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An update on the Current approaches for the enhancement of virility and the models for preclinical evaluation

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Abstract:-

Aphrodisiacs can be categorized into drugs that act on sexual desire, affect the potency and ones that affect the sperm viability. According to statistics, it is seen that patients with end stage renal disease face sexual disturbances. Everolimus, sirolimus, tacrolimus these are mostly used drug which causes this condition. Here we are elaborating a promising approach to restore the virility by using the test drug *Vidarikand.* In this context the wister albino rats are used to study this kind of condition, divided in various groups as well to prove regenerative effect of *Vidarikand* on infertility in specifically male rat models. The results that are obtained can be used in preclinical studies and the proper criteria to apprise the activity of regenerative drugs to treat immunosuppressant induced infertility has been chosen.

Key words: aphrodisiacs, animal models, infertility drugs

1. INTRODUCTION:-

According to the mode of action aphrodisiacs can be divided in to three categories –

- Act on sexual desire
- Affect potency (effectiveness of erection)
- Affecting sperm viability.

Basically for effective intercourse the sexual desire originates from central nervous system, where spinal or supra spinal mechanism plays a major role in erectile function. It is initiates in the brain, which is the result of sexual stimuli which might be visual, olfactory,tactile or imaginative. MPOA (Medical Pre-optic Area) it is recognize ing the sensory stimuli from higher portion of brain into sexual motivation and copulatorybehavior, where PVN (Para Ventricular Nuclei) does different work in erectile response. It is producing the seminal discharge (PVN stimulated pharmacological or through electrical stimulation) when the rats are unanesthetised, and when rats are anesthetized it causes erection and ejaculation.

Patients with end stage renal disease are treated with the help of renal transplantation for survival benefit and for better quality of life. For this reason ESRD patients facing the sexual disturbance and reduced fertility, renal transplantation is the most obvious treatment which can restore these functions again in both the genders. It is important to understand the immunemechanisms for allograft rejection and simultaneously the development of the novel immunosuppressant drugs which can remarkably produce the curative effect. Therefore prolonging the reproductive period in renal transplant recipient takes place. For example the use of sirolimus, everolimus, cyclosporine as immunosuppressive agents, which has tremendous effect in allograft rejection curative regimen but at the same time it causes toxicity to the germ cells and impair both spermatogenesis and male gonadal function. It is known that sperm concentration and motility are inversely correlated with the immunosuppressive agents whole blood trough levels and gonadal dysfunction, infertility which is associated with the sirolimus that is already reported in male renal transplant recipients.

2. EPIDEMIOLOGY:

Infertility is a significant issue now a days, influencing roughly 15 percent of couples of regenerative age. It is assessed that universally, 60-80 million couples experience the ill effects of this, consistently, of which around 15-20 million are in India alone. Male factor adds to barrenness as much as 51.2% agreeing WHO examines, because of the debate of idea of male climateric or "andropause". Developing proof shows that some maturing men have diminished generation of testosterone can caused because of the decreased libido, impotence, decreased growth of body hair, decreased muscle mass, fatigue, increased risk of myocardial infarction and decreased muscle mass in conjuction with osteoporosis. It is all around set up that smoking effectsly affects spermatogenesis as it has connected with lower sperm tallies, diminished motility and impeded morphology. Weakness, testicular decay and loss of sexual intrigue are related with liquor abuse and decreased FSH, LH and testosterone levels have been found because of over the top drinking.

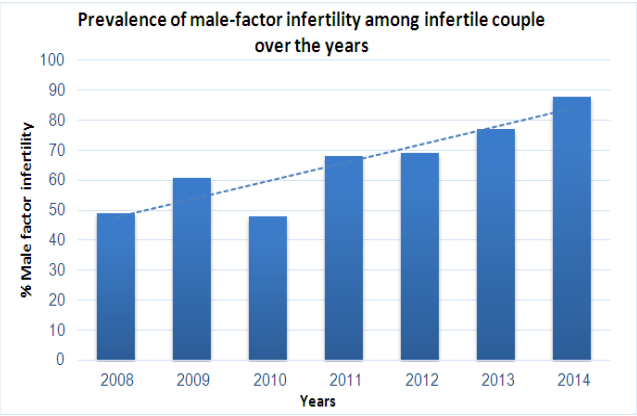

Fig No. 2.1 Bar graph indicating the prevalence of male factor infertility among infertile couple over the years (Source: Clinical Medicine and Diagnostics; p-ISSN: 2163-1433;e-ISSN2163-1441;2018; 8(1): 14-20)

3. FACTORS AFFECTING MALE FERTILITY:-

- **Age :**

Testosterone level in men decreases with age, this can takesplace in the age of thirty even the person is healthy[16]. It is reported that per year around 1% of this hormone level declines, can cause andropause (the more accurate term will be 'symptomatic hypogonadism)[18]. The other symptoms are loss of libido, muscle mass decrement, decreased in bone mineral density, fat mass increment, obesity, emotional irritability also dysphoria and erectile dysfunction. A study in Belgium by Mahmoud et al. showed that testicular volume of elderly males in their eighth decade was significantly less with 31% when compared with the young control group of 18 to 40 years old[21].

- **Smoking**

Smoking can affect male fertility. The decrement in percentage of motility of sperm cells as well the morphology is correlated with the number of cigarettes smoked per day. This activity increases the reactive oxygen species levels, which can cause oxidative stress in the environment[22]. The antioxidant capacity of seminal plasma may exceed due to oxidative stress, which can be toxic for the sperm causing oxidative damage. One of the studies revealed that smoking leads to a decrease in semen parameters such as viability, sperm concentration, motility and morphology[23].

- **Reactive Oxygen Species (ROS)**

Reactive oxygen species have dangerous effect on sperm parameter. ROS (contains abundant unsaturated fatty acids) affect the sperm plasma which is sensitive in nature. So increment in the level of ROS in semen leads to an imbalance between the production of the species and the antioxidant system. Increased ROS level can lead to damage with subsequent sperm dysfunction or cell death. These free radical or oxidative damage to sperm is thought to be responsible for many cases of idiopathic oligospermia[32].

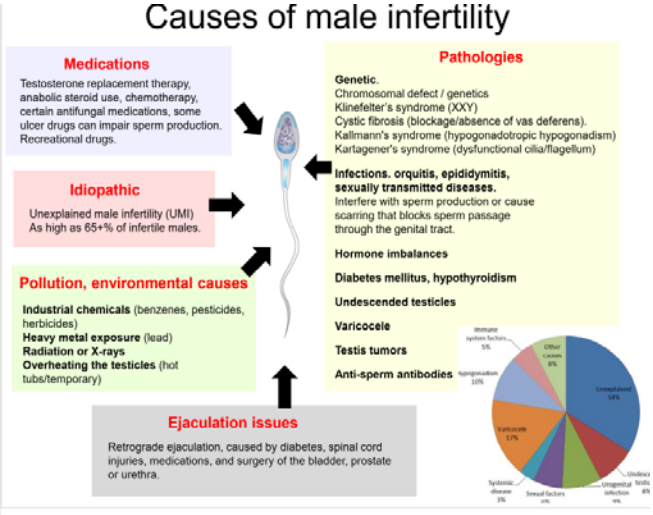

Fig No.3.1 Causes of male infertility
(Source:https://www.memorangapp.com/flashcards/241053/CBCL%3A+Male+Infertility/)

- **Therapeutic drugs:**

Administration of various drugs can cause primary infertility. This phenomenon may be the result of an effect on the hypothalamicpituitary- gonadal axis or a direct toxic effect on the gonads. The drugs are antineoplastic agents (cyclophosphamide, chlorambucil, busulphan, and methotrexate), glucocorticosteroids, hormonal steroids (diethylstilbestrol, medroxyprogesterone acetate, estrogen, and the constituents of oral contraceptives), antibiotics (sulfasalazine and cotrimoxazole), thyroid supplements, spironolactone, cimetidine, colchicine, marijuana, opiates, and neuroleptic agents[41].

- **Stress**:

Though this stress factor is rapidly rejected in studies but there is growing evidence to prove the theory of stress as a risk factor for male fertility. The analysis of semen parameters, which significantly decreases in men, is the main evidence are under stress. There are different forms of stress including psychological, which can affect male fertility and reproduction[34]. He autonomic nervous system and the adrenal hormones participate in the classic stress response while also affecting the reproductive system[23].

- **Scrotal temperature:**

Types of under trousers affect the scrotal temperature, and semen quality. Wearing tight fitting under trousers is associated with increased scrotal temperature[24]. Also the position or activity has its impact on increasing the scrotal temperature, walking is associated with significantly lower scrotal temperature than sitting. Continuous driving (two hours or more than two hours) can elevate the scrotum temperature[25]. Basically spermatogenesis is temperature dependent process, where 1°C to 28°C below core body temperature is suitable, as the differentiation and maturation of spermatocytes and spermatids takes place. This is supported by studies in humans that artificially brought testicles near or into the inguinal canal and induced high scrotal and consequently testicular temperatures near core body temperature[26]. Consequently, spermatogenesis was impaired and semen quality reduced.

4. CAUSES OF MALE INFERTILITY:

Varicocele

Dialation of veins present in pampiniform plexus of scrotum, known as varicocele. It is the frequent cause of male infertility. Certain cases are reported regarding varicocele, that it can cause testicular damage that further inference loss of volume of testicles. It has multifactorialetiology, among this, most common is anatomical difference of right and left spermatic vein, absence of the valves present in the spermatic vessels leads to retrograde of blood flow and compression of left renal vein, which causes a partial obstruction. This condition found in 15% of the population According to human report update(2001)[13].

Endocrinal disorders:

Either deficiency in levels of hormone or excessive levels of hormone causes male infertility. Decreased sperm count is caused as a result of inability of the pituitary gland to

produce enough levels of hormone. 11% of men experiences infertility due to increased levels of prolactin as because hyperprolactenimea causes reduced levels of FSH, LH finally leading to male infertility, sperm motility and sperm quality is also reduced. It later produces secondary hypogonadism and infertility[16,12].

Male reproductive tract infection

Male accessory glands cannot function well as the quality of spermatozoa detoriation takes palce through the reproductive infection. This infection can decline the semen volume level, α-glucosidase, fructose and zinc in seminal plasma suggesting impairment of the secretary function of the epididymis, seminal vesicles and prostate. So it is one of the potentially correctable causes of male infertility[48].

Ejaculatory disorders

The ejaculation process is based on central and peripferal nervous system control. It includessemmen deposition in prostatic urethra, bladder neck closure and also there is contraction of pelvic and periurethral muscles that finally leads to semen ejaculation. Due to neurologic, anatomic as well as psychologic conditions there is lack of emission ejaculation and retrograde ejaculation. Ejaculatory dysfunction is also caused by lesions in CNS. α- blockers, antidepressants, antipsychotics and antihypertensive can also affect ejaculation[41].

Immunological factor [41]

Around 9-33% of couples carries antisperm antibodies which causes infertility for both of them. In this case antisperm antibodies are found in men about 8-19% and 1-21% in the female partner is contributing. Risk factors may be vasectomy, epididymitis. Though how and why this antibodies are forming still under study as the concept is not very clear. These antibodies functions by agglutinising the sperm cells as well as hindering the sperm motility[]. Sperm penetration through cervical mucus is also impaired. This type of antibodies with sperm have no or rare interactions with egg. These antibodies diminishes the acrosome reaction and zona pellucid binding, which leads to decline the potency of fertility.

Genetic and chromosomal defects[41]

According to the recent studies around 10-15% of the male population suffering from genetic and chromosomal defects (chromosomal abbressions and single gene mutations) which can cause severe male infertility.. Due to this condition spermatogenesis may alter. Causing a dysfunction in the normal development of the genital tract and declines the sperm motility as well as fertilization capacity, any of these leads to male infertility

5. PATHOPHYSIOLOGY (INFERTILITY) [40] :-

Hormonal regulation takes place in spermatogenesis. The pulsatile release of hypothalamic GnRH stimulates pituitary gonadotrophin secretion: resultant in Leydig cells LH predominantly acts, promoting spermatogenesis, whilst FSH acts predominantly on sertolicells. Here testosterone mimics as androgen which is important in the negative feedback controlof both gonadotrophins also hypothalamic aromatization to estradiol increase the degree of inhibition. Initiation of successful spermatogenesis in primates require FSH and LH, which is secreted from pituitary gland. The importance of FSH, it is particularly maintaining the spermatogenesis, and thus remains controversial. When LH is stimulated intratesticular testosterone levels and two orders of magnitude higher than those of circulating testosterone, which is sufficient to maintain spermatogenesis. In sertoli cells the androgens acts on, since germ cells are not expressing the androgen receptors. Sertoli cells/Leydig cells producing intratesticular proteins, involved in the local paracrine regulation of spermatogenesis. Although there is an evidence that estradiol having a physiological role in spermatogenesis, in addition to its better known inhibitory role in hypothalamus- pituitary- testicular axis.

Clinically male infertility takes place following the rare deficiencies in gonadotrophin induction and maintenance of spermatogenesis. Direct testicular injury to the germ cells or the supporting somatic or steroidogenic cells can cause male infertility. Currently however, some evidence has recognized the paradoxical fact that higher intratesticular testosterone may inhibit recovery of spermatogenesis, meaning that this reflex compensatory mechanism may be partially counterproductive. These intriguing observations from animal experiments require further evaluation in primates, including humans, but if they are confirmed then novel (possibly counter-intuitive) methods of enhancing spermatogenesis may be developed. Additional novel post-receptor hormonal approaches could be developed with suffcient progress in understanding the pathways of androgen and FSH action and interaction within Sertoli cells and their fostering of germ cell development.

6. DIAGNOSIS OF INFERTILITY[39]

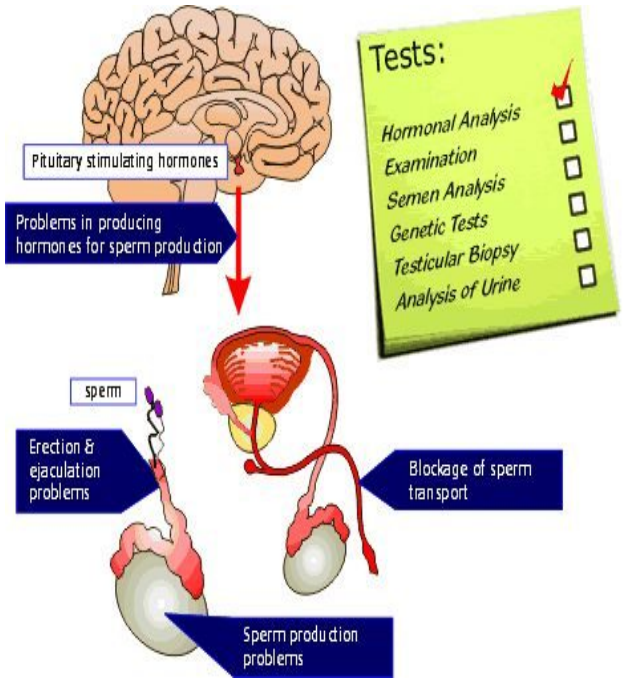

Fig 6.1. Diagnostic parameters for confirming male infertility

(Source: https://laparoscopysurgeries.com/male-infertility-diagnosis-and-treatment/)

Endocrine tests

Hormones including FSH, LH, testosterone prolactin and also sperm count assessments are done in men to confirm infertility. If the testosterone level is low and the LH and FSH levels are high it represents testicular disappointment. Low testosterone with decreased FSH and LH demonstrates focal imperfection with optional hypogonadism. Azoospermia in mix with typical hormone levels recommends an obstructive reason. Other indications for serum endocrinology testing include evidence of impaired sexual function (e.g. impotence, reduced libido) and clinical symptoms of endocrine disease (e.g. hypothyroidism).

Imaging

If physical examination report detects the presence of tumours in testicles then it is recommended to perform scrotal ultrasound. For diagnosis of variocele also ultrasound is required in combination with the use of colour flow. Men with renal abnormalities needs to get a renal ultrasound done.

Testicular biopsy

Patients with oligospermia and azoospermia are recommended to get testicular biopsy done.

7. CURRENT THERAPEUTIC APPROACHES:-

7.1 SPECIFIC HORMONAL THERAPIES:

✓ **Gonadotrophin deficiency** –

it is the reason for male infertility which isn't frequent, gonadotrophin supplanting treatment involving hCG with or without FSH, for gonadotrophin-inadequate men stays one of only a specifc and effective treatments of male infertility. Gonadotrophin treatment is successful for initiating spermatogenesis and fertility in gonadotrophin insufficiency. Explicit treatment, for example, the exhaustion of iron overload in hemochromatosis and surgery or radiotherapy for pituitary tumors viable for this situation[3].

✓ **Pulsatile GnRH therapy**

Pulsatile GnRH is a compelling treatment of non-pituitary gonadotrophin lack, both for prompting androgenization and spermatogenesis (Typically, GnRH 5 to 20mg/120min is taken subcutaneously through an inhabiting butterfly needle)[4]. Intranasal GnRH can keep up effectively incited spermatogenesis, however the requirement for a 2-h dosing routine makes this clinically impracticable. Thus, intravenous GnRH with its unrivaled pharmacokinetic profile can effectively induce spermatogenesis and pregnancy, but on the other hand is considered clinically impracticable and expensive. Pulsatile GnRH isn't viable at supporting gonadotrophin secretion in men with loss of pituitary gonadotrophin function.

✓ **GnRH treatment:**

A single randomized controlled study has examined the effects of stimulation with both gonadotrophins, although there are other uncontrolled reports. The possibility that FSH therapy might improve spermatogenesis or sperm structure and function in subgroups of infertile men, who are selected according to specific features of sperm ultra structure, testicular or oligozoospermia with normal spermatogenic hormones, has been proposed based on posthoc analysis of responders in other studies[5].

✓ **Androgen treatment**

Depends on the fundamental importance of testosterone to spermatogenesis, androgen administration was among the first treatments proposed for idiopathic male infertility. One rationale is based upon a direct `stimulatory' effect using low-dose androgens via a hypothetical selective improvement in epididymal maturation. Since it is well known that exogenous androgen therapy suppresses spermatogenesis, this rationale depends upon a selective tissue-specific effect of androgen therapy which appears a remote physiological possibility. Another rationale is based upon a `rebound' effect One rationale is based upon a direct `stimulatory' effect using low-dose androgens via a hypothetical selective improvement in epididymal maturation. Since it is well known that exogenous androgen therapy suppresses spermatogenesis, this rationale depends upon a selective tissue-specific effect of androgen therapy which appears a remote physiological possibility. Another rationale is based upon a `rebound' effect whereby cessation of androgen-induced gonadotrophin and spermatogenic suppression results in transient reflex rebound increase in circulating gonadotrophins, which might then stimulate spermatogenesis[8].

✓ **Cryptorchidism:**

Hormonal treatment of cryptorchidism was recently reviewed (Pyorala et al., 1995), and the suggestion made that GnRH analogue treatment is effective in causing testicular descent, and that hCG is more effective than placebo, though data remain scant and confounded by testes which are retractile rather than truly cryptorchid. Data concerning the impact of hormonal treatment of cryptorchidism on fertility is even more limited. Unilateral cryptorchidism per se may not adversely affect fertility at all, either because the remaining testis can compensate or because available treatments are effective. Men with bilateral cryptorchidism have lower semen counts and reduced fertility (Lee et al., 1995b). By promoting testicular descent, GnRH or hCG improves sperm output, presumably by rectifying elevated testicular temperature. However, whilst it is plausible that this may increase pregnancy rates this remains to be proven.

7.2 Counseling and Treatment:

one of the primary factor which can impair the semen quality is ‘ lifestyle’ i.e., chain smoking, alcohol abuse, frequent use of anabolic steroids, extreme sports (marathon training, excessive strength sports etc.), and also increment in scrotal temperature through thermal underwear, excessive use of hot tub, or occupational pressure. Secondary factors followed by the use of certain drugs that can affect the spermatogenesis. Treatment measurements can be the hormonal treatment, antioxidant treatment because they have positive influence on semen quality (except in idiopathic OAT)[9]. Some endocrinological pathologies has to be treated with the following medications –

Sno	Condition	Drug ,Dose and Route	Results
1.	Low testosterone	Clomiphene citrate – 50mg/day Tamoxifen 20mg/day Oral route.	It increases the level of sperm count, morphology and motility by triggering the release of luteinizing hormone through pituitary gland.
2.	Hypogonadotrophichypogon-adism	start HCG 1500 IU subcutaneously 3 times per week, and add HMG or FSH 75–150 IU intramuscularly 3 times per week, until spermatogenesis occurs	More prominent effect for the patients who are not responding to clomiphene citrate.
3.	Hyperprolactinaemia	dopamine agonists. (In patients with sperm autoantibodies), high-dose corticosteroids.	Pulsetile the release of GnRH.

Table No. 7.2.1: medicines used to treat male infertility

7.3 Surgical treatment

✓ **Varicocelectomy:**

Dialation of veins present in pampiniform plexus of scrotum, known as varicocele. It is the frequent cause of male infertility. Certain cases are reported regarding varicocele, that it can cause testicular damage that further inference loss of volume of testicles.

The treatment of varicocele is a controversial subject, mainly based on whether there is an actual need to treat this condition in infertile men. There is evidence of improved semen parameters after successful treatment. Current information supports the hypothesis that in some men, the presence of varicocele is associated with progressive testicular damage from adolescence onwards and consequent reduction in fertility. Although treatment of this condition in adolescents may be effective, there is a significant risk of over-treatment. In cases of normal semen analysis and in men with a subclinical varicocele, there appears to be no benefit from treatment compared with observation. Condition [repair], however, seems effective in couples in whom the men has oligozoospermia, a clinical varicocele and otherwise unexplained infertility[13].

✓ **MESA/TESE:**

MESA in combination with ICSI is indicated in men with obstructive azoospermia when reconstruction (vasovasostomy, vaso-epididymostomy) cannot be performed or is unsuccessful. An alternative would be percutaneous aspiration of spermatozoa from the caput epididymis (PESA). If a MESA or PESA procedure does not produce spermatozoa, testicular sperm extraction (TESE) can be applied. In about 50–60% of men with non-obstructive azoospermia (NOA), spermatozoa can be found in the testis. Some authors recommend taking several testicular samples, while others advocate microsurgical harvesting of spermatozoa. So far, no clinical or laboratory parameter has been shown to be useful in predicting sperm harvesting in men with NOA. In case of AZFa and AZFbmicrodeletions, no spermatozoa can be retrieved[11].

✓ **Transurethral incision of ejaculatory ducts or midline prostatic cyst:**

Distal obstructions of the genital tract are commonly caused by infections of the prostatic urethra and the accessory glands or by a cyst in the midline of the prostate. Treatment of the obstruction by transurethral incision of the cyst or the ejaculatory ducts (TURED) may lead to an increase in semen quality and, occasionally, spontaneous pregnancy. Long-term results, however, are disappointing.

8 Ayurvedic concept about prevention of male infertility :-

In the classic of Ayurveda the following charya's have been mentioned with which a person will be able to be healthy and can cure infertility i.e., Dinacharya, Ritucharya, Thrayopasthambha palana, Sadvritha, Achara rasayana, Ashtanga yoga's[10].

Herbs traditionally used for vajikarana and shukral purposes –

S. no	***Herbs***	***Uses***
1.	Kapikacchu (Mucuna Pririens Bak)	Increase sperm concentration and motility.
2.	Gokshura (Tribulus terrestris)	Raises testosterone levels.
3.	Ashwagandha (Withania somnifera Dunal)	enhances spermatogenesis via a presumed testosterone like effect.
4.	Shatavari (Asparagus racemosus Wild)	Enhance fertility by reducing oxidative stress.
5.	Yasthimadhu (Glycyrrhiza glabra)	Improve semen quality.

Table No.8.1: Ayurvedic medicines to treat male infertility

9 Different types of experimental animal models:

Given the multifaceted nature of male infertility, the whole procedure can't be completely displayed in vitro. As such, animal models give a practical option in contrast to experimentation. Mice are the most usually utilized animal models in biomedical research, including conceptive science, in view of their short regenerative cycle with huge litter size and moderately modest lodging (housing) conditions. Above all, mice are hereditarily fundamentally the same as human and their undeveloped organisms are moderately simple to control at the hereditary level. In addition, mouse spermatogenesis and oogenesis are equivalent to human. More than a very long while, numerous sorts of mouse models have been made accessible for biomedical research, including knockout/knockin and transgenic models (using the reverse genetic or candidate gene approach). All the more as of late, chemical mutagenized mutant mouse models (utilizing the forward hereditary methodology) have been progressively used to uncover infection-related qualities, including reasons for male infertility[5].

10 Current medicines under investigation:-

Currently Folic acid is also used for treating infertility. Folic acid treats infertility by increasing cellular turnover associated with increased or decreased side effects. According to studies done, it was seen that the cellular cohesion improved and also the presence of immature cells in plasma was reduced.

Dietary supplements are also helpful in cases of infertility. Recently spermotrend, a dietary supplement came into the market which is seen to have a positive effect on spermatogenesis.

Antioxidants like Fairhaven Pro is also effective for treating condition like infertility.

Mixture of myo-inositol and antioxidant is used as dietary supplement.

11 Techniques undergoing investigation:-

- **Treatment of severe Male Infertility – Predictive Factors (imaging) (TESE-MRI)** - Diagnostic Test: Pelvis MRI (The major outcome will be functional difference in testicular MRI depending on MD-TESE)
- I**ntra-Testicular Transplantation of Autologous Stem Cells for Treatment of Non-Obstructive Azoospermia male infertility.** - Biological: Stem Cell Transplantation.

CONCLUSION:

Male infertility is common issue that requires specialist referral, that too when males are administering an immunosuppressive agent infertility takes place, which take lots of time to investigate as well as to treat. Semen analysis remains the main initial investigation that is the ultimate diagnosis for further assessment of the infertile male. Recently, the pathophysiological mechanisms of male infertility are not so well understood. Furthermore, hormonal overdrive is not feasible, suggesting that the hormonal regulation of spermatogenesis, sperm maturation and function is complex and probably already functioning maximally. For male infertility, hormonal therapy has a limited but important role as an effective and specific treatment of gonadotrophin deficiency, but no established role in empirical therapy.

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