 5 | 1.3 | 20 | 7.2 | 6 | 2.2 | 31 |
| Oliogasthenozoos-spermia | 10 | 3.6 | 25 | 9.1 | 6 | 2.2 | 41 |
| Oliogasthenteratonecrozoospermia | 10 | 3.6 | 38 | 13.8 | - | - | 48 |
| Oliogasthenonecrozoospermia | 3 | 1.1 | 12 | 4.4 | 2 | 0.7 | 17 |
| Oligoasthenoteratoz-oospermia | 1 | 0.36 | 3 | 1.1 | 1 | 0.36 | 5 |

**Table 5 : Body mass index(BMI) and frequency of infertile conditions among all male subjects(n= number of study subjects).**

| BMI Conditions | <20 (n=36) | >20<25 (n=180) | >25<30 (n=139) | >30 (n=49) |
|---|---|---|---|---|
| **Normal (n=130)** | 15 | 54 | 49 | 12 |
| **Aspermia (n= 14)** | 2 | 6 | 3 | 3 |
| **Azoospermia (n= 98)** | 11 | 51 | 25 | 11 |
| **Oligospermia (n=20 )** | - | 8 | 11 | 1 |
| **Asthenospermia (n=31)** | 1 | 13 | 11 | 6 |
| **Oligoasthenospermia (n=41)** | 3 | 17 | 16 | 5 |
| **Oligoasthenoteratonecrospermia (n=48)** | 2 | 22 | 19 | 5 |
| **Oligoasthenonecrospermia (n=17)** | 2 | 4 | 5 | 6 |
| **Oligoasthenoteratospermia (n=5)** | - | 5 | - | - |

**Table 6: Distribution of different semen parameters with respect to BMI in both groups.**

**(Azoospermia and Aspermia were excluded)**

| BMI | No. of Subjects | Volume | Count | Total Count | Motility | Vitality |
|---|---|---|---|---|---|---|
| **<20** | Normal-15 | 2.07±0.77 | 64.64±24.33 | 133.03±71.18 | 56.14±11.31 | 70.21±11.57 |
| | Infertile-8 | 1.13±0.67 | 25.75±32.89 | 15.16±18.03 | 17.5±11.63 | 48.5±23.77 |
| **>20-<25** | Normal -54 | 2.03±0.61 | 59.65±21.18 | 120.93±58.88 | 56±12.76 | 73.75±10.54 |
| | Infertile- 69 | 1.69±0.79 | 15.78±17.9 | 23.18±24.43 | 15.34±15.01 | 47.33±22.16 |
| **>25-<30** | Normal-49 | 1.95±0.75 | 60.61±22.56 | 116.53±65.82 | 53.38±10.78 | 71.87±10.85 |
| | Infertile-62 | 1.62±0.75 | 16.28±16.16 | 23.91±24.64 | 18.74±17.33 | 49.85±20.94 |
| **>30** | Normal-12 | 2.35±1.11 | 60.5±13.35 | 138.64±63.47 | 58.7±9.61 | 73.2±7.84 |
| | Infertile-23 | 1.77±0.70 | 16.14±15.55 | 27.00±25.56 | 17.33±15.00 | 46.58±22.12 |

**Table 7: Mean and standard deviation of spermiogram among both groups.**(CF=Coital Frequency, BMI= Body mass index, SV=Seminal volume, SC=Sperm count, TSC= Total sperm count, TSM= Total sperm motility, Liq= Liquefaction, n= number of study subjects).

| | Groups | n | Mean± Std. Deviation | |
|---|---|---|---|---|
| **Age** | Infertile | 274 | 34.15 | 4.87 |
| | Normal | 130 | 33.43 | 5.61 |
| **C.F.** | Infertile | 274 | 2.71 | 1.29 |
| | Normal | 130 | 2.35 | 1.37 |
| **BMI** | Infertile | 274 | 25.26 | 4.26 |
| | Normal | 130 | 25.16 | 4.25 |
| **SV** | Infertile | 274 | 1.56 | 0.90 |
| | Normal | 130 | 2.01 | 0.73 |
| **SC** | Infertile | 274 | 9.44 | 15.72 |
| | Normal | 130 | 59.42 | 22.01 |
| **TSC** | Infertile | 274 | 13.61 | 22.25 |
| | Normal | 130 | 118.68 | 63.76 |
| **TSM** | Infertile | 274 | 9.59 | 14.46 |
| | Normal | 130 | 54.41 | 12.25 |
| **Morphology** | Infertile | 274 | 4.50 | 6.74 |
| | Normal | 130 | 17.55 | 11.03 |
| **Viability** | Infertile | 274 | 27.82 | 28.98 |
| | Normal | 130 | 72.05 | 10.55 |
| **pH** | Infertile | 274 | 8.29 | 0.13 |

| | | | | |
|---|---|---|---|---|
| | Normal | 130 | 7.74 | 0.32 |
| **Pus Cell** | Infertile | 262 | 2.15 | 2.92 |
| | Normal | 130 | 3.19 | 4.34 |
| **Liq. Time** | Infertile | 274 | 24.63 | 13.68 |
| | Normal | 130 | 27.23 | 11.95 |

**Table 8: Independent t-test between spermiogram in infertile and control groups** (CF=Coital Frequency, BMI= Body mass index ,SV= Seminal volume, SC=Sperm count, TSC= Total sperm count ,TSM= Total sperm motility, Liq= Liquefaction, CI=Confidence Interval).

| | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | |
|---|---|---|---|---|---|---|---|
| | F | Sig. | t | Df | Sig. (2-tailed) | 95% CI | |
| | | | | | | Lower | Upper |
| **Age** | 2.192 | 0.140 | 1.318 | 402 | 0.188 | -.353 | 1.791 |
| **C.F.** | 3.305 | 0.070 | 2.539 | 402 | 0.011* | 0.081 | 0.635 |
| **BMI** | 0.142 | 0.706 | 0.224 | 402 | 0.823 | -.7905 | .9938 |
| **SV** | 9.029 | 0.003 | -4.939 | 402 | 0.0001* | -.6251 | -.2692 |
| **SC** | 33.039 | .0001 | -26.100 | 402 | 0.0001* | -53.746 | -46.216 |
| **TSC** | 131.212 | .0001 | -24.354 | 402 | 0.0001* | -113.551 | -96.588 |
| **TSM** | 2.461 | 0.117 | -30.513 | 402 | 0.0001* | -47.700 | -41.926 |
| **Morphology** | 35.350 | .0001 | -14.646 | 402 | 0.0001* | -14.793 | -11.292 |
| **Viability** | 416.618 | .0001 | -16.869 | 402 | 0.0001* | -49.391 | -39.081 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **pH** | 17.233 | .0001 | -.734 | 402 | 0.464 | -.4404 | .2010 |
| **PusCell** | 22.635 | .0001 | -3.133 | 402 | 0.002* | -1.857 | -.425 |

*** Significant at the P< 0.05**

**Table 9: Pearson correlation between age and quantitative semen parameters among cases and controls.**(NS=non significant, r= Correlation coefficient, *. Correlation is significant at the 0.05 level (2-tailed).**Correlation is significant at the 0.01 level (2-tailed).

| | | C.F. | BMI | SV | SC | TSC | TSM | Morphology | Viability | pH | Pus cell | Liq Time |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Age** | r | -.073 | 0.151** | 0.059 | -.057 | -.024 | -.076 | -.025 | .022 | .060 | .018 | -.049 |
| **C.F.** | r | | 0.023 | -.004 | -.113* | -.131** | -.101* | -.029 | -.086 | 0.009 | -.077 | 0.016 |
| **BMI** | r | | | 0.052 | -.041 | -.021 | -.018 | 0.064 | 0.015 | 0.006 | -.054 | -.016 |
| **SV** | r | | | | 0.152** | 0.395** | .202** | 0.185** | 0.204** | -.098 | 0.054 | 0.081 |
| **SC** | r | | | | | .883** | .817** | 0.588** | 0.753** | -.065 | 0.089 | 0.100* |
| **TSC** | r | | | | | | 0.751** | 0.569** | 0.666** | -.060 | 0.095 | 0.116* |
| **TSM** | r | | | | | | | 0.653** | 0.823** | -.075 | 0.091 | 0.126* |
| **Morphology** | r | | | | | | | | 0.681** | -.062 | .130** | 0.169** |
| **Viability** | r | | | | | | | | | -.092 | .151** | 0.096 |
| **pH** | r | | | | | | | | | | -.006 | -.087 |
| **PusCell** | r | | | | | | | | | | | 0.004 |

**Table 10 : Pearson correlation between Body mass index and different parameters among cases and controls.**

(NS=non significant, r= Correlation coefficient, *p*= significant value)

| | | LiqTime | C.F. | SV | SC | TSC | TSM | Morphology | Viability | pH | PusCell |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BMI | r | -0.016 | 0.023 | 0.052 | -.041 | -.021 | -.018 | .064 | 0.015 | .006 | -.054 |
| LiqTime | r= | | 0.016 | 0.081 | 0.100* | 0.116* | 0.126* | 0.169** | 0.096 | -.087 | 0.004 |
| C.F. | r= | | | -.004 | -.113* | -.131** | -.101* | -.029 | -.086 | 0.009 | -.077 |
| SV | r= | | | | 0.152** | 0.395** | 0.202** | 0.185** | 0.204** | -.098 | 0.054 |
| SC | r= | | | | | 0.883** | 0.817** | 0.588** | 0.753** | -.065 | 0.089 |
| TSC | r= | | | | | | 0.751** | 0.569** | 0.666** | -.060 | 0.095 |
| TSM | r= | | | | | | | 0.653** | 0.823** | -.075 | 0.091 |
| Morphology | r= | | | | | | | | 0.681** | -.062 | 0.130** |
| Viability | r= | | | | | | | | | -.092 | 0.151** |
| pH | r= | | | | | | | | | | -.006 |

* Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

**Table 11: Description of the infertile and controls with respect to the hormones** (n= number of study subjects, LH=Luteinizing hormone, FSH= Follicle stimulating hormone, PROL= Prolactin, TESTO=Testosterone, ESTRAD=Estradiol).

| | Condition | n | Mean | Std. Deviation |
|---|---|---|---|---|
| **LH** | Infertile | 222 | 5.59 | 5.07 |
| | Control | 104 | 4.55 | 3.13 |
| **FSH** | Infertile | 222 | 10.28 | 10.65 |
| | Control | 104 | 4.89 | 3.05 |
| **PROL** | Infertile | 222 | 12.48 | 9.95 |
| | Control | 104 | 11.85 | 7.17 |
| **TESTO** | Infertile | 222 | 5.94 | 26.74 |
| | Control | 104 | 12.74 | 67.60 |
| **ESTRAD** | Infertile | 222 | 41.35 | 39.97 |
| | Control | 104 | 33.71 | 16.08 |

**Table 12: Independent t-test between different hormones in both infertile and control groups** (LH=Luteinizing hormone, FSH= Follicle stimulating hormone, PROL= Prolactin, TESTO=Testosterone, ESTRAD=Estradiol).

| | **Levene's Test for Equality of Variances** | | **t-test for Equality of Means** | | | | | |
|---|---|---|---|---|---|---|---|---|
| | F | Sig. | t | df | Sig. (2-tailed) | Std. Error Difference | 95% CI | |
| | | | | | | | Lower | Upper |
| **LH** | 5.88 | 0.016 | 1.91 | 324 | 0.057 | 0.54 | -0.03 | 2.09 |
| **FSH** | 54.84 | 0.000 | 5.05 | 324 | 0.000 | 1.06 | 3.29 | 7.48 |
| **PROL** | 6.40 | 0.012 | 0.57 | 324 | 0.564 | 1.08 | -1.51 | 2.77 |
| **TESTO** | 5.83 | 0.016 | -1.29 | 324 | 0.195 | 5.23 | -17.10 | 3.49 |
| **ESTRAD** | 11.66 | 0.001 | 1.87 | 324 | 0.061 | 4.06 | -0.36 | 15.64 |

**Table 13: Testicular volume and semen parameters in both study groups excluding aspermic patients**

| Groups | Right testis | Left testis | Total testis volume | Semen volume | Sperm count | Total sperm count | Sperm motility |
|---|---|---|---|---|---|---|---|
| **Controls** (n=130) | 11.83±3.20 | 11.08±2.84 | 22.91±5.38 | 2.0±0.76 | 60.19±20.57 | 119.13±62.43 | 55.42±11.79 |
| **Infertile** (n=274) | 7.62±4.056 | 6.99±3.60 | 14.61±7.21 | 1.64±0.85 | 23.93±16.65 | 40.16±54.80 | 22.73±19.81 |
| ***p* Value** | 0.0001** | 0.0001** | 0.0001** | 0.0001** | 0.0001** | 0.0001** | 0.0001** |

(Patients with aspermic condition were excluded for all semen parameters and Azoospermic cases were included only for semen volume). **significant at $p<0.01$.

**Table 14: Testicular volume according to distribution of the subjects based on the seminal characteristics in infertility and control group (OA=oligozoospermia, OAT=Oligoasthenoteratozoospermia, OAN=Oligoasthenonecrospermia, OATN= Oligoasthenoteratonecrospermia).**

| Condition | No. of cases % (n=404) | Testicular volume (ml) Mean±SD |
|---|---|---|
| **Normal** | 130 (22.2%) | 22.91 ±5.38 |
| **Aspermia** | 14 (3.66 %) | 16.97±9.59 |
| **Azoospermia** | 101( 28.7 % ) | 12.11±8.11 |
| **Oligozoospermia** | 19 ( 5.4% ) | 16.1±4.67 |
| **OA** | 43 (12.21 %) | 16.35±4.93 |
| **OAT** | 7 (2 %) | 17.97±4.45 |
| **OATNE** | 48 (13.6%) | 14.45±6.75 |
| **Asthenozoospermia** | 28 (8 %) | 16.35±4.93 |
| **OANE** | 14 (4 %) | 13.60±4.93 |

**Table 15:Pearson correlation between Testicular Volume and semen parameters. (r=correlation coefficient, p=significant value,)**

| | | Semen volume | Sperm Count | Total sperm count | Sperm motility (% of motile sperm) |
|---|---|---|---|---|---|
| **Total testicular volume (ml)** | r | 0.172** | 0.501** | 0.508** | 0.476** |
| **Semen volume (ml)** | r | | 0.131* | 0.373** | 0.173** |
| **Sperm count (million per ml of semen)** | r | | | 0.879** | 0.816** |
| **Total sperm count (million)** | r | | | | 0.741** |

**Significant at p<0.01, * Significant at p< 0.05.

**Table 16 : Description of the study groups for prostate volume assessment(N= Number of study subjects.).**

| | condition | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| **Prostate volume** | **Infertile** | 274 | 12.38 | 3.98 | 0.25 |
| | **Normal** | 130 | 14.16 | 4.16 | 0.38 |

**Table 17: Independent t-test between infertile and control groups with respect to the Prostate volume.**

| | **Levene's Test for Equality of Variances** | | **t-test for Equality of Means** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | 95% Confidence Interval of the Difference | |
| | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | Lower | Upper |
| **Prostate** | 0.004 | 0.95 | -3.89 | 364 | 0.0001 | -1.77 | 0.45 | -2.66 | -0.87 |

**Table 18 : Pearson correlation between Prostate volume and different parameters.**

| | | BMI | SV | SC | TSC | TSM | Morphology | Viability | pH | LiqTime | Prostate |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **age** | r= | .151** | .059 | -.057 | -.024 | -.076 | -.025 | .022 | .007 | .043 | .139** |
| **BMI** | r= | | .052 | -.041 | -.021 | -.018 | .064 | .015 | -.017 | -.021 | .163** |
| **SV** | r= | | | .152** | .395** | .202** | .185** | .204** | .314** | -.098 | .216** |
| **SC** | r= | | | | .883** | .817** | .588** | .753** | .076 | -.041 | .154** |
| **TSC** | r= | | | | | .751** | .569** | .666** | .055 | -.079 | .247** |
| **TSM** | r= | | | | | | .653** | .823** | .076 | -.073 | .176** |
| **Morphology** | r= | | | | | | | .681** | .079 | -.062 | .155** |
| **Viability** | r= | | | | | | | | .137** | -.044 | .174** |
| **pH** | r= | | | | | | | | | .164** | -.040 |
| **LiqTime** | r= | | | | | | | | | | -.026 |

**. (* Significant at p<0.05, ** Significant at p<0.01)**

**Table 19 : Description of the study groups for Seminal Vesicle volume assessment.**

| | Conditions | N | Mean | Std. Deviation |
|---|---|---|---|---|
| **Right Seminal Vesicle volume** | Infertile | 248 | 0.97 | 1.09 |
| | Normal | 116 | 1.05 | 0.93 |
| **Left seminal vesicle volume** | Infertile | 246 | 0.95 | 1.12 |
| | Normal | 115 | 1.05 | 0.95 |

**Table 20: Independent t-test between infertile and control groups with respect to the Seminal Vesicle volume. CI= Confidence Interval**

| | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | |
|---|---|---|---|---|---|---|---|---|
| | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | 95% CI | |
| | | | | | | | Lower | Upper |
| **Right Seminal Vesicle volume** | 0.14 | 0.70 | -0.69 | 362 | $0.48^{NS}$ | -0.08 | -0.31 | 0.15 |
| **Left seminal vesicle volume** | 0.12 | 0.72 | -0.80 | 359 | $0.42^{NS}$ | -0.09 | -0.33 | 0.14 |

**NS= Non-significant**

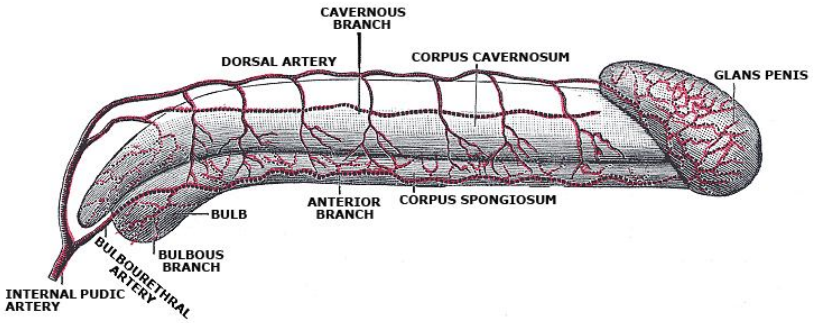



Figure 1: Anatomy of a human penis showing blood supply.

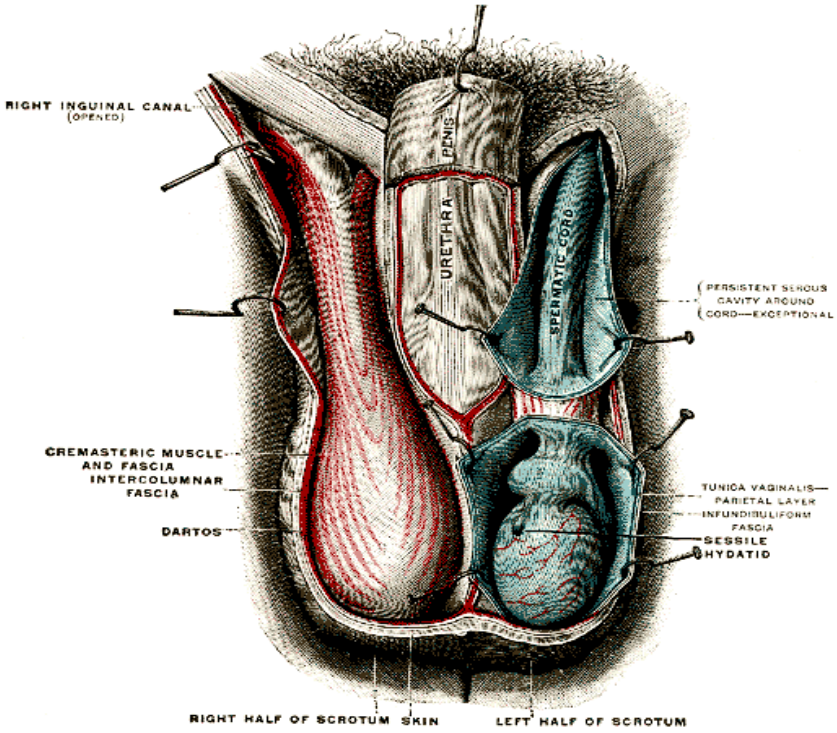



Figure 2: Anatomy of  scrotum, showing varios internal structures.

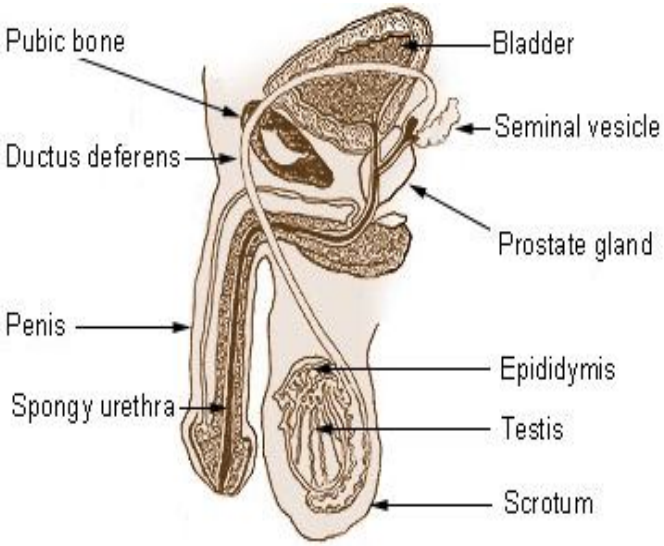



Figure 3: Anatomy of the Human Reproductive system.

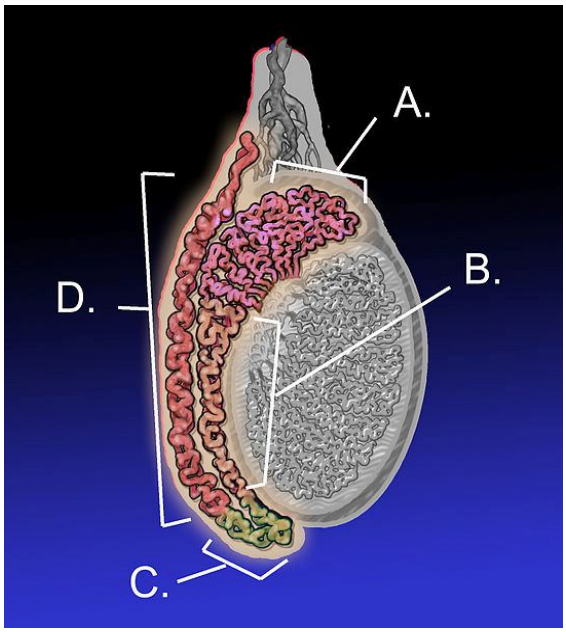



Figure 4: Anatomy of epididymis ( A= Head of epididymus, B= Body of epididymus, C= Tail of epididymis, and D= Vas deferens).

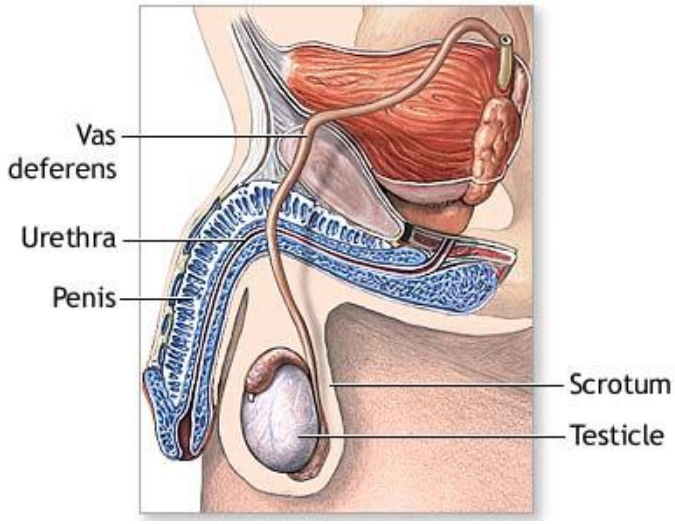



Figure 5: Anatomy of Vas deferens.

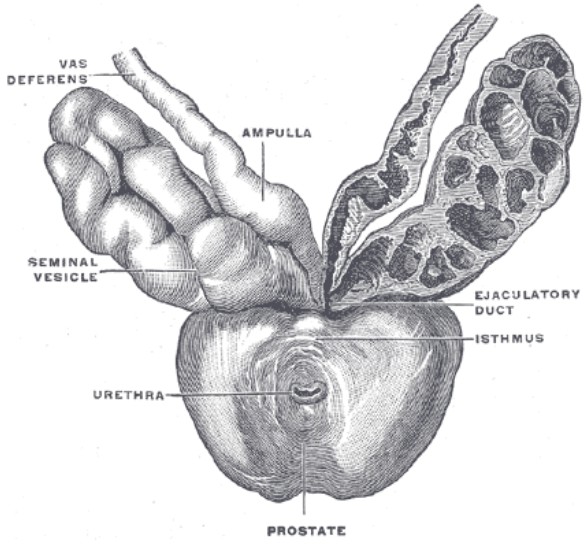



Figure 6: Anatomy of prostate with seminalvesicles and Vas deferens, viewed from in front and above .

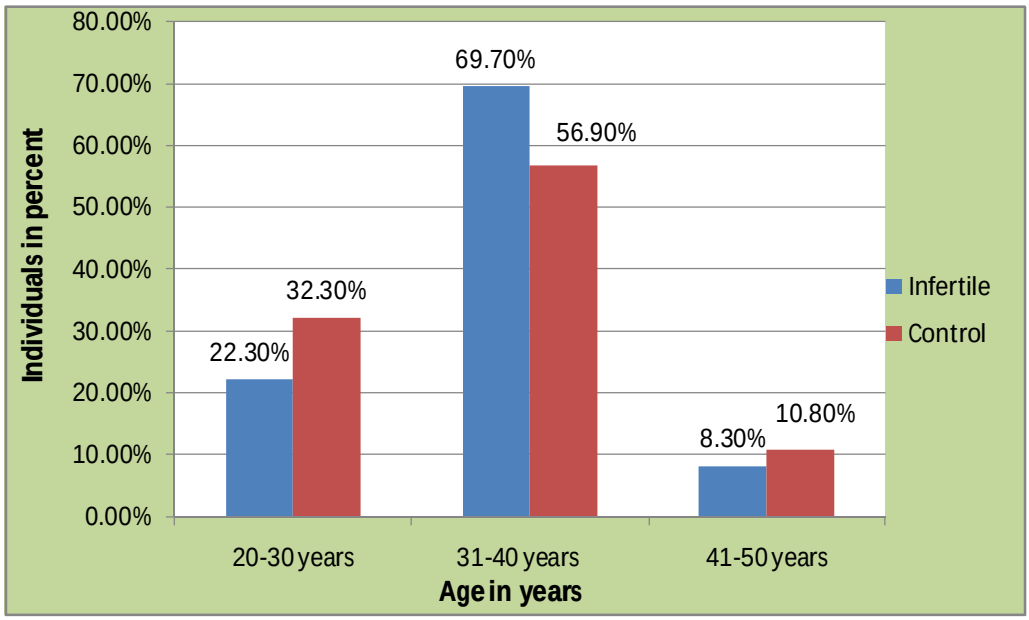



Figure 7 : Distribution of different subjects with respect to the age in both groups.

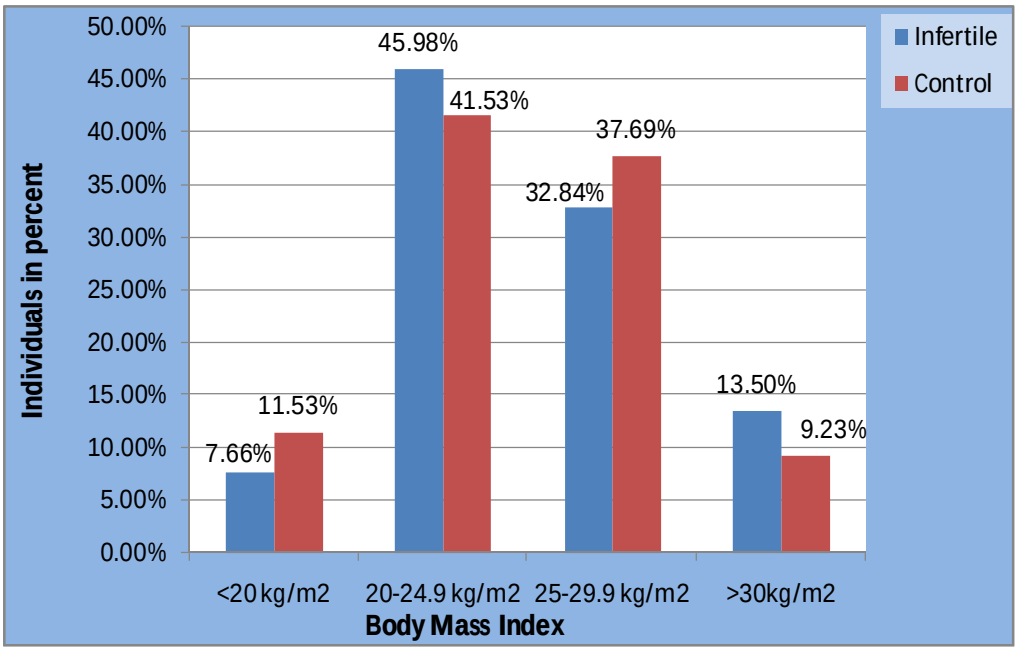



Figure 8 : Distribution of different subjects with respect to the BMI in both groups.

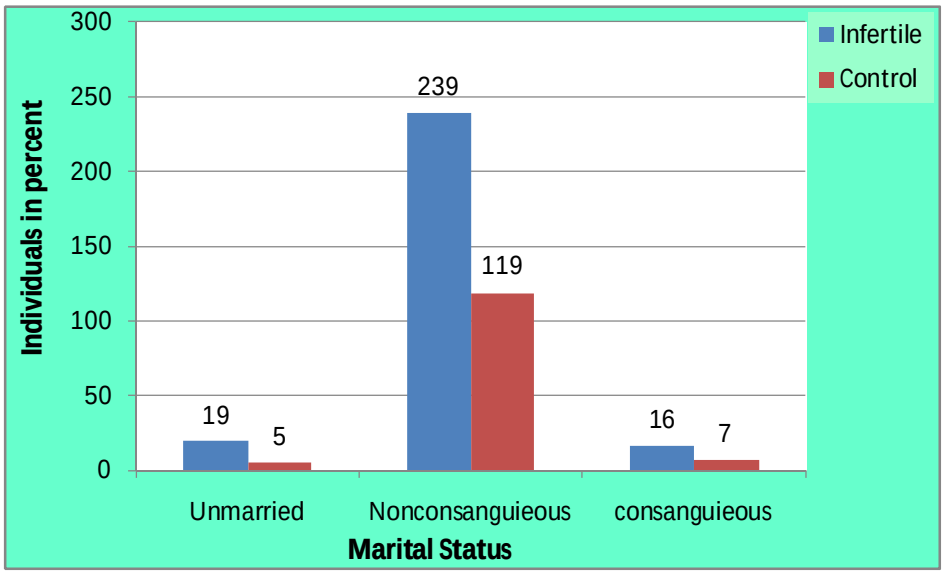



Figure 9 : Distribution of different subjects with respect to the type of marriage in both groups.

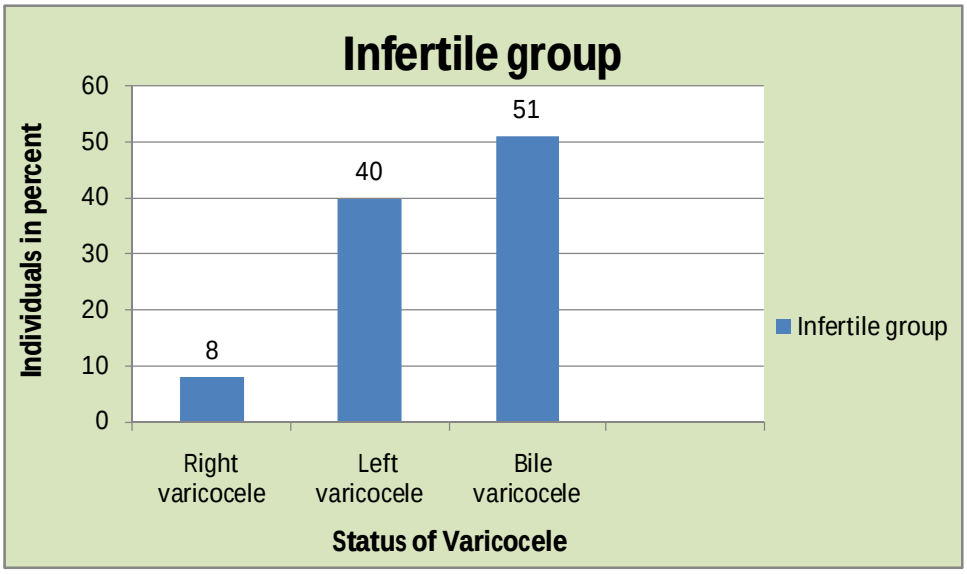



Figure 10 : Distribution of infertile subjects with respect to varicocele.

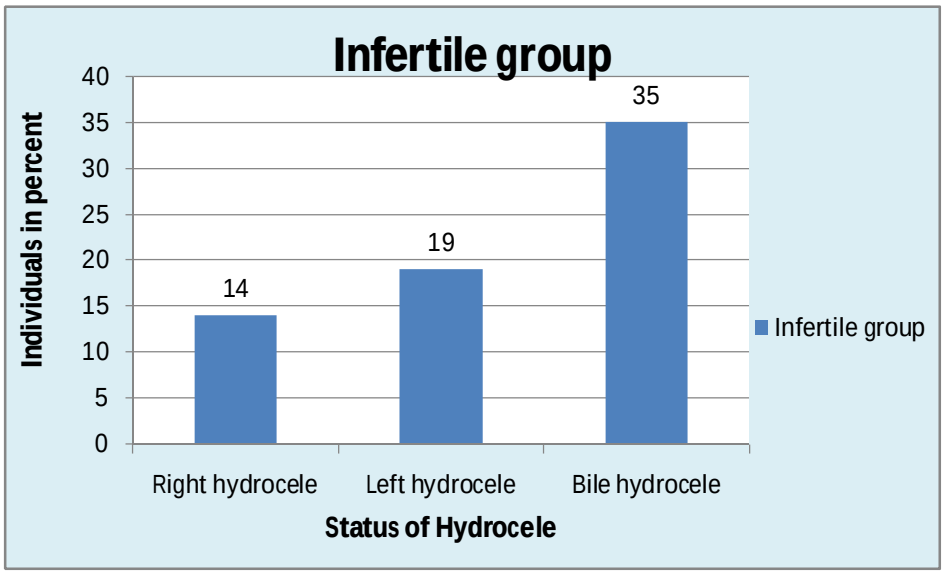



Figure 11 : Distribution of infertile subjects with respect to hydrocele.

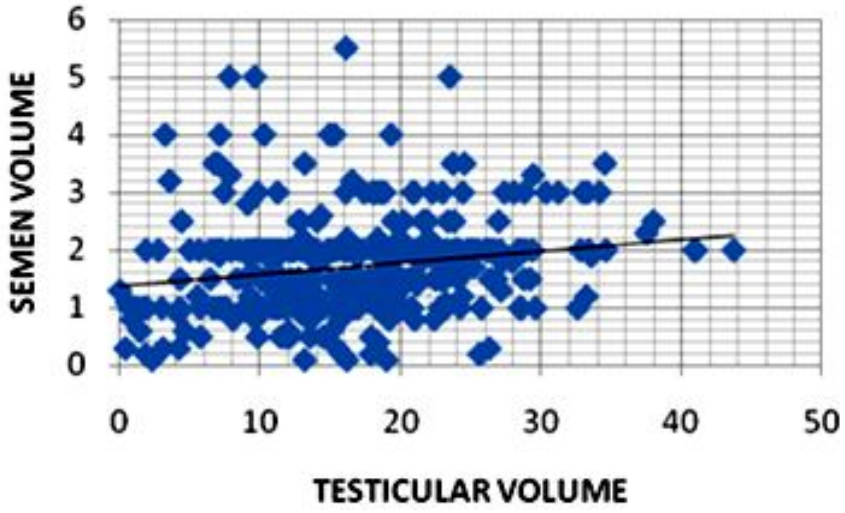



Figure12: Scatter diagram of semen volume vs. testicular volume excluding aspermia.

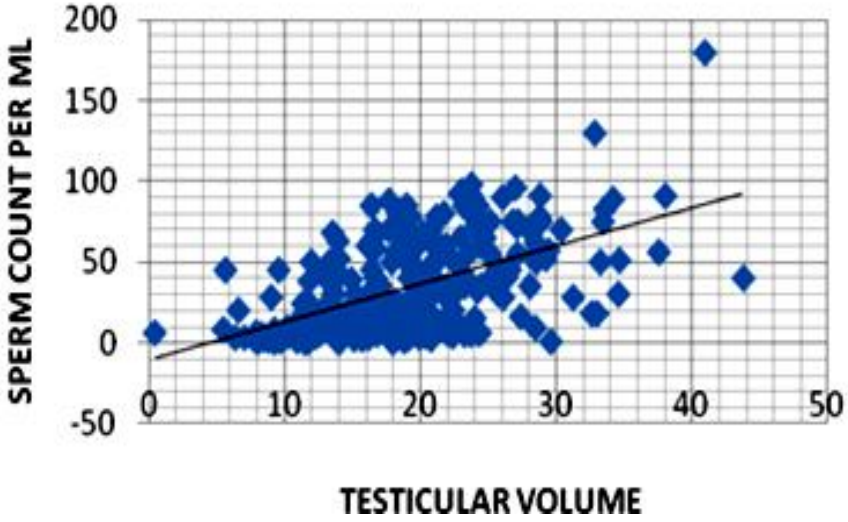



Figure 13 : Scatter diagram of Testicular volume vs. sperm count per ml excluding patients with azoospermia and aspermia.

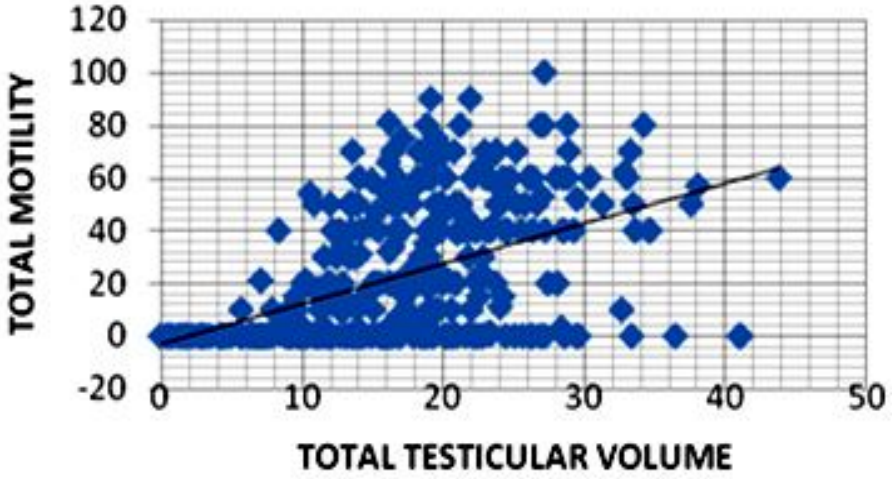
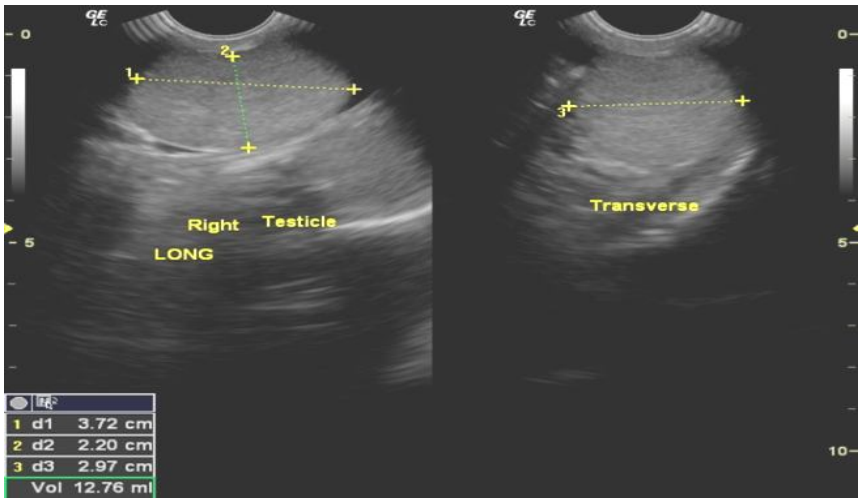
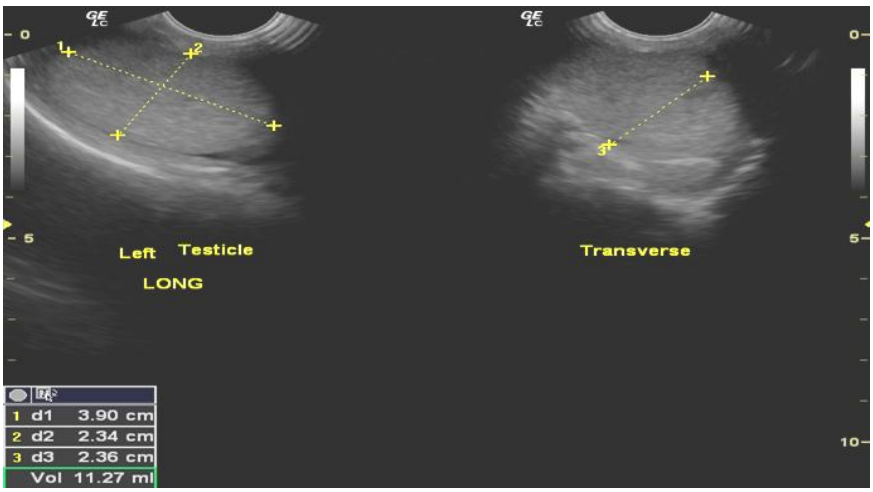



Figure 14: Scatter diagram of Total motility vs. total testicular volume excluding azoospermia and aspermia.

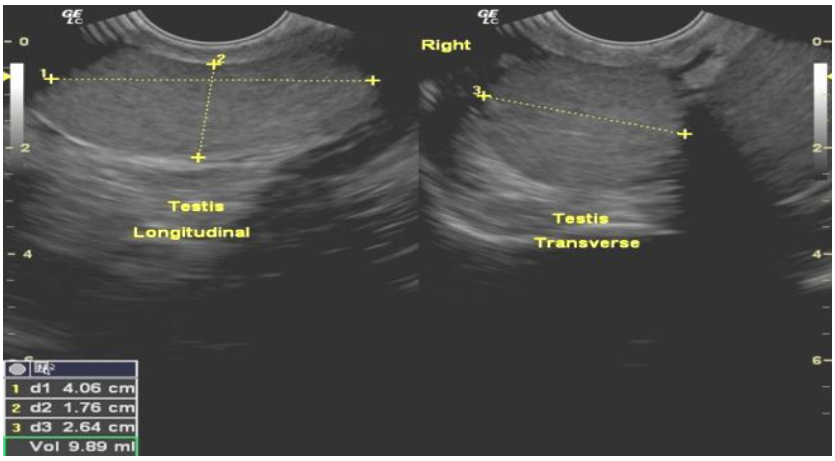



**A**

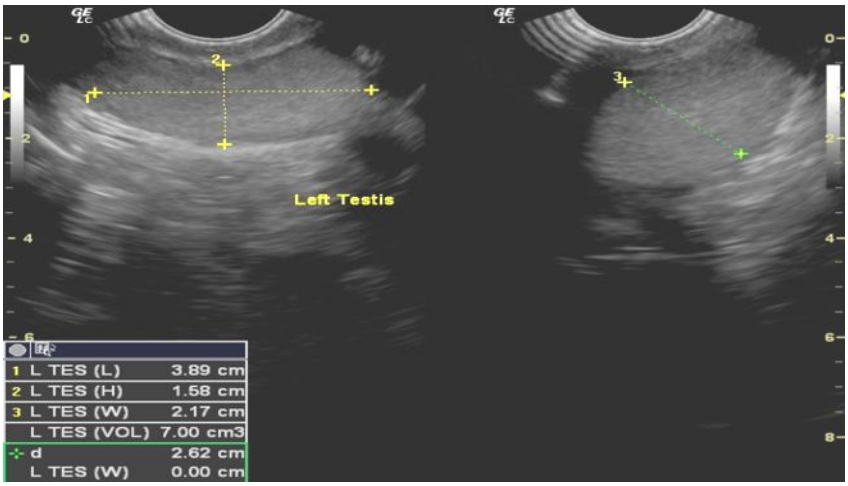



**B**

Figure 15: Images of ultrasound scanning of the testis with measurementsin longitudinal and transverse view .A:Right tests, B: Left testis

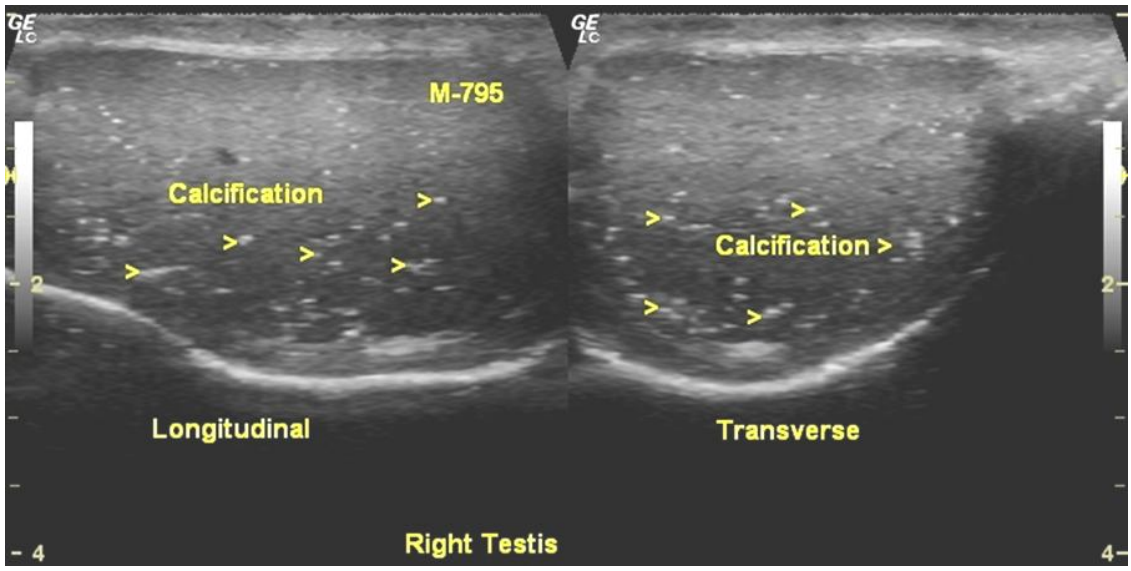



A

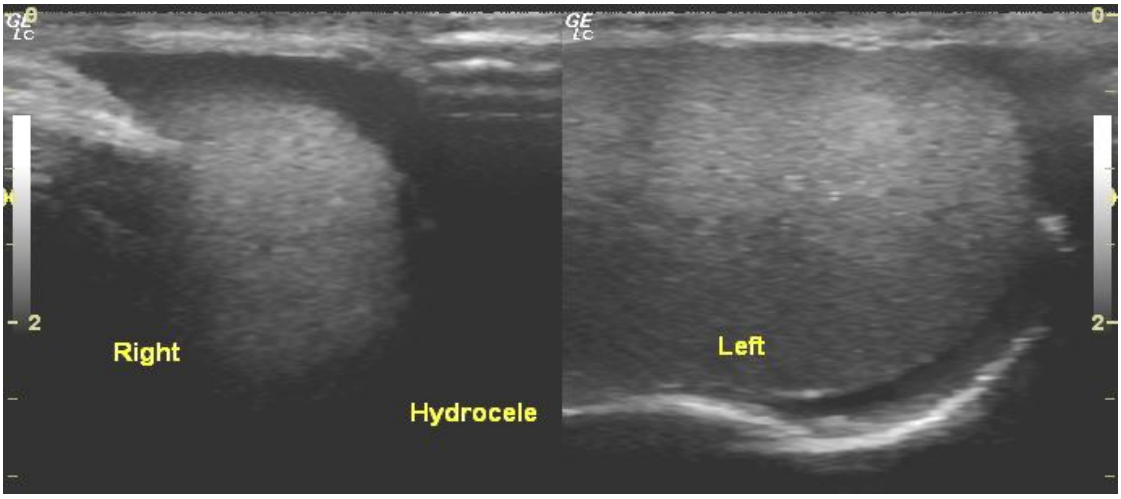



B

Figure 16: Image of ultrasound scanning of infertile man with Bilateral testicular Hypoplasia. A:Right testis, B: Lestft testis




A:




B:

Figure 17: Image of ultrasound scanning of the testisA: Right tesits in longitudinal and Transvers section showing Testicular calcification B: Testicular scanning showing Bilateralhydrocele.

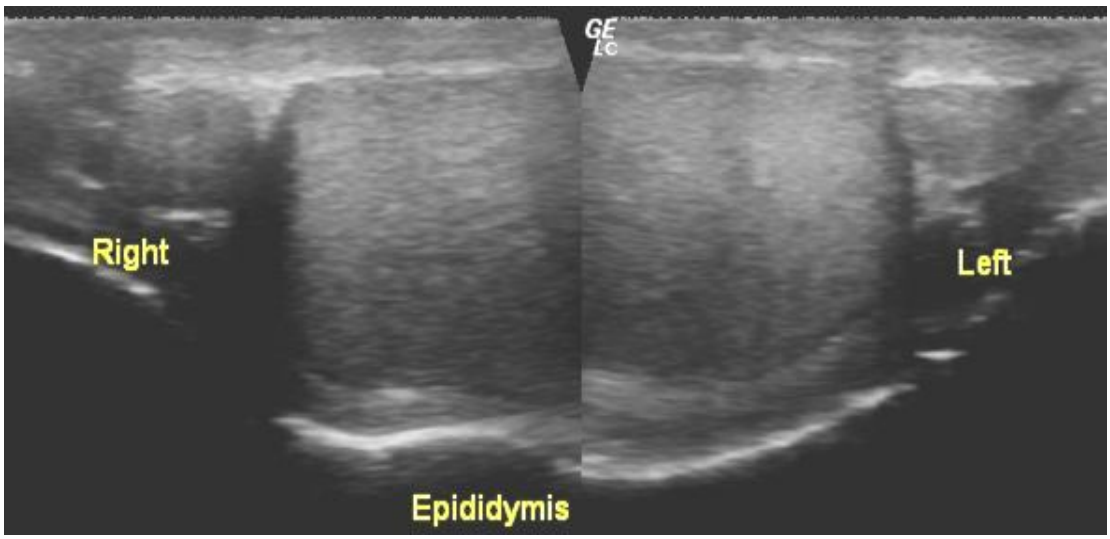



Figure 18: Image of ultrasound scanning of the normal epididymis.

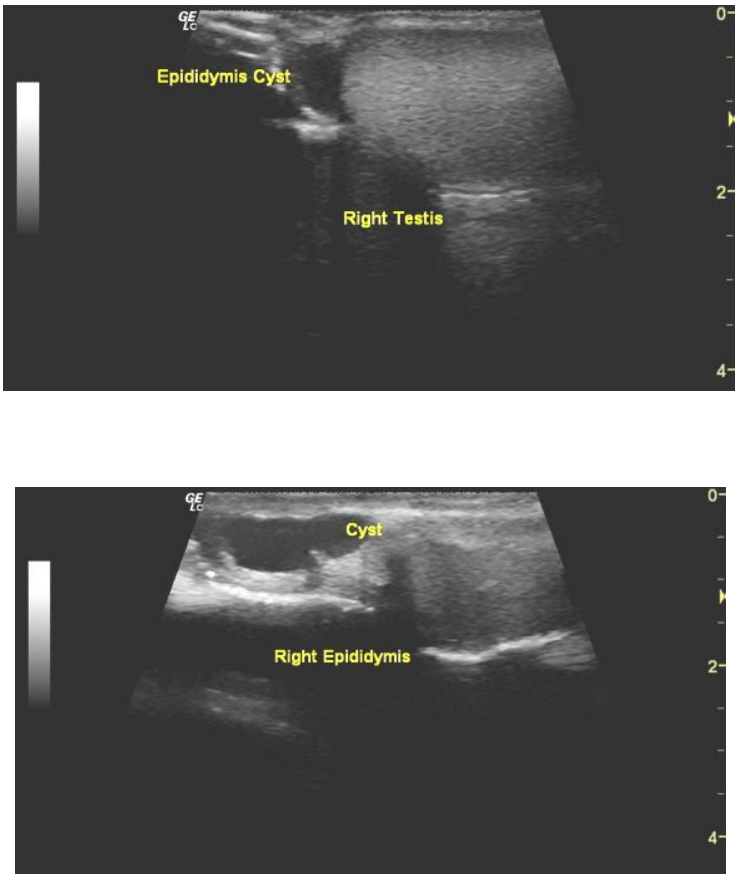



Figure 19:Images of ultrasound scanning of epididymis with cyst in two different subjects.

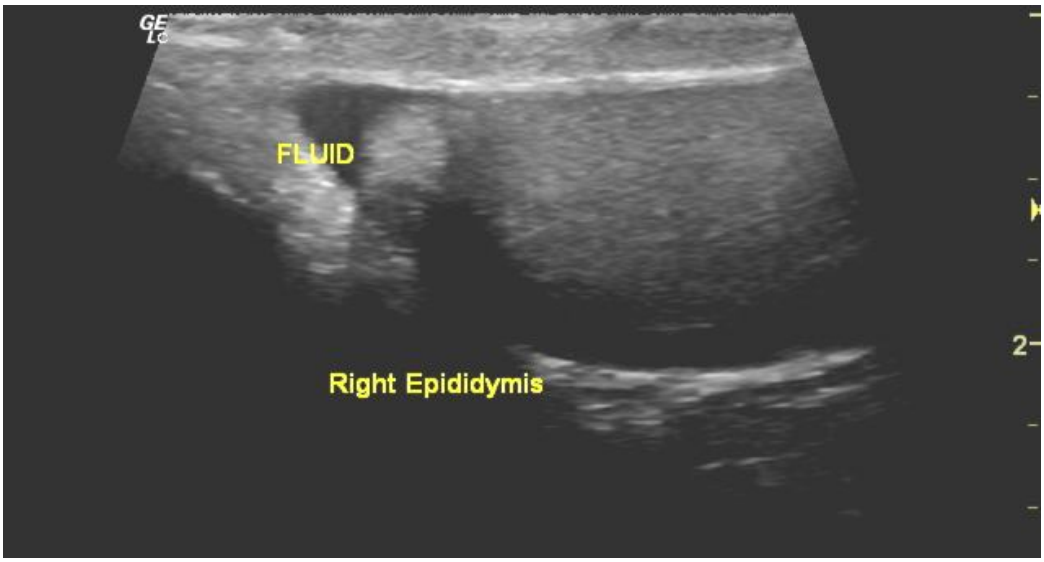



Figure 20: Image of ultrasound scanning of epididymis with fluid accumulation.

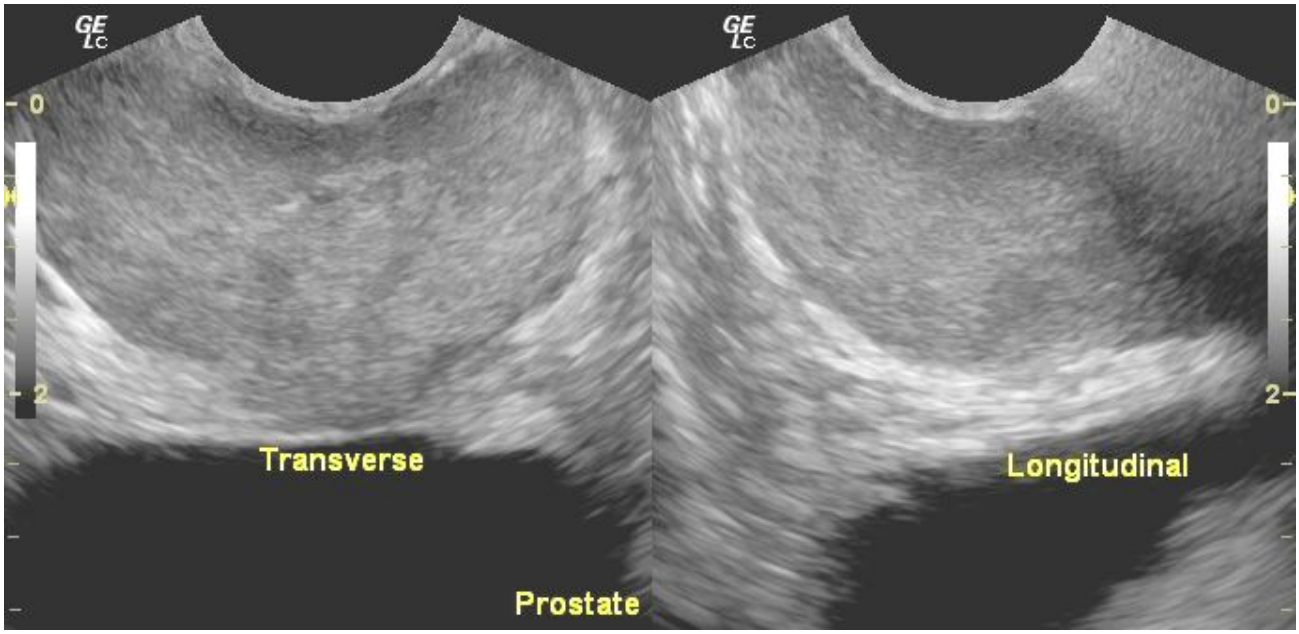



Figure 21: Image of ultrasound scanning of the normal prostate

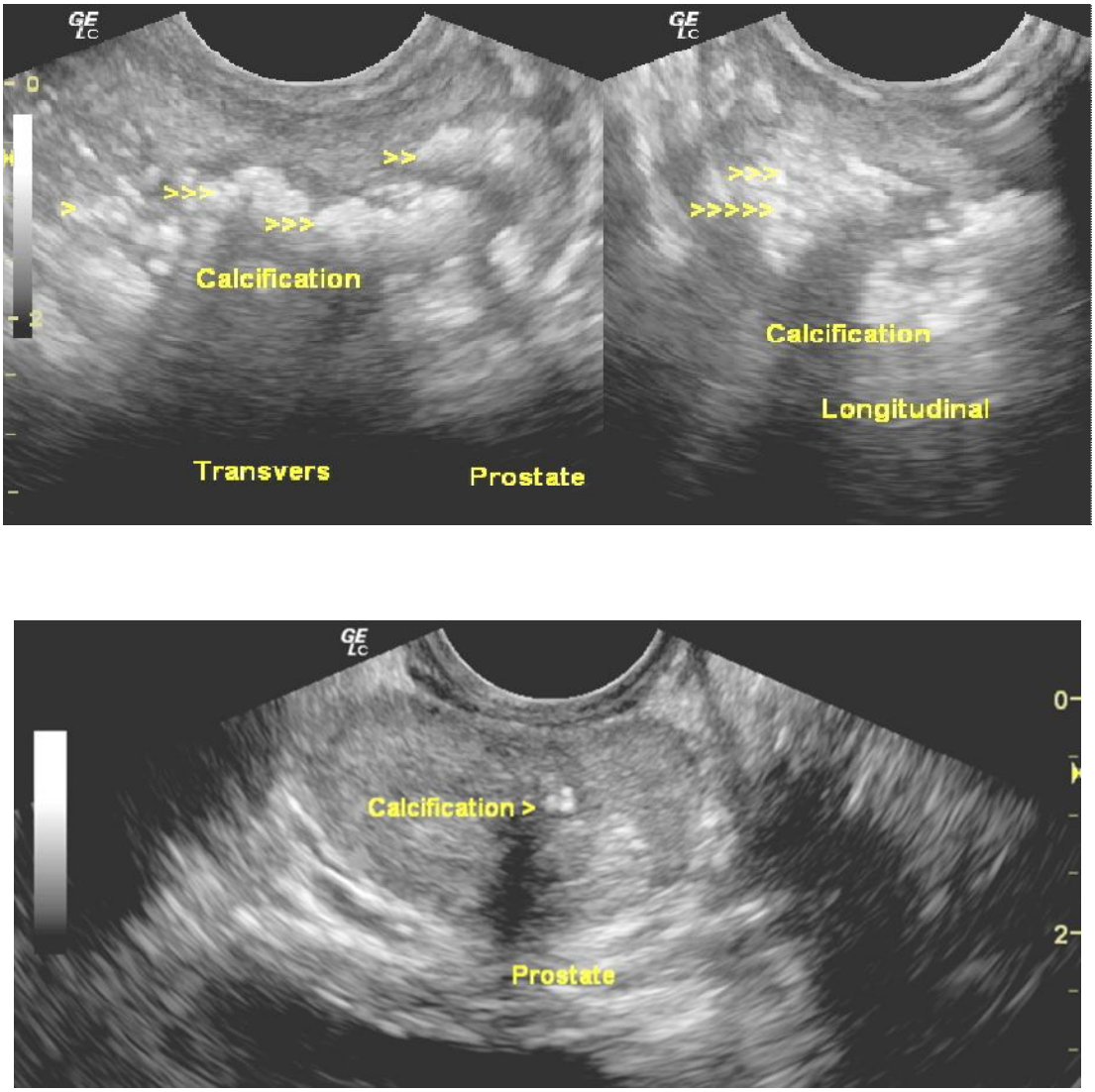



Figure 22: Images of ultrasound scanning of the prostate with calcification in two different subjects.

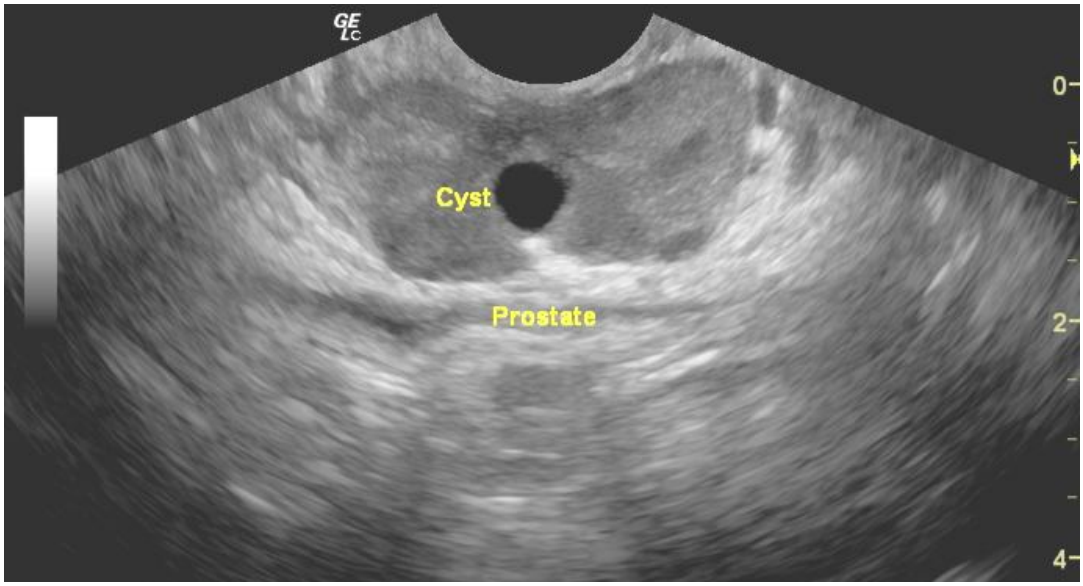



Figure 23: Image of ultrasound scanning of the prostate with cysts.

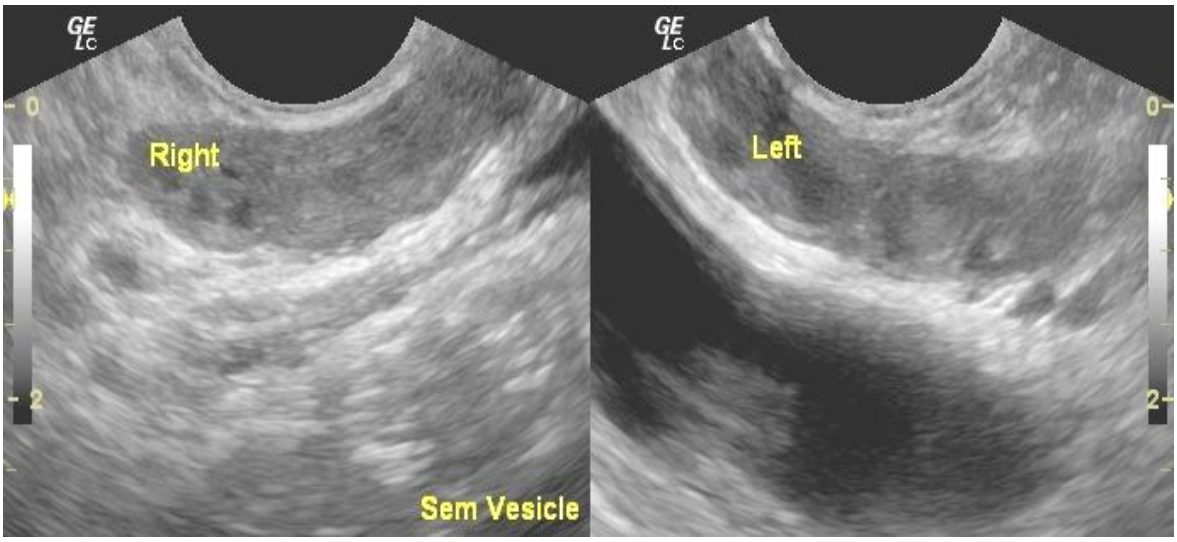



Figure 24: Image of ultrasound scanning of normal seminal vesicle Right and Left.

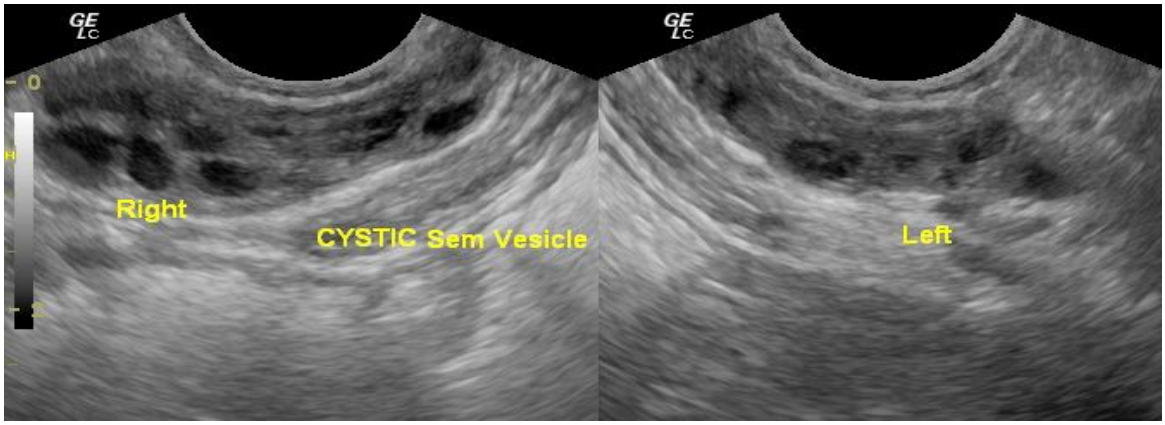



Figure 25: Image of ultrasound scanning of Seminal vesicle with Bilateral cysts.

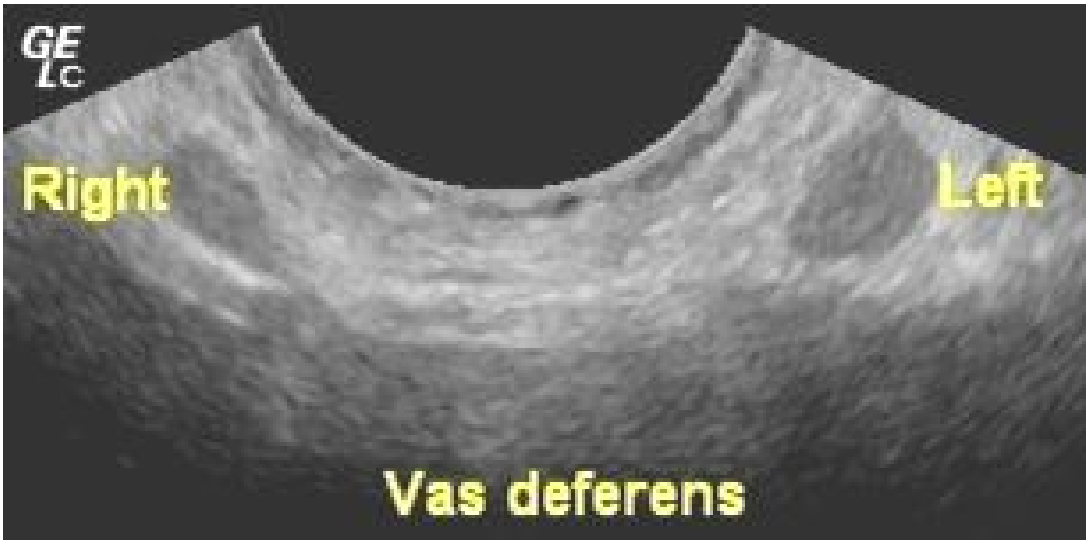



Figure 26: Image of ultrasound scanning of normal Vas deferens

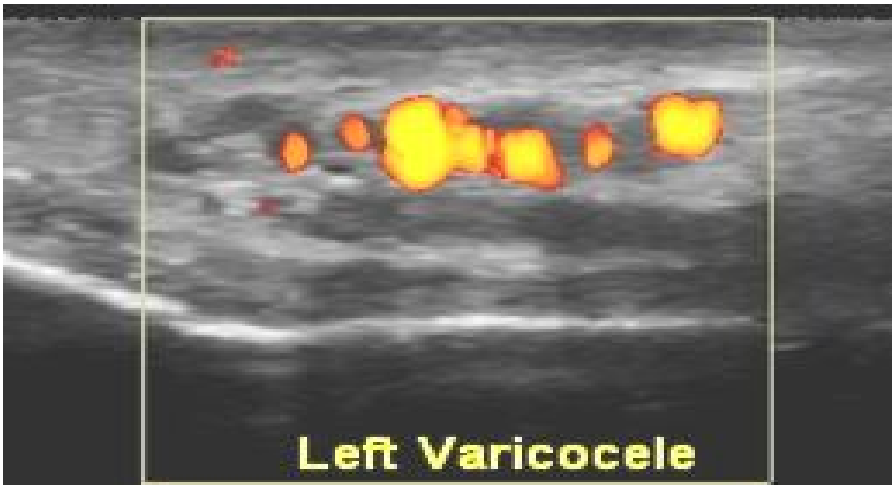



Figure 27: Image of ultrasound scanning of left testis with varicocele

## ***SECTION IV***

# *DISCUSSION*

Infertility is a common up coming problem wherein approximately 8% of men of reproductive age seek medical attention for infertility problems. Of these, up to 10% are with reversible causes affecting their fertility potential; varicocele represents 35% of these cases (Esteves *et al.,* 2011). As such, the male partner must be systematically evaluated in every investigation of an infertile couple (Esteves*et al.,* 2011). Because 80% of couples are able to achieve pregnancy within the first year of attempting, a couple should only be diagnosed as infertile after one year of regular sexual activity without using a contraceptive method. Investigation is initiated earlier when risk factors are present, including advanced maternal (>35 years) or paternal age (>45 years), a history of urogenital surgery, cancer, cryptorchidism, varicocele, orchitis, use of gonadotoxins or genital infections, etc. (Esteves*et al.,* 2011). The Andrologist is responsible for diagnosing, counseling and treating the underlying cause whenever possible. When there is no specific treatment he/she is still responsible for referring the patient to specialized assisted reproductive techniques (ART) center or, if the Andrologist is a member of an ART center's multi-professional team, for extracting the male gamete from the testicle or epididymis (Esteves*et al.,*2011).

For healthy young couples, the probability of achieving pregnancy per reproductive cycle is approximately 20 to 25%. The cumulative probabilities of conception are 60% within the first 6 months, 84% within the first year and 92% within the second year of fertility-focused sexual activity (Kamel, 2010). Infertility is a common clinical problem affecting 13 to 15% of couples worldwide (WHO, 1984). The prevalence varies throughout different countries, being higher in the underdeveloped nations where limited resources for diagnosis and treatment exist (Cates *et al.,*1985). In the United

Kingdom, infertility is believed to affect one in six couples (Zargar *et al.*,1997). According to Kamel *et al.*,(2010), it should be regarded as a public health problem, as it affects not only the health care system but also the social environment (Kamel, 2010).

In most of the studies, the research was concentrated either only on reproductive organs or on hormones in infertile males. **This is the first study that was conducted on anatomical, pathological and hormonal differences among fertile and infertile males using semen parameters as criteria and compared the fertility potential among different infertile subgroups.**

**Seminal manifestation**

In the present study we detected a decrease in semen volume among infertile subgroups evaluated. Indeed, published reports showed a decrease in semen volume with ageing (Ford *et al.,* 2000; Kidd *et al.,* 2001; Ng *et al.,* 2004). In the studies where the analyses were adjusted for the period of abstinence there was a decrease in semen volume of 3–22% (Ford *et al.,* 2000).

In our present study, sperm count was found to be significantly decreased in infertile males when compared with the controls and the negative correlation was also observed between age, BMI and total sperm count but not significant (**Table 4 and Table 6**). This finding reveals that our data accord with the previous studies on the effect of age and BMI on male fertility potential. Jensen *et al.,* (2004) reported a higher prevalence of oligozoospermia in overweight and obese men compared with normal-weight men (24.4% vs. 21.7%). Kort *et al*.. (2006) found that BMI correlated negatively with the total number of normal spermatozoa. Najafi *et al.,* (2011, 2012) showed the similar result in

Mysorepopulation. Central obesity in particular appears to be associated with a decrease in circulating androgen levels proportional to the degree of obesity. Data on the effect of age factor on sperm count accords with the similar results obtained in previous studies (Eskenazi*et al.,* 2006; Dunson *et al.,* 2004). The main reason for non-significant values obtained for these factors in the present study could be due to a randomized sampling rather than a purposeful sampling.

In the present study sperm motility tended to decrease with age, indeed most studies have found a decrease in sperm motility with increasing age (Mladenovic *et al.*,1994; Auger *et al.*,1995; Hassan and Killick, 2003; Ng *et al.*,2004). Those studies that adjusted the results for the duration of abstinence reported statistically significant effects, such as negative linear relationships and decreases in motility of 0.17–0.6% for each year of age (Berling and Wolner-Hanssen, 1997;Kidd *et al.,* 2001). Thus, the present study supports the conclusion based on the data from most others, that there is consistent evidence for a decrease in sperm motility with increasing age although this correlation is not significant.

**Sperm viability**

Present investigation showed that semen samples containing higher percentage of viable sperms were mostly with normal physical profile (Normozoospermia) where as abnormal semen samples were with poor viable sperms. Thus, viability of sperm may be considered as an authentic and handy tool to assess male fertilizing potential specially when facility for other sophisticated techniques if not available.

.Sperm morphology is a good indicator of the status of the germinal epithelium (Mladenovic *et al.*,1994; Bujan *et al.*,1996). Degenerative changes in the germinal

epithelium may be due to ageing which may affect spermatogenesis and thus sperm morphology. The results of our study clearly demonstrate that there is a significant increase in the frequency of sperm morphological defects among infertile males when compared with the control group (**Tables 8 and 9**).

The biochemical constituents of seminal plasma was routinely studied are fructose and citric acid. Fructose a readily glycolysable sugar is produced in humans mainly by the seminal vesicle with a small contribution from the ampulla of the ductus deferens and is essential for spermatozoal metabolism and motility as an energy source (Schoenfeld *et al.,* 1979). Absence of fructose indicates congenital absence of seminal vesicle mainly in case of azoospermia. In a patient with a low volume ejaculate, the absence of fructose indicates ejaculatory duct obstruction, seminal vesicle dysfunction or hypoplasia (Aumuller and Riva, 1992). In the present study fructose value and semen volume is normal for almost all infertile cases. This indicates neither of seminal vesicle agenesis in azoospermic cases nor ejaculatory duct obstruction in other infertile conditions.

**Age as a risk factor:**

Hassan and Killick (2003) stated that increased male age is associated with a significant decline in fertility (five times longer to paternity when aged> 45 years), which is independent of the woman's age, coital frequency, and life-style effect, as well as the effect of other subfertility risk factors. In addition, paternity at older ages may have significant effects on the viability and genetic health of human pregnancies and offspring, primarily as a result of structural chromosomal aberrations in sperm. The evidence for sex chromosomal aneuploidy suggests that there may be about a doubling of the risk at the age of 50 years (Sloter *et al*., 2004).

Decreased fertility rates in aged males could be due to cellular, biochemical and molecular changes in spermatozoa that affect the fertilization by decreasing sperm motility and hampering the potential for undergoing the acrosome reaction and penetrating an oocyte. Robertson *et al.,* (1982) have reported a gradual decline of male fertility associated with the age factor. Autopsies of men who died from accidental causes showed narrowing and sclerosis of the testicular lumen, decreased in spermatogenic activity, increased degeneration of germ cells and decreased number and function of Leydig cells with increase age (Bishop,1970; Johnson, 1986). Clinical studies suggest that age was associated with diminished semen volume, sperm motility and sperm normal morphology, but sperm concentration had minor affected by age (Schwatz *et al.,* 1983; Kidd *et al.,* 2001). Increasing paternal age was also found to be associated with delayed conception in fertile couples, especially after age of 25 years (Hull *et al.,* 1996). In our study, the prevalence of azoospermia and oligospermia was higher in subjects between 41-50 years indicating that age can be a causative factor for such conditions that brings about diminished sperm count and this is in agreement with previous studies.

**Effect of obesity as a lifestyle Factor:**

Obesity is a major health issue and the relationship between obesity and male infertility has been described recently in many reports(Oliva *et al.,* 2001; Magnusdottir *et al.,* 2005; Nguyen *et al.,* 2007; Mara *et al.,* 2008). Also, men with high BMIs typically are found to have an abnormal semen analysis as well. Jensen et al. reported a higher prevalence of oligozoospermia in overweight and obese men compared with normal BMI (Jensen *et al.,* 2004). However, they did not find any relationship between increasing male BMI and percentage of motile sperm. Kort *et al.,* (2006) found that BMI correlated

negatively with the total number of normal spermatozoa. They did not report on sperm count or morphology. In a recent study in India, the negative correlation was found between male BMI and sperm parameters like sperm count and motility (Najafi *et al.*, 2011) and stated that obesity may lead to male infertility by increasing lipid peroxidation (Najafi *et al.*, 2011).

In a trial to improve the semen quality it was reported that; reproductive hormone levels have been shown to normalize after weight loss (Norman *et al.*, 2004; Koloszar *et al.*, 2005). It remains, however, to be seen whether weight loss may also improve semen quality. Sallmen *et al.*, (2006) stated that, hormone irregularities in men affect stimulation of the testicles that inhibit sperm production (Shome and Parlow, 1974) Several studies have reported reductions in testosterone with obesity (Jensen *et al.*, 2004; Roudebush *et al.*, 2005; Fejes *et al.*, 2006). In massively obese individuals, reduced spermatogenesis associated with severe hypotestosteronemia may favor infertility (Ratcliffe *et al.*, 1988;Sallmen *et al*., 2006). In another study, overweight and obese men had reduced sperm motility and increased sperm DNA fragmentation (Kort *et al*., 2006). Contrary to other recent studies, no increased risk was observed in the present study among obese men, could be due to the less sample size.

Many studies lacked information on frequency of sexual intercourse so obesity-related changes in sexual function could not be distinguished from obesity related effects on fertility (Sallmen *et al.*, 2006), because obesity has been associated with both sexual and erectile dysfunction (Esposito and Giugliano, 2005). Therefore, reduced intercourse frequency could be a mediating factor by which obesity produces infertility. In this study we observed the relationship between frequency of intercourse and men's BMI. But the

association was negative thus; the mechanism that explains the BMI effect is likely to involve hormones rather than semen changes or sexual function. All previous studies mainly focused on the effect of obesity on male infertility but none of them assessed the incidence of obesity among infertile couples and as per our knowledge this study is the first in India which analyzed the prevalence of overweight and obesity in infertile couples.

**Endocrine evaluation**

Androgens play a crucial role in the development of the male reproductive organs, such as the testis, the epididymis, the vas deferens, the seminal vesicle, the prostate and the penis. The role of androgens is an important topic in the study of puberty, male fertility and male sexual function. The effects of androgen withdrawal have been well established through the experimental model of orchiectomy. A decrease in the weight of the epididymis has been commonly observed in animals that have had their testicles removed. In these cases, androgen replacement, even at supraphysiological levels, only partially restored the weight of the epididymis. The removal of the testicles causes the loss of androgens, but it is clear that this approach affected estrogen levels and other testicular factors that may affect the maintenance of epididymis (Robaire *et al.*, 2006).

The formation and function of the epididymis is androgen-dependent. The principal androgen, testosterone is essential for the development of the internal sex organs and is derived from the Wolffian duct system, which consists of the epididymis, the vas deferens, and the seminal vesicle (Umar *et al.*, 2003). Dihydrotestosterone (DHT), the 5α-reduced form of testosterone is involved in the development of the prostate and the external genitalia. Although testosterone is the predominantly active androgen during the first phase of the postnatal development of the epididymis, it is the effects of DHT that are

important in the epididymal fluid of the mature epididymis. DHT can be produced locally in the epididymis by principal cells and is primarily found in the initial segment of the duct (Dacheux *et al.*, 2005; França *et al.*, 2005; Robaire & Henderson, 2006).

The actions of both testosterone and DHT are initiated through the intracellular receptor known as the androgen receptor (AR). DHT is the more potent androgen among them. The AR is found in all male reproductive organs and can be stimulated by either testosterone or its more potent metabolite, DHT. The binding of either testosterone or DHT to the AR may regulate distinct androgenic effects in target tissues. Clinical syndromes, such as androgen insensitivity (AIS), illustrate the differential actions of testosterone and DHT (Umar *et al.*, 2003). AR expression in the developing male genital tract occurs in a strict temporal pattern. It is first detected in the mesenchymal cells, then in the epithelial cells and then in both the epithelial and stromal compartments of the epididymis (Umar *et al.*, 2003; O'Hara *et al.*, 2011).

**Endocrine evaluation is suggested when the following scenarios are present:**

a) A sperm concentration of less than 10 million/mLb) Erectile dysfunction c) Hypospermia (volume 1 mL) or d) Signs and symptoms of endocrinopathies or hypogonadism. The minimal evaluation includes the assessment of serum FSH and testosterone levels, which reflect germ cell epithelium and Leydig cell status, respectively. If the testosterone level is low, a second collection is recommended along with free testosterone, LH and prolactin measurements. Isolated FSH elevation is usually indicative of severe germ cell epithelium damage.

Highly elevated FSH and LH levels, when associated with low-normal or below normal testosterone levels, suggest diffuse testicular failure and may have either a

congenital (e.g., Klinefelter syndrome) or acquired cause. Concomitant low levels of FSH and LH may implicate hypogonadotropic hypogonadism. This condition may be congenital or secondary to a prolactin-producing pituitary tumor. Gonadotropin values within the normal range suggest an extraductal obstruction in azoospermic subjects. However, azoospermic patients with testicular failure and testis histology exhibiting sperm maturation arrest and 10% of those diagnosed with Sertoli-cell-only syndrome may present with non-elevated FSH levels. Serum estradiol levels should be determined in patients presenting with gynecomastia. Infertile patients with a testosterone to estradiol ratio less than 10 can harbor significant but reversible seminal alterations (Raman and Schlegel, 2002). Vaucher *et al.*, (2009) suggested that hyperestrogenism secondary to a higher conversion rate of testosterone into estradiol in Klinefelter syndrome (KS) patients inhibits testosterone production via a negative feedback pathway and may indicate the over expression of aromatase *CYP19* in the testis. As such, there would be a scientific rationale for the use of aromatase inhibitors in KS patients (Raman and Schlegel, 2002). In azoospermic men with a normal ejaculate volume, FSH serum level greater than two times the upper limit of the normal range is reliably diagnostic of dysfunctional spermatogenesis (Technical Bulletin - American Society for Reproductive Medicine, 2008). Serum prolactin levels should be determined in infertile men with a complaint of concomitant sexual dysfunction or when there is clinical or laboratory evidence of pituitary disease; however, hyperprolactinemia is rarely a cause of infertility in healthy men (Sigman and Jarow, 1997). Although hormonal alterations may be present in approximately 10% of men who undergo assessment, clinically significant changes affect less than 3%.

In our study, decreased LH level was observed in 21 subjects of the infertile group and 6 subjects of the control group. Moreover, 43 infertile subjects and 11 subjects from control group expressed higher levels of LH values when compared with the normal ranges.

FSH levels was observed to be decreased in 19 infertile subjects and 3 control males while an increase of FSH were found in 56 infertile cases indicating more frequent germ cell epithelium damage than normal males.

In the present work we observed that the level of serum estradiol was found to be lower than normal values in 8 infertile subjects while 43 infertile subjects showed a significant increase in estradiol levels compared to the normal values that may indicate the higher conversion of testosterone to estradiol due to over expression of aromatase *CYP19* in the testis.

With respect to the serum prolactin level, 4 infertile and 3 control males showed an increased levels of prolactin compared to the normal values. Higher number of infertile subjects (30 males) with decreased values for serum prolactin indicates more sexual dysfunction among infertile males.

In the present study, testosterone levels was found to be decreased in 61 cases of the infertile group and 10 cases showed an increase in testosterone level stating that most of the infertile cases show tendency towards decrease in testosterone level than increase. Among control group, 8 subjects showed a decreased value in testosterone levels and only 3 subjects showed increased testosterone level when compared with the normal range of

the laboratory. All the data obtained in our study accord with the previous studies conducted for hormonal assessment of male infertility.

**Physical Examination**

Appropriate sexual development was assessed in physical examination. In the presence of diminished body hair distribution, gynecomastia or eunuchoid proportions, androgen deficiency was suspected. Genital examination in our study revealed the presence of a hypospadiac urethral meatus, pathologic curvature of the phallus or an active sexually transmitted disease. Normal adult testicles should have a length of 3.5- 4.0 cm, breadth of 2.0-2.5cm and thickness of 2.5-3.0 cm, resulting in a volume of approximately 12-18cc. Testicular volume can be estimated using a pachymeter or an orchidometer. Testicles should present with a firm consistency. Approximately 85% of the testicular parenchyma is involved in spermatogenesis, but there is no lower limit for testicular volume to exclude the presence of spermatozoa.

**Colour Doppler Ultrasound scanning and Trans Rectal Ultrasound Scanning (TRUS) of subjects for reproductive organs**

Physical deformities of the male reproductive tract are structural abnormalities that can damage or block the testes, epididymis, seminal ducts, or prostatic utricles and ultimately decrease fertility. These deformities differ in their pathological impact on male reproductive function; some render men totally sterile (such as bilateral absence of the vasa deferentia) while others produce only mild alterations in semen parameters (such as hydrocele). Other physical abnormalities, such as inguinal hernia, may not result in male infertility directly, but are commonly associated with other fertility-threatening conditions. Moreover, surgical repair of inguinal hernia can also result in male infertility.

**Colour Doppler scanning of the Scrotum Testicular scanning**

**Testes**

Assessment of testicular volume in relation to spermiogram in a sizable group of infertile men when compared to healthy fertile individuals once again confirms our insight into the changes of the testicular function in infertile males.

This study is, to our knowledge, the first to analyze in detail relationship between testicular volume, measured by ultrasonography, and spermiogram in large group of young infertile males. Testicular volume was significantly lower not only in infertile individuals but in the elderly compared with the young control group, due to aging process through which the spermatogenesis is compromised. This might reflect the existence of testicular pathology distinct from the normal aging, even though subjects with detectable causes of testicular failure were excluded. It is also possible that these changes represent a range of age-related alterations in testicular function. In the present study, the most common age group affected was 25–35 years comprising around 70 % of the cases similar to findings of Cardona *et al.,* (2009) and 25 % of the patients were below 25 years, whereas Cardona *et al.,* (2009) found only 8 % of the patients in this age group. This can be due to early age of marriage in India.

In the present study the ultrasonic testicular volume was positively correlated with sperm count, which is in accordance with previous studies on sperm quality and measurements of the testes (Takihara*et al.,* 1987).

**Varicocele**

Varicocele is defined as abnormal dilatation and elongation of the internal spermatic veins and pampiniform plexus of the spermatic cord. Varicocele affects about 15% of the men in US population (Steeno *et al.*, 1976). Despite the fact that most adult varicoceles (>80%) have no effect on male infertility (Green *et al.*,1984; Sylora and Pryor, 1994). Several studies suggest that a man with varicocele is at risk of subsequent loss of testicular function and fertility, regardless of normal semen analysis or documentation of previous fertility (Cozzolino and Lipshultz, 2001; Marmar, 2001). At the same time, varicocele is the most common correctable cause of male infertility, present in 40% of men with primary infertility and in up to 70% of men with secondary infertility (Kursh, 1987; Jarow, 2001). The WHO has reported that 1 in 4 men with abnormal semen parameters have a varicocele, compared with 1 in 10 men with normal semen parameters (WHO, 1992). Significant improvement in semen parameters after varicocele repair has been achieved in more than 50% of affected men (Dubin and Amelar, 1971).

Unilateral or bilateral clinical varicocele is associated with defective endocrine and exocrine testicular and epididymal functions, manifested by disordered semen parameters include asthenozoospermia, teratozoospermia, oligozoospermia and azoospermia. Several studies have shown testicular endocrine abnormalities in infertile men with varicocele, marked by low serum inhibin B levels and low serum testosterone, although these endocrine changes have not been reproduced in others (Pirke *et al.*,1983; Younes, 2000; Mormandi *et al.*, 2003; Goulis *et al.*, 2011) Sperm dysfunction in patients with varicocele is characterized by elevated sperm DNA fragmentation index, a build-up of oxidative stress markers, inactive mitochondrial activity and abnormal acrosome

reaction (Lacerda *et al.,* 2011). Leydig cell dysfunction has been demonstrated in patients with varicocele, in correlation with a reduction in serum testosterone levels (although the levels remained within normal limits) (Hudson, 1996). Animal studies have demonstrated reduced intratesticular testosterone, despite normal serum testosterone level, which may jeopardize the functional and proliferate activity of androgen-dependent cells along the genital ducts, such as epididymal principle cells, seminal vesicle cells and prostate cells (Luo *et al.,* 2011).

The pathophysiological effects of varicocele on testicular function are incompletely understood, although the rise in scrotal temperature attributed to poor venous return has been suggested to be an important mechanism. Adequate venous return is an important mechanism for testicular cooling, which is essential for the process of spermatogenesis (Goldstein and Eid, 1989). Testicular thermal injury occurs via alterations in RNA binding proteins and DNA within the sperm, leading to an increased rate of apoptosis (Fujisawa *et al.,* 1989;Yin *et al.,* 1997; Nishiyama *et al.,* 1998). Experimental elevation of epididymal temperature enhances apoptosis and diminishes the storage capacity of this structure resulting in impaired spermiogenesis and changes in sperm count, motility and morphology (Bedford and Yanagimachi, 1998;Ozturk *et al.,* 2008). Oxidative stress has been suggested as another major mediator of varicocele-induced testicular injury. 80% of infertile men with varicocele and 77% of men with incidental varicocele have elevated seminal ROS concentrations (Sharma *et al.,* 1999; Hendin *et al.,* 1999). Excessive ROS generation associated with varicocele has been attributed to an increase in nitric oxide, superoxide anion and hydrogen peroxide production, released by inducible nitric oxide synthase and xanthine oxidase in the dilated

spermatic veins, (Mitropoulos *et al.,* 1996; Romeo *et al.,* 2003), which could cause the high levels of sperm DNA damage commonly seen in patients with varicocele (Fujisawa *et al.,* 1989;Yin *et al.,* 1997; Nishiyama *et al.,*1998; Smith et al., 2006). Oxidative stress has also been linked to a decrease in the antioxidant defense system in seminal plasma observed in varicocele (Barbieri *et al.,* 1999; Chen *et al.,* 2001).

Some investigators suggest that varicocele causes increased hydrostatic pressure in the pampiniform plexus and venous stasis, which leads to testicular hypoperfusion and, consequently, testicular hypoxia and progressive atrophy (Benoff and Gilbert, 2001). Venous stasis also results in the insufficient removal or backflow of toxic substances from the kidney or adrenal glands. Testicular hypoperfusion and hypoxia can lead to release of vascular endothelial growth factor (VEGF) from Sertoli cells, Leydig cells, vascular endothelial cells and epididymal principal cells (Shiraishi and Naito, 2008), which can then inhibit spermatogonial proliferation and lead to increased vascular permeability, capillary angiogenesis and thickening of basement membrane and interstitial tissue, interfering with regulation of microcirculation (Korff and Augustin, 1999).

Larger varicoceles (grade II and III) are associated with a higher incidence of testicular growth arrest (Zenke and Turek, 2005) and higher levels of oxidative stress markers (Mostafa *et al.,* 2006). Nevertheless, no significant correlation has been demonstrated between varicocele grade and the severity of semen quality impairment (Diamond *et al.,* 2007).

The impact of varicocele repair on male fertility has been assessed in many retrospective and prospective studies. A recent meta-analysis reported that varicocele repair—whether by microsurgical varicocele vein ligation, macroscopic open inguinal

procedure, laparoscopic vein ligation or embolization of the varicocele veins—can significantly improve sperm count, sperm progressive motility and sperm ultrastructure (Baazeem *et al.*, 2011). Moreover, varicocele repair can enhance sperm function through reduction in oxidative stress markers and DNA fragmentation index (Baazeem *et al.*, 2011). Nevertheless, this meta-analysis failed to show significant improvement in spontaneous pregnancy rates after repair. Previous Cochrane meta-analyses have also failed to demonstrate improved paternity rates (Evers and Collins, 2001; Evers and Collins, 2007). Marmar *et al.*, (2007) on the other hand, reported a significant improvement in pregnancy rate, which has been attributed to the inclusion of men with varicocele who were normospermic or had subclinical varicocele, a high patient dropout rate resulting in loss of paternity information, limited period of follow-up after repair, inclusion of prospective and observational studies in the same meta-analysis, and heterogeneity between studies. Future prospective studies are certainly required to critically assess the effect of varicocele repair on pregnancy rate, taking into account all these confounding factors.

Varicoceles are present in 15% of the normal male population and in approximately 40% of men presenting with infertility (Nagler, 1997). The preponderance of experimental data from clinical and animal models demonstrates a deleterious effect of varicoceles on spermatogenesis. Testicular temperature elevation and venous reflux appear to play an important role in varicocele-induced testicular dysfunction, although the exact pathophysiology of varicocele induced damage is not yet completely understood.In our study, out of 274 infertile subjects, 8 (2.9%) of them were detected with right varicocele, 40 (14.6%) of them were found to be associated with left varicocele and 51

(18.6%) of them were detected with bile varicocele. In total 98 infertile subjects (36%) were diagnosed with different types of varicocele that accords with the previous studies.

**Hydrocele**

Hydrocele is an abnormal collection of fluid between the parietal and visceral layers of the tunica vaginalis. It is the most common cause of painless scrotal swelling (Rubenstein *et al*., 2004) with an incidence of 1–3% in full-term infants (Baskin and Kogan, 1999) and up to 30% in premature infants and those with delayed testicular descent. The incidence in adult males is approximately 1%, (Esposito *et al.,* 2004; Al-Kandari *et al.,* 2007;Lipshultz *et al.,* 2007) although prevalence varies according to country. Hydroceles are bilateral in approximately 7–10% of affected men (Mihmanli *et al*., 2004). The imbalance between fluid production and absorption through tunical mesothelial cells is the underlying mechanism that is responsible for the formation of hydroceles. Hydroceles are classified as communicating or noncommunicating based on the patency of the processus vaginalis—a peritoneal pouch that invades and migrates with the gubernaculum to provide the potential space for the testis to descend into the scrotum (Heyns, 1987). The processus vaginalis normally closes after complete descent of the testis, within 18 months of birth. However, autopsy findings suggest that a patent processus vaginalis is present in 80–94% of infants and in 15–30% of adults (Skoog, 1997;Barthold and Kass, 2002). In the presence of a unilateral patent processus vaginalis, the incidence of a contralateral patent processus vaginalis has been found to be 15–22% (Schneck and Bellinger, 2007). Hydrocele constitutes the third most common ultrasonographically-detected scrotal abnormality after varicocele and epididymal cyst (Pierik *et al.,* 1999).

Communicating hydroceles occur when the processus vaginalis is persistently patent. They are commonly diagnosed in the pediatric age group and are frequently associated with indirect inguinal hernia when the patent processus vaginalis is wide. Diurnal variation in the size of hydrocele occurs owing to gravity-induced movement of the peritoneal fluid (Schneck and Bellinger, 2007). Although communicating hydroceles are less common in adults, they are sometimes observed in patients with a patent processus vaginalis accompanied by increased intra-abdominal fluid or pressure owing to shunts, peritoneal dialysis, or ascites (Barthold and Kass, 2002; Schneck and Bellinger, 2007).Adults with connective tissue disorders have a high risk of communicating hydrocele owing to attenuation of tissue support to the inguinal openings (Baskin and Kogan, 1999). Intrauterine exposure to polybrominated biphenyl, a brominated flame retardant and endocrine disruptor, is a risk factor for pediatric hydrocele (Small *et al.*, 2009). Closure of the processus vaginalis results in a noncommunicating hydrocele. Depending on location, noncommunicating hydroceles are referred to as simple scrotal hydrocele (limited to the area surrounding the testis) or hydrocele of the cord (surrounding an isolated part of the spermatic cord). Noncommunicating hydroceles are more common in adults than children. Primary adult hydrocele is usually of idiopathic etiology, whereas secondary hydrocele can be caused by testicular torsion, tumor, infection, trauma or varicocelectomy (Kogan *et al.*, 2002).

The impact of hydrocele on testicular geometry and size, spermatogenesis, scrotal temperature and testicular blood flow dynamics has been evaluated. Dandapat *et al.*, (1990) assessed the pressure effect of hydroceles in 120 men with unilateral idiopathic hydrocele, finding no pressure effect in 70% of men, testicular flattening in 22% of the

cohort and pressure-induced testicular atrophy in 8% of patients. Turgut *et al.,* (2006) noted time-related testicular size declines in patients with hydrocele and described a rounding rather than flattening effect of hydrocele on testicular shape (Turgut *et al.,* 2006). By contrast, Mihmanli *et al.,* (2004) found that testicular volume was larger in men with hydrocele and that the testis returned to normal size after hydrocele excision. They propose that this increase in size is due to hydocele pressure-induced obstruction in the vessels of the testis, which creates stasis in the venous and lymphatic outflow resulting in testicular vascular edema (Mihmanli *et al.,* 2004). Some investigators have shown that hydrocele can affect spermatogenesis, which may be partially or totally absent (Dandapat *et al.,* 1990; Mangoud *et al.,* 1993). For example, Dandapat *et al.,* (1990) reported normal spermatogenesis in 82% of the cohort, partial arrest of spermatogenesis in 10% and a total arrest in 8% (Dandapat, *et al.,* 1990).The possible pathophysiologic mechanisms that underly impaired spermatogenesis include the pressure effect of the hydrocele on the testis, (Turgut *et al.,* 2006) the reaction of testicular cells to the highly proteinaceous fluid, and raised intrascrotal temperature (Mihmanli *et al.,* 2004).

The hydrostatic pressure of a hydrocele exceeds the pressure in blood vessels within the scrotum, which interferes with arterial blood flow and might have an ischemic effect on the testicle. Histopathologic testicular changes observed in patients with hydrocele include interstitial fibrosis, thickening of the basement membrane, and disorganization of spermatogenic cells (Bhatnagar *et al.,* 1970; Singh *et al.,* 1989; Dandapat *et al.,* 1990; Mangoud *et al.,* 1993). Testicular blood flow dynamics reveal an increase in the resistive index of the subcapsular arteries of the ipsilateral testis, compared to those in the normal testis. Mihmanli *et al.,* (2004) used color Doppler ultrasonography

to assess blood flow before and after surgical excision of hydrocele, and found that a high-resistance flow in the intratesticular arteries before surgery was replaced by a low-resistance flow after hydrocele repair and elimination of the pressure. Nye *et al.,* (1997) on the other hand, observed a lack of testicular diastolic flow ipsilateral to the hydrocele. Altered blood flow dynamics clearly indicate that hydrocele causes an ischemic insult to testicular tissue. Besides that, hydrocele repair may inadvertently injure the epididymis and vas deferens (Zahalsky *et al.,* 2004).

**In the present study,** 68 infertile subjects (25%) were found with hydrocele out of 274 infertile subjects. Wherein 14 (5.1%) of them were detected with right hydrocele, 19 (6.9%) of them were found to be associated with left hydrocele and 35 (12.7%) of them were detected with bile varicocele. Certainly, controlled randomized trials are required to prove or disapprove such a relationship and to verify the usefulness of hydrocele repair for improving paternity rates in infertile men. In India and other tropical countries the incidence of hydrocele is much higher due to the high prevalence of filarial infections. In one review of 500 cases of hydrocele from India almost 43% were due to filarial infections (Kumar et al., 2006). Filarial infections are known to infect 120 million people worldwide and of these 25 million suffer from urinary and genital region infections. Hydrocele can also develop as a result of inflammation or injury within the scrotum. Inflammation may be the result of infection of the small coiled tube at the back of each testicle (epididymitis) or of the testicle (orchitis). The imbalance exist between fluid production and absorption through tunical mesothelial cells is responsible for the formation of hydroceles in few infertile patients in the present study. Apart from this

Congenital hydrocele, scrotal injury and radiotherapy could be other causative factor in our study.

## Epididymal deformities

Epididymal cysts are the most common epididymal mass, occurring in 20–40% of asymptomatic men (Leung *et al.,* 1984). 75% of epididymal cysts are true cysts, meaning they are lined with epithelial cells and contain lymphatic fluid. The remaining are spermatoceles, commonly formed from obstruction of the efferent ductal system, which leads to cystic dilatation with fluid containing spermatozoa, lymphocytes, and cellular debris. True epididymal cysts can arise throughout the epididymis before and after puberty whereas spermatoceles almost always occur in the epididymal head of postpubertal men (Dogra *et al.,* 2003) The two types are indistinguishable on ultrasonography, so the only means of differentiating epididymal cysts from spermatoceles is aspiration of the cystic fluid to assess for the presence of sperm (Munden and Trautwein, 2000).

The exact etiology of epididymal cysts is unknown; however, Wollin *et al.,* (1987) have suggested they arise from vestigial remnants of the epididymis that no longer communicate with epididymal tubules. Cysts have been linked to diethylstilbestrol exposure, testicular dysgenesis syndrome and cryptorchidism. Because the epididymis is an androgen-dependent structure, it has been assumed that fetal exposure to diethylstilbestrol, dietary ingestion of phytestrogen and cannabis intake have a role in causing not only epididymal cyst but also other genital anomalies, such as hypospadias and undescended testicles (Paulozzi *et al.,* 1999; Baskin *et al*., 2001). Others have hypothesized that vasal or epididymal obstruction leads to epididymal congestion,

swelling and secondary cyst formation, (Jarvis and Dubbins, 1989) although direct measurement of hydrostatic pressure in the epididymis after vasectomy does not support this theory. Epididymal cysts can occur in association with genetic syndromes such as von Hippel–Lindau and cystic fibrosis (Leung *et al.*, 1984). The etiology of spermatocele is also unknown, but is thought to be the result of a focal weakening of the external layer of an epididymal tubule, leading to formation of diverticula. The clinical significance of epididymal cysts and spermatoceles, as well as their association with male infertility has not yet been resolved.155 Spermatoceles have been described as 'sperm retrieval reservoirs' in men with obstructive azoospermia (Rados *et al.*, 1996) but there have been no reports of a correlation between epididymal cysts and male infertility, even in those with bilateral epididymal cysts. Watchful waiting with regular follow-up has been suggested for both epididymal cyst and spermatocele, as long as they are small in size and produce no symptoms. Cyst excision and spermatocelectomy are recommended for abnormally large and painful lesions, although surgery is not without complications. Epididymal injury is a primary concern during excision surgery and has been diagnosed or suggested in 17–50% of patients who undergo spermatocelectomy (Chiari and Drujan, 1980; Zahalsky *et al.*, 2004). Such injury can lead to epididymal obstruction (Chiari and Drujan, 1980). Additional postoperative complications are those typical of scrotal surgery, including hematoma, hydrocele, hematocele, infection and testicular atrophy due to vascular injury. Kauffman *et al.*, (2011) suggested the use of microscopic surgery to reduce the incidence of injury to the epididymis, especially during spermatocelectomy (Kauffman *et al*., 2011). Percutaneous aspiration and sclerotherapy have been attempted

but are not advocated due to the risk of epididymal obstruction, chemical epididymitis and recurrence (Beiko and Morales 2001).

**In the present study,** infertile males were found with right epididymal cyst while 16 subjects were diagnosed with left cyst and 11 subjects with bile cyst. 11 infertile subjects showed an atrophy of left epididym that could be due to genetic factors or torsion, may be caused by obstruction of the tubes that carry sperm from the testicles. The reason for these cysts development is unknown, but they usually develop as a result of sperm and/or other fluids accumulating at the head of the epididymis. An epididymal cyst is often preceded by either an injury to the groin area or infection called epididymitis.

**Vas deferens**

Congenital absence of the vas deferens (CAVD) is an uncommon entity with a reported prevalence range of 1%- 2% in the male population (Durieu *et al.*, 1997). Most of these cases are due to bilateral vas agenesis (1%–6%). Only 0.4% of male infertility cases have been attributed to CUAVD. The infertility in CUAVD patients is often due to obstruction of the contralateral vas deferens (Weiske et al., 2000). Renal agenesis is more commonly associated with unilateral vasal agenesis (73.7%) compared to the bilateral form (11.8%)(Weiske et al., 2000). CUAVD occurring with renal agenesis is due to an intrinsic Wolffian duct defect. Other renal anomalies associated with CUAVD are malrotation of the solitary kidney, multicystic kidney, ectopic kidney, and horseshoe kidney (Khan 2001). Anomalies of the seminal vesicles, ejaculatory ducts, cryptorchidism, and inguinal hernia have also been reported in association with CUAVD (Kolettis and Sandlow). It is commonly discovered either during an evaluation for infertility or during a vasectomy. Casals *et al.*, (2000) showed that 38% of congenital

unilateral absences of the vas deferens cases are associated with mutations in the CFTR gene. About 45% of these mutations were specific to congenital absence of the vas deferens (CAVD) and were not found in cystic fibrosis patients. In the present study out of 274 infertile cases only 3 cases were reported with congenital absence of vas deferens. Of theses, all three of them are due to bilateral vas agenesis. Hence our data accords the previous work. CFTR gene mutational analysis was not done in the present study.

**Prostate abnormalities**

Widespread implementation of imaging techniques such as TRUS and endorectal MRI has increased the detection of cystic lesions of the prostate, which are thought to affect 0.5–7.9% of men (Hamper *et al.*, 1990; Dik *et al.*, 1996). Various methods of classifying prostatic cysts have been reported, such as whether they are congenital or acquired, or based on their position within the prostate (midline, paramedian or lateral). Most recently, Galosi *et al.,* (2009) suggested a new model based upon anatomical site, embryological origin and pathological characteristics that classify cysts into six major types. There are two types of cyst (midline prostatic cysts and ejaculatory duct cysts) that can obstruct the ejaculatory ducts and lead to male infertility.

Midline prostatic cysts can be divided into three types: prostatic utricle cysts (previously called Müllerian duct cysts), cystic dilatation of the prostatic utricle and enlarged prostatic utricles. A prostatic utricle cyst results from failure of the Müllerian ducts to regress causing focal saccular dilatation (Mayersak*et al.,* 1989) Located at the region of the verumontanum, these cysts may extend above the prostate or slightly lateral to the midline, and may grow into a large mass. Prostatic utricle cysts do not communicate with the urethra, therefore aspirations do not contain spermatozoa (Kato

*etal.,* 2002). This type of cyst affects 5% of men with obstructive azoospermia (Li *etal.,* 2010). The condition is usually asymptomatic, but patients in the third or fourth decade of life (Nghiem *et al.,* 1990) may develop irritative and obstructive urinary symptoms as well as hematuria, hematospermia, bloody urethral discharge, ejaculatory pain, urinary tract infection, epididymitis, infertility and constipation (Shabsigh *et al.,*1989). Cystic dilatation of the prostatic utricle (cystic utricle) arises due to obstruction of the junction between the utricle and the urethra (Kato *et al.,* 2002; Kato *et al.,* 2005). Such cysts are therefore able to communicate with the posterior urethra (Nghiem *et al.,* 1990). Typically, cystic utricles are smaller than prostatic utricle cysts, are strictly localized to the midline, and measure no more than 15 mm (along the longest axis). Both prostatic utricle cysts and cystic utricles can enlarge and compress both ejaculatory ducts resulting in altered semen parameters, and sometimes azoospermia. The third type of midline prostatic cyst is not technically a cyst but rather an enlarged or hypertrophied prostatic utricle that communicates freely with the prostatic urethra. Mainly detected in children and adolescents, enlarged prostatic utricles are frequently found in children with urogenital malformations, such as proximal hypospadias or virilization defects (Hinman, 1993). TRUS and cystourethrography usually reveal an enlarged prostatic utricle that is midline and posterior—the wide opening into the posterior urethra can be easily identified. This type of cyst does not typically obstruct the ejaculatory ducts (Mayersak, 1989).

Ejaculatory duct cysts originate from the Wolffian ducts and occupy a paramedian or median position in the prostatic gland above the level of the verumontanum (Galosi, *et al.,* 2009). Such cysts can be congenital or acquired, with etiologies including partial distal obstruction caused by chronic infection, transurethral manipulation, tuberculosis or

urethral foreign body (Ardill *et al.,* 1990). Ejaculatory duct cysts can be unilateral or bilateral and are associated with obstructive azoospermia. When small, these cysts appear on TRUS as intraprostatic masses just lateral to the midline at the base and midline at the level of the verumontanum (Nghiem *et al.*, 1990). When large, however, these lesions can mimic cystic utricles and prostatic utricle cysts. Clinical presentation depends on the size of the cyst; small cysts are usually asymptomatic while large ones can cause hematospermia, ejaculatory pain, azoospermia and male infertility (Littrup, *et al.* , 1988; Nghiem *et al.,* 1990).

In the present study, a considerable number of infertile subjects, 137 (50%), were found with a decreased values (< 12 cc) for prostate volume when compared to the normal ranges (12-20 cc) with different value of deviation from 0.2 cc to 8 cc. In control group also 14 subjects were diagnosed with prostatic hypoplasia with lower volume than normal ranges which needs further investigation to understand the mechanism leading to this condition. Although these control subjects have normal semen parameters, they were associated with some other clinical symptoms like hydrocele or testicular hypoplasia. **As per our knowledge, this is the first report in south India revealing these conditions in infertile and fertile population.** Causative agents include bacterial infections similar to those causing urinary tract infections, as well as *Neisseria gonorrheae*. A related complication of prostatic abscess is uncommon. Prostatic cysts usually result from an obstruction of prostatic ducts and fluid retention within the prostatic parenchyma. There are usually multiple cavitary areas within the gland which potentially connect with the urethra. Paraprostatic cysts are thought to originate from the blind-ended uterus masculinus, formed from the müllerian duct system. Microscopically,

nodular prostatic hyperplasia consists of nodules of glands and intervening stroma. Most of the hyperplasia is contributed by glandular proliferation, but the stroma is also increased, and in rare cases it may predominate. The glands may be more variably sized with larger glands have more prominent papillary in folding. Nodular hyperplasia is not a precursor to carcinoma. (Homma *et al.*, 1996). The mechanism for hyperplasia may be related to accumulation of dihydrotestosterone in the prostate, which then binds to nuclear hormone receptors which then trigger growth.

## Seminal vesicle

The function of seminal vesicle is important for fertility. Estimation of fructose level is a simple method for the assessment of the seminal vesicular function. Measurement of seminal fructose has been used in almost all laboratories across the globe as a marker of the seminal vesicular function. The WHO includes the measurement of this sugar to assess the function of these glands (WHO, 2001). After ejaculation, fructose is consumed by the spermatozoa in a process named fructolysis. At higher sperm counts, the process will be stronger resulting in a low seminal fructose concentration. That is the reason that seminal fructose is higher in azoospermic and oligozoospermic than in normozoospermic or polyzoospermic men. This can be seen that the value of seminal fructose concentration is not appropriate as a marker of the secretory activity of the seminal vesicles, unless the influence of sperm count on the fructose concentration can be excluded. A lower level of corrected seminal fructose were observed in men with hypofunction of the seminal vesicles (Gonzales and Villena 1997) and either low serum testosterone levels or with an obstructive process at the seminal vesicles has been related to male infertility (Gonzales et al., 1988; WHO 2001). Subjects with hypofunction of the

seminal vesicles have low sperm motility, which itself may cause infertility (Gonzales and Villena 1997). In the present study no significant difference exists between controls and infertile subject with reference to fructose level indicating seminal fructose is not involved in male infertility in the present study.

Ultrasonographic examinations are useful for the diagnosis of seminal vesicle dysfunction because it is one possible cause of sexual impairment. Schultheiss *et al.*, (2008) showed that urogenital infections may affect sexual function by causing premature ejaculation and erectile dysfunction. However, there is no direct evidence regarding the influence of seminal vesicle dysfunction on ejaculation and erection (Schultheiss *et al.*, 2008). Nevertheless, some studies have indicated that prostate inflammation often involves both seminal vesicle (Vicari 1999; Vicari et al., 2006) and this could have multiple effects on sexual function.

In the present study, Ultrasonographic examination of seminal vesicle has revealed that the mean value of right seminal vesicle volume was .97±1.09cc in infertile group that was lower when compared with the control subjects (1.05±0.93) but the difference was not significant ($p>0.05$). Similarly, the left seminal vesicle was also found to be insignificantly higher in controls (1.05±0.95) than infertile group (0.95±1.12, $p>0.05$). The presence of cysts in the seminal vesicle is extremely rare, they must be considered in patients presenting with symptoms of chronic prostatitis, recurrent epididymitis and recurrent UTIs**.**

To our knowledge, this is the first study exploring the ultrasound characteristics of the seminal vesicle in infertile individuals in relation to control group. The lack of

ultrasound data on seminal vesicle function in infertile subjects with control is of particular relevance given the increasing interest of the negative impact of seminal vesicle on sperm parameters and consequently on male reproductive functions.

Hence Ultrasonography (US) is a simple and noninvasive method of imaging wherein imaging of the upper urinary tract is extremely important in these patients.

## ***SECTION V***

# *SUMMARY*

1. Male infertility refers to the inability of a male to contribute to a pregnancy in a fertile female within one year of married life with regular intercourse not using any method of contraception.
2. In human population malefactor contributes for 40-50% of infertility in married couple . Male infertility is commonly due to deficiencies in the semen, and semen quality is used as a surrogate measure of male fecundity. In Western countries one in four men consulting fertility clinics has specific condition like low sperm count, motility or/ and morphology, causing infertility. In India about 15–20% of married couples known to be sub/ infertile category, selected for medically Assisted reproductive technology (ART). However, a substantial portion of infertile patients still remain without help for various reasons such as lack of adequate treatment options and their accessibility, high cost and fear of conceiving and bearing potentially abnormal offspring. This is despite the fact that over the years ART has become useful for couples with infertility, with a good success rate of about 20 to 30% globally.
3. The basic diagnosis of male fertility is best described by the World Health Organization (WHO) laboratory manual for the examination for the human semen and sperm-cervical mucus interaction (WHO, 2010). Other complementary non invasive method for diagnosing the anatomical pathology of male infertility is Ultrasound scanning and Transrectal Ultrasound Scanning (TRUS) of the reproductive organs.

4. The present investigation was undertaken in infertile men in Mysore, with objectives of analysis of reproductive hormones, examination of internal and external reproductive organs in association with semen profile.
5. The literature survey about the known pathophysiology of male infertility and possible physiological and negative impact of anatomical changes on fertility pathways in men has been reviewed.
6. A total 274 confirmed subjects with infertility were considered for the present study from Medivawe IVF and fertility research hospital in Mysore. The age of the patients ranged from 21 to 50 years. Aged match controls consisting 130 males with normal semen parameters and proved fertility were also randomly selected from different locations of Mysore city, irrespective of their ethnic background. The informed written consent letter was obtained from the participants before including them in the study. This study was approved by the Institutional Human Ethical Committee of University of Mysore.
7. The diagnosis of infertile patients based on their semen characteristics were classified as aspermia, azoospermia, oligozoospermia, asthenozoospermia, teratozoospermia, oligoasthenozoospermia, oligoasthenoteratozoospermia etc. according to WHO guidelines (2010).
8. The incidence of consanguineous marriage among infertile group was not significant when compared with the control group.
9. Coital frequency was found to have no significant difference between infertile and control groups. No significant relationship was observed between coital frequency, age and BMI.

**10.** Analysis of clinical manifestation of all conditions revealed the following information:

a. Azoospermia was most prevalent condition among infertile groups.

b. More than 50% of the infertile subjects were found with impaired semen liquefaction time and the number was significantly higher (7.6%) when compared with the control group.

c. Among infertile individuals 67.5% had abnormal semen pH values.

d. Independent t-test with respect to motility as a variable revealed that the infertile males showed a lower progressive motility values with significant difference compared to controls.

e. Comparison of sperm viability between study groups showed a significantly decrease in infertile group compared to control group.

f. Independent t-test for sperm and germ cell morphology assessment showed the higher values in control group with a significant difference with infertile group.

g. Pearson correlation analysis showed a negative relationship between BMI and all the quantitative semen parameters but the relationship between age and quantitative semen characteristics was negatively significant only for sperm viability and morphology.

h. Fructose was present in all control group **is 13 µmol per ml**. In infertile group out of 258 subjects fructose was completely absent in 3 (1.2%) subjects and partially detected in 19 (7.4%) subjects.

**11.** In the present study, data obtained on assessment of hormones revealed the following results:

a. LH levelwas higher in infertile males when compared to the controls.

b. FSHlevels was also found to be higher in infertile males when compared to controls and the difference was significant.

**c.** Datashowed thatProlactinlevels lower controls when compared with the infertile but the value was not significantly different between groups.

d. Testosterone levels showed higher value in control group when compared to the infertile subjects and the difference was significant at 0.01 level.

e. Non significant increase of estradiollevelswas found in infertile group when compared to controls.

f. Assessment of reproductive organs using Ultrasound scanning and TRUS revealed the following data:

**g.** Mean value of both right and left testicular volume was significantly higher when compared with the infertile subjects than infertile group.

h. Significant positive correlation was seen between total testicular volume and semen volume.

i. Highly positive relationship was observed between testicular volume and sperm motility.

j. Testicular volume was also observed to be significantly lower in men with low semen volume.

k. Both sperm count per ml and total Sperm count were directly related to total testicular volume.

l. Around 69.9% (267) of men had normal total sperm count per ejaculate (39 million and above) demonstrating the mean total testicular volume of 20.63 ml.

m. Significant positive correlation was seen between prostate volume and age, BMI semen volume, sperm count, total sperm count, sperm motility, normal sperm morphology and sperm vitality.

n. Pearson correlation test values were not significant between prostate volume and semen pH.

o. Non significant negative relationship was observed between prostate volume and semen liquefaction time.

**p.** Mean value of both right and left seminal vesicle volume was lower in infertile subjects when compared with the control subjects but the difference was not significant.

q. Pearson correlation test revealed a significant positive relationship between left seminal vesicle volume and BMI.

**r.** No significant relationship was observed between age and both right and left seminal vesicle volume.

s. Around 18.5% of infertile subjects showed abnormalities in both right and left epididymis.

t. 36.1% of infertile subjects were associated with different grade of varicocele.

u. Among infertile subjects 24.7% were found to be associated with Hydrocele.

**Appendices**

ADD- Ampulla ductus deference

ANOVA-Analysis of Variance

AR – Artificial Reproduction

ART- Assisted Reproductive Technology

BMI- Body Mass Index

BPH- Benign Prostatic Hyperplasia

CAP - Carcinoma of the Prostate

CAVD-Congenital Absence of the Vas deferens

CBVAD -Congenital Bilateral Absence of the Vas deferens

CF=Coital Frequency

CFD - Color Flow Doppler

CFTR - Cystic fibrosis Transmembrane conductance Regulator

CI - Confidence Interval

CYP-Cytochrome 450

DHT- Dihydrotestosteron

DNA- Deoxy -ribose nucleic acid

EDO- Ejaculatory Duct Obstruction

ELISA- Enzyme Linked Immune Sorbent assay

FSH- Follicle Stimulation Hormone

GnRH- Gonadotrophin-Releasing Hormone

HBS- Hepatitis B Virus

HRP - Horseradish Peroxidase

HIV – Human Immune Virus

IVF - Invitro Fertilization

ICSI- Intra Cytoplasmic Sperm Injection

LH - Leutinizing hormone

Liq= Liquefaction

IHEC - Institutional Human Ethical Committee

MART- Medically Assisted Reproductive Technology

MRI - Magnetic Resonance Imaging

µl – Micro liter

M – Molarity

mM- Milli molar

ml- milli liter

NS=non significant,

NaOH- Sodium hydroxide

OAT- OligoAsthenoTeratozoospermia

OD - Absorbance

PD - Penile Duplication

PSA - Prostate-Specific Antigen

pH- Percentage of hydrogen

*p*= Significant value

ROS- Reactive Oxygen Species

RNA- Ribose nucleic acid

KS-Klinefelter Syndrome

r= Correlation coefficient

SV=Seminal volume,

SC=Sperm count,

SPSS-Statistical Package for the Social Sciences

STD - Sexual Transmitted Diseases

TESE - Ttesticular Sperm Extraction

TRUS -Transrectal Ultrasonography,

TURED - Transurethral Resection of Ejaculatory Ducts

TSH -Thyroid Simulating Hormone

TSM= Total Sperm Motility,

TCA - Trichloroacetic Acid

UMI- Unexplained Male Infertility

US-United States

VEGF - Vascular Endothelial Growth Factor

WHO – World Health Organization

$ZnSO_4$ - Zinc Sulphate.

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