IJBPAS, March, 2020, 9(3): 494-516

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

AN OVERVIEW ON CAUSES, DIAGNOSIS, MANAGEMENT AND BIOLOGICAL SCREENING METHODS OF INFERTILITY

VINCIYA T*[1] AND RANI S[2]

1: Research Scholar, Department of Pharmacy, Annamalai University, Annamalainagar, Chidambaram - 608002, Tamilnadu, India

2: Associate Professor, Department of Pharmacy, Annamalai University, Annamalainagar, Chidambaram - 608002, Tamilnadu, India

***Corresponding author details: T. Vinciya: E Mail Id: veenusvinciya1995@gmail.com; Mobile No.: 9790854146**

Received 10th Oct. 2019; Revised 8th Nov. 2019; Accepted 9th Dec. 2019; Available online 1st March 2020

https://doi.org/10.31032/IJBPAS/2020/9.3.4992

ABSTRACT

Infertility is the disorder which can change the mankind and also the man mind to cause most important problems. Infertility i.e. childlessness causes physical and mental worries which can frustrate the family happiness. Male and female fertility can be incomplete or diminished by number of factors such as hormone imbalance, illness and infections on reproductive organs, obstruction or sexual dysfunction. In this scientific period, infertility is also caused by lack of healthy food, stressful world, excess radiation, changing lifestyle, exposure to various toxins, smoking, addiction to alcohol and drugs. This review deals with fertilization, implantation, infertility and biological screening methods. It also deals with the factors which cause infertility and diagnosis and management of infertility in both male and female. This review will be more useful not only to the scientific people and also normal peoples.

Keywords: Fertilization, Implantation, Infertility, Causes, Diagnosis, Treatments, biological screening

INTRODUCTION

Pregnancy is the most intimate and heart- warming experience in the life of married couples. The personal events do not lead up, but the actual incident represents a merging of the essential features of both partners. It is the beginning

of a new stage in every women's life, and acts as a climatic point in the life of every couple. Today, the most of the couples are showing up at fertility centre to attain fertility. Due to childlessness they are socially isolated and thus emotionally distressed in their existing life which leads to divorce. It may be due to male or female infertility.

Infertility represent a major predicament for most of the couples, with both partners experiencing failure in ways that affect them as individuals, as family members and as members of society as a whole.

Infertility:

Infertility is defined as the inability to conceive naturally after one year of regular unprotected intercourse. In majority of the cases, infertility is some extent of sub fertility, in which 1 in 7 couples needs specialist help to conceive. Sub fertility may be either primary or secondary.

Primary sub fertility:

Primary sub fertility is a delay for a couple who have no previous pregnancies.

secondary sub fertility:

Secondary sub fertility is a delay for a couple who have conceived earlier, even if the pregnancy may not have been successful for example: miscarriage, and ectopic pregnancy.

The chance to conceive may be depends on the length of sexual contact, regularity of coitus, and couple's age. The normal young aged couples have 25% chance to conceive after 1 month of unprotected intercourse,70% of the couple's have conceive by 6 months, and 90% of the couples have a probability to conceive by 1 year. Only 5% of the couples will conceive after one and a half year or two years. Both males and females are equally responsible for fertile. Most of the infertile couples have affected by one of these three major causes. The male factor include ovulatory dysfunction, or tubal-peritoneal disease. The Literature shows that vaginismus and dyspareunia are more common in 20-24 years aged females, that influence the fertility. The sexual response cycle plays an important role to support fertility, because it comprises of chronological physical and emotional changes that occur as a person becomes sexually aroused.

Physiology:

In normal physiology, the two gonadotropin hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH) are produced in the pituitary gland and their secretion is controlled by gonadotropin releasing hormone (GnRH) that is released by the hypothalamus.

At the start of a new cycle, the hypothalamus begins to release GnRH that acts on the pituitary gland to release FSH and LH. These two hormones stimulate the ovary and cause the follicles to develop.

Every month about 30-40 follicles start to grow in response to FSH, but only a single mature egg is released every month. This involves messages transmission in the form of hormones from the ovary, the pituitary and the hypothalamus. When the egg is ripe, the mature follicle releases an increasing amount of estrogen, that is produced by the granulosa cells lining the follicle. The estrogen produced by the dominant follicle gradually increases in quantity as the egg matures, until a pour of estrogen is released into the blood.

Causes of infertility in men:

The following are the common causes of infertility in men.

Semen and sperm:

Semen is the milky fluid that is released by man's penis during orgasm. Semen consists of fluid and sperm. The fluid comes from the prostate gland, the seminal vesicle, and other sex glands. The sperm is produced in the testicles. When a man ejaculate and releases the semen through penis; the seminal fluid, or semen, helps transport the sperm toward the egg.

The following problems are feasible for men infertile:

- Low sperm count: The man ejaculates a low number of sperm. A sperm count of under 15 million is considered low. Approximately one third of couples have difficulty in conceiving due to a low sperm count.
- Low sperm mobility (motility): The sperm cannot "swim" as well as they should to reach the egg.
- Abnormal sperm: The sperm may have an abnormal in shape, so making it harder to move and fertilize an egg.

If the sperm do not have the right shape, or they cannot travel rapidly and accurately towards the egg, conception may be difficult. Abnormal semen may not be able to carry the sperm effectively.

This can result from:

- A medical condition: This could be a testicular infection, cancer, or surgery.
- Overheated testicles: This include an undescended testicle, a varicocele, or varicose vein in the scrotum, the use of saunas or hot tubs, wearing tight clothes, and working in hot environments.
- Ejaculation disorders: If the ejaculatory ducts are blocked, semen may be ejaculated into the bladder
- Hormonal imbalance: Hypogonadism, for example: it can lead to a testosterone deficiency.

Other causes may include:

- Genetic factors: A man must have an X and Y chromosome. If he has

two X chromosomes and one Y chromosome, as in Klinefelter's syndrome, the testicles will develop abnormally and there will be low testosterone and a low sperm count or no sperm.

- Mumps: If this occurs after puberty, the inflammation of the testicles may affect sperm production.
- Hypospadias: The urethral opening is under the penis, instead of its tip. This deformity is generally corrected by surgically in childhood. If the correction is not done, it may be harder for the sperm to get to the female's cervix. Hypospadias affect concerning 1 in every 500 infant boys.
- Cystic fibrosis: This is a chronic illness that results in the formation of a sticky mucus. This mucus mainly affects the lungs, but males may also have a missing or obstructed vas deferens. The vas deferens carries sperm from the epididymis to the ejaculatory duct and the urethra.
- Radiation therapy: This can impair sperm production. The severity usually depends on how near to the testicles the radiation was aimed.
- Some diseases: Conditions that are occasionally related to lower fertility in males are anemia, Cushing's syndrome, diabetes, and thyroid disease.

Some medications can increase the risk of fertility problems in men.

- Sulfasalazine: This is an anti-inflammatory drug, that can drastically lower a man's sperm count. It is frequently prescribed for Crohn's disease or rheumatoid arthritis. Sperm count often returns to normal after stop the medication.
- Anabolic steroids: fashionable with bodybuilders and athletes, long-term use can seriously diminish the sperm count and mobility.
- Chemotherapy: Some types may extensively reduce the sperm count.
- Illegal drugs: Consumption of marijuana and cocaine can lower the sperm count up.
- Age: Male fertility starts to fall after 40 years.
- Exposure to chemicals: Pesticides, for example: it increase the risk.
- Excess alcohol utilization: This could lower male fertility. Reasonable alcohol consumption has not been revealed to lower fertility in most men, but it may affect those who already have a low sperm count.
- Overweight or obesity: This may reduce the probability of conceiving.

- Mental stress: Stress can be a cause, especially if it leads to reduced sexual activity.

Causes in women:

Infertility in women can have a numerous range of causes.

Risk factors:

Risk factors that increase the risk include:

- Age: The ability to conceive starts to fall around the age of 32 years.
- Smoking: Smoking significantly increases the risk of infertility in both men and women, and it may weaken the effects of fertility treatment. Smoking during pregnancy increases the possibility of pregnancy loss. Passive smoking has also been linked to lower fertility.
- Alcohol: A few quantity of alcohol utilization can affect the chances of conceiving.
- Being obese or overweight: This can increase the possibility of infertility in women as well as men.
- Eating disorders: If an intake disorder leads to severe weight loss, fertility problems may occur.
- Diet: A lack of folic acid, iron, zinc, and vitamin B-12 can influence the fertility. Women who are at risk, including those who are a vegetarian diet, should ask the doctor about supplements.
- Exercise: Individually too much and too little exercise can lead to fertility problems.
- Sexually transmitted infections (STIs): Chlamydia can harm the fallopian tubes in a woman and cause inflammation in a man's scrotum. Different STIs may also cause infertility.
- Exposure to various chemicals: Some pesticides, herbicides, metals, such as lead, and solvents have been related to fertility problems in both men and women. A mouse study has suggested that ingredients in some household detergents may reduce the fertility.
- Mental stress: This could affect the female ovulation and male sperm production and it can lead to reduced sexual activity.

Medical conditions:

Some medical conditions can affect fertility.

Ovulation disorders: The most common cause of infertility in women. Ovulation is the monthly release of an egg. The eggs may never be released or they may only be released in some cycles.

Ovulation disorders can be due to:

- Premature ovarian failure: The ovaries stop functioning before the age of 40 years.
- Polycystic ovary syndrome (PCOS): The ovaries function abnormally and ovulation may not take place.
- Hyperprolactinemia: If prolactin levels are elevated, and the woman is not pregnant or breastfeeding, it may affect ovulation and fertility.
- Poor egg quality: Eggs that are damaged or develop genetic abnormalities cannot maintain a pregnancy. The older a woman is the chance of higher risk.
- Thyroid problems: An overactive or underactive thyroid gland can direct to a hormonal imbalance.
- Chronic conditions: These comprise AIDS or cancer.

Problems in the uterus or fallopian tubes can prevent the egg travelling from the ovary to the uterus, or womb. If the egg does not travel means it can be harder to conceive naturally.

Causes include:

- Surgery: Pelvic surgery can occasionally reason for scarring or damage to the fallopian tubes. Cervical surgery can sometimes cause scarring or shortening of the cervix. The cervix is the neckline of the uterus.
- Sub mucosal fibroids: Benign or non-cancerous tumors take place in the muscular wall of the uterus. They can interfere with the implantation or block the fallopian tube, preventing sperm from fertilizing the egg. Large sub mucosal uterine fibroids may create the uterus cavity improved, increasing the distance the sperm has to travel.
- Endometriosis: Cells that usually occur within the lining of the uterus start growing in a different place in the body.
- Previous sterilization treatment: In women who have selected to have their fallopian tubes blocked, the process can be reversed, but the chances of becoming fertile another time are not high.

Medications, treatments, and drugs:

Various drugs can affect fertility in a woman.

- Non-steroidal anti-inflammatory drugs (NSAIDs): Long-term use of aspirin or ibuprofen may create it harder to conceive.
- Chemotherapy:
  Some chemotherapy drugs can result in ovarian failure. In some cases, this may be permanent.
- Radiation therapy: If this is aimed near the reproductive organs, it can

increase the risk of fertility problems.

- Illegal drugs: Some women who use marijuana or cocaine may have fertility problems.
- **Cholesterol**
  Individual study has found that high cholesterol levels affect the fertility in women.

Structural problems:

Structural problems generally involve the occurrence of abnormal tissue in the fallopian tubes or uterus. If the fallopian tubes are blocked, eggs are not able to move from the ovaries to the uterus and sperm is not able to reach the egg for fertilization. Structural problems with the uterus may interfere with implantation and also cause infertility.

Some specific structural problems that can cause infertility include:

- Endometriosis: when tissue that normally lines the inside of the uterus is found in other places, such as blocking the fallopian tubes.
- Uterine fibroids: growths that appear within and around the wall of the uterus, even though most women with fibroids do not have problems with fertility and can get pregnant. However, some women with fibroids may not be able to get pregnant naturally or may have multiple miscarriages or preterm labor.
- Polyps: These are noncancerous growths on the inside surface of the uterus. Polyps can interfere with the function of the uterus and make it difficult for a woman to remain pregnant after conception. Surgical removal of the polyps can increase the chances for a woman to get pregnant.
- Scarring in the uterus from previous injuries, infections, or surgery. Scarring may increase the risk of miscarriage and may interfere with implantation and thus leading to infertility.
- An abnormally shaped uterus, which can affect implantation and the ability to carry a pregnancy is difficult

The factors responsible to become pregnant:

- **Need to ovulate.** To obtain pregnant, the ovaries must produce and release an egg, a process known as ovulation. The doctor can help to evaluate the menstrual cycles and prove ovulation.
- **The partner needs sperm.** For most couples, this isn't a problem except the partner has the past of illness or surgery. The doctor can sprint a number of easy tests to evaluate the health of the partner's sperm.

- **Need to have regular intercourse.** Need to comprise a regular sexual intercourse during the fertile time.
- **Need of fallopian tubes open and a normal uterus.** The egg and sperm get together in the fallopian tubes, and the embryo desires a healthy uterus in which to grow. For pregnancy to occur, every step of the human reproduction process has to take place correctly.

The steps in this process are:

- One of the two ovaries releases a mature egg.
- The egg is picked up by the fallopian tube.
- Sperm swim up the cervix, through the uterus and into the fallopian tube to reach the egg for fertilization.
- The fertilized egg travels downwards the fallopian tube to the uterus.
- The fertilized egg implants and grows in the uterus.

In women, a number of factors can interrupt this process at any step. Female infertility is caused by one or more of the factors below.

Treatment:

Treatment resolve depend on numerous factors, including the age of the person who needs to conceive, how long the infertility has lasted, personal preferences, and their general state of health.

Frequency of intercourse:

The couple may be advised to have sexual intercourse more frequently around the instance of ovulation. Sperm can stay alive inside the female for up to 5 days, whereas an egg can be fertilized for up to 1 day after ovulation. In theory, it is possible to conceive on any of these 6 days that occur before and during ovulation.

However, a examination has suggested that the 3 days most likely to recommend a fertile window are the 2 days before ovulation plus the 1 day of ovulation. Some suggest that the number of times a couple has intercourse must be reduced to increase sperm supply, but this is doubtful to make a difference.

Fertility treatments for men:

Treatment will depend on the essential cause of the infertility.

- Erectile dysfunction or premature ejaculation: Medication, behavioral approaches, or together may assist to recover fertility.
- Varicocele: Surgically removing a varicose vein in the scrotum may help to improve fertility.
- Blockage of the ejaculatory duct: Sperm can be extracted directly from the testicles and injected into an egg in the laboratory.
- Retrograde ejaculation: Sperm can be taken directly from the bladder

and injected into an egg in the laboratory.

- Surgery for epididymal blockage: A blocked epididymis can be surgically repaired. The epididymis is a coil-like structure in the testicles which helps to store and transport sperm. If the epididymis is blocked, sperm may not be ejaculated accurately.

Fertility treatments for women:

Fertility drugs may be prescribed to normalize or encourage ovulation.

They include:

- Clomifene (Clomid, Serophene): This encourages ovulation in those who ovulate either irregularly or not at all, because of PCOS or another disorder. It makes the pituitary gland release more follicle-stimulating hormone (FSH) and luteinizing hormone (LH).
- Metformin (Glucophage): If Clomifene is not effective, Metformin could help women with PCOS, particularly while linked to insulin resistance.
- Human menopausal gonadotropin, or hMG (Repronex): This contains both FSH and LH. Patients who do not ovulate because of a imperfection in the pituitary gland may possibly receive this drug as an injection.
- Follicle-stimulating hormone (Gonal-F, Bravelle): This hormone is formed by the pituitary gland that controls estrogen production by the ovaries. It stimulates the ovaries to mature egg follicles.
- Human chorionic gonadotropin (Ovidrel, Pregnyl): Used together with clomiphene, hMG, and FSH, this can stimulate the follicle to ovulate.
- Gonadotropin-releasing hormone (Gn-RH) analogs: These can help women who ovulate too early—before the lead follicle is mature—during hmG treatment. It delivers a constant supply of Gn-RH to the pituitary gland, which alters the production of hormone, allowing to induce follicle growth with FSH.
- Bromocriptine (Parlodel): This drug inhibits prolactin production. Prolactin stimulates milk production during breastfeeding. Outside pregnancy and lactation, women with high levels of prolactin may have irregular ovulation cycles and fertility problems.

Reducing the risk of multiple pregnancies:

Injectable fertility drugs can sometimes effect in multiple births, for example: twins or triplets. The possibility of a multiple birth is lower with an oral fertility drug.

Careful monitoring for the duration of treatment and pregnancy can help reduce the risk of complications. The more foetuses there are, the higher the risk of premature labor.

If a woman wants an HCG injection to stimulate ovulation and ultrasound scans show that too several follicles have developed, it is achievable to withhold the HCG injection. Couples may decide to go forward in any case if they desire to become pregnant is very strong. If too many embryos develop, one or more can be removed. Couples will have to consider the ethical and emotional aspects of this procedure.

Surgical procedures for women:

If the fallopian tubes are blocked or scarred, surgical repair may compose it easier for eggs to pass through. Endometriosis may be treated through laparoscopic surgery. A small incision is made in the abdomen, and a thin, flexible microscope with a light at the end, called a laparoscope, is inserted through it. The surgeon can remove implants and scar tissue, and this may reduce pain and assist fertility.

Assisted conception:

The subsequent methods are presently obtainable for assisted conception.

Intrauterine insemination (IUI): At the time of ovulation, a fine catheter is inserted through the cervix into the uterus to place a sperm sample directly into the uterus. The sperm is washed in a fluid and the best specimens are selected. The woman may be specified a low dose of ovary stimulating hormones. IUI is more frequently done when the man has a low sperm count, decreased sperm motility, or when infertility does not have an main cause. It can also help if a man has severe erectile dysfunction.

In-vitro fertilization (IVF): Sperm are placed with unfertilized eggs in a petri dish, where fertilization can take place. The embryo is then placed in the uterus to start on a pregnancy. Sometimes the embryo is frozen for future use.

Intra cytoplasmic sperm injection (ICSI): A single sperm is injected into an egg to achieve fertilization during an IVF procedure. The possibility of fertilization improves considerably for men with low sperm concentrations.

Sperm or egg donation: If essential, sperm or eggs can be received from a donor. Fertility treatment with donor eggs is usually done using IVF.

Assisted hatching: The embryologist opens a small hole in the outer membrane of the embryo, known as the zona pellucid. The opening improves the ability of the embryo to implant into the uterine lining. This improves the chances that the embryo will implant at, or attach to, the wall of the uterus.

This may be used if IVF has not been effective, if there has been poor embryo growth rate, and if the woman is older. In several women, and especially with age, the membrane becomes harder. This can make it complicated for the embryo to implant.

Electric or vibratory stimulation to achieve ejaculation: Ejaculation is achieved with electric or vibratory stimulation. This can help a man who cannot ejaculate normally, for example: because of a spinal cord injury.

Surgical sperm aspiration: The sperm is removed from part of the male reproductive tract, such as the vas deferens, testicle, or epididymis.

Types:

Infertility can be primary or secondary.

Primary infertility is when a couple has not conceived after trying for at least 12 months without using birth control.

Secondary infertility is when they have previously conceived but are no longer able to continue the pregnancy.

Diagnosis:

Most people will visit a physician if there is no pregnancy after 12 months of trying.

If the woman is aged over 35 years, the couple may wish to see a doctor earlier, because fertility testing can take time, and female fertility starts to drop when a woman is in her 30s. A doctor can provide advice and carry out some beginning assessment. It is better for a couple to see the doctor together. The doctor may ask about the couple's sexual routine and make recommendations regarding these. Tests and trials are available, but testing does not always reveal a specific cause.

Infertility tests for men: The doctor will ask the man in relation to his medical history, medications, and sexual habits and carry out a physical examination. The testicles will be checked for lumps or deformities, and the shape and structure of the penis will be examined for abnormalities.

- Semen analysis: A sample may be taken to test for sperm concentration, motility, color, quality, any infections, and whether any blood is present. Sperm counts can fluctuate, so that several samples may be necessary.
- Blood test: The lab test for the levels of testosterone and other hormones.
- Ultrasound: This may reveal issues such as ejaculatory duct obstruction or retrograde ejaculation.
- Chlamydia test: Chlamydia can affect fertility, but antibiotics can treat it.

Infertility tests for women

A woman will experience a general physical examination, and the doctor will ask about her medical history, medications, menstruation cycle, and sexual habits.

She will also undergo a gynecologic examination and a number of tests:

- Blood test: This can assess hormone levels and whether a woman is ovulating.
- Hysterosalpingography: Fluid is injected into the woman's uterus and X-rays are taken to determine whether the fluid travels properly out of the uterus and into the fallopian tubes. If a blockage is present, surgery may be necessary.
- Laparoscopy: A thin, flexible tube with a camera at the end is inserted into the abdomen and pelvis, allowing a doctor to look at the fallopian tubes, uterus, and ovaries. This can reveal signs of endometriosis, scarring, blockages, and some irregularities of the uterus and fallopian tubes.

Other tests include:

- ovarian reserve testing, to find out how effective the eggs are after ovulation
- genetic testing, to see if a genetic abnormality is interfering with fertility
- pelvic ultrasound, to produce an image of the uterus, fallopian tubes, and ovaries
- Chlamydia test, which may indicate the need for antibiotic treatment
- thyroid function test, as this may affect the hormonal balance

Complications:

Several complications can end result from infertility and its treatment. If conception does not occur after many months or years of trying, it can lead to stress and perhaps depression.

Some physical effects may also result from the treatment.

Ovarian hyper stimulation syndrome:

The ovaries can swell, leak excess fluid into the body, and produce too many follicles, the small fluid sacs in which an egg develops. Ovarian hyper stimulation syndrome (OHSS) typically results from taking medications to encourage the ovaries, such as clomifene and gonadotrophins. It can also develop after IVF.

Symptoms include bloating, constipation, dark urine, diarrhoea, nausea, abdominal pain and vomiting. They are generally mild and easy to treat. Rarely, a blood clot might develop in an artery or vein, liver or kidney problems can arise, and respiratory distress may develop. In severe cases, OHSS can be fatal.

Ectopic pregnancy:

This is after a fertilized egg implants outside the womb, frequently in a fallopian tube. If it stays in there, complications can develop, such as the rupture of the fallopian tube. This pregnancy has no chance of

continuing. Immediate surgery is needed and sadly, the tube on that side will be lost. However, future pregnancy is possible with the other ovary and tube.

Women receiving fertility treatment have somewhat upper risk of an ectopic pregnancy. An ultrasound scan can detect an ectopic pregnancy.

Coping mentally:

It is impractical to recognize how long treatment will go on for and how successful it will be. Coping and persevering can be stressful. The emotional toll on both partners can affect their relationship. Some people recover that joining a support group helps, as it offers the chance to talk to others in a similar situation. It is essential to tell a doctor if excessive mental and emotional stress develop. They can regularly recommend a counselor and others who can offer appropriate support. Online support from organizations such as Resolve can be helpful.

BIOLOGICAL SCREENING METHODS FOR INFERTILITY IN ANIMAL MODELS:

INVIVO METHODS FOR MALE:

Measurement of testosterone: Blood samples were collected from the eye by plain capillary tube and centrifuged at 3000 rpm for 10 min by using hematocrit centrifuge and then kept in freezer until use. Total serum concentration of testosterone was measured using a double-antibody radioimmunoassay kit as manufacturer protocol.

Evaluation of infertility organs: At the end of the experiment, animals were anaesthetized with sodium pentobarbital (40 mg kgG1) administered intra peritoneally and the peritoneal cavity was opened and the epididymis was immediately removed then weighed.

Sperm count, motility and morphology: For sperm counting, the cauda epididymis was cut and smashed with forceps to discharge sperms into 2 mL of medium ham's containing 0.5% bovine serum albumin. After 10 min of incubation at 37EC (with 5% CO2), sperm count was achieved by using the standard hemocytometric method. Sperm motility was analyzed with a microscope at 10x magnification and was reported as the mean of motile sperm according to the world health organization method. Morphology of sperms was detected.

Seminal analysis: At the end of the experiment, epididymis and vas deferens of all groups were excised, torn with a dissecting needle in 2 ml of 0.9 % of NaCl and incubated at 35 oC for sperm quality evaluation. Sperm concentration was determined in terms of total sperm count per individual, viable sperms, progressive moving sperms and abnormal morphology sperms and expressed as percentage of incidence.

Sperm quality assessment: To measure fertility of animals based on sperm quality parameters sperms count, viability, motility and normal morphology, semen in the epididymis was squeezed and diluted with physiological saline. Spermatozoa was counted using a Neubauer's haemocytometer under a light microscope at 400x magnification and expressed as million/ml of suspension. Quantitative epididymal sperm motility expressed as an index determined by counting both motile and immotile spermatozoa per unit area. Sperm morphology was assessed from a smear of the epididymal filtrate prepared on a clean glass slides by addition of a drop of 1% eosin. After the object dried observation done under a light microscope at 400x magnification and abnormalities of either head or tail were noted.

Determination of Sperm count:

At the end of the treatment rats were sacrificed, seminal fluid was collected from epididymis using forced extraction method. Sperm count was determined by Neubaure's ruling chamber method. Sperm count was calculated as per the formula.

$$\text{Sperm count per ml} = \frac{\text{Sperm count x 10 X 1000}}{\text{4 X 0.1}}$$

Sperm count was expressed in terms of the number of sperms per ml.

Upon scarification, left and right epididymes and testes were removed and cleaned to eliminate all the fat bodies. Epididymal and testicular weight were recorded using an electric balance.

Preparation of Sperm Suspension:

Epididymis was separated carefully from testis and divided into 2 segments: caput (the head) and cauda (the tail). These parts from both epididymes were put into different vials and minced with scissors and gently homogenized with 2mL mammalian saline solution. Approximately 100µL of this concentrated sperm suspension was pipetted out into another vial and mixed with 900 µL of mammalian saline solution. Evaluation of sperm count, an hour after the sperm diffusion in the solution 10µL aliquot of the epididymal sperm suspension was transferred to each counting chamber of the hemocytometer and allowed to stand for 5min. Total sperm count of corpus and cauda epididymis regions were estimated using a light microscope at ×40 magnification. Sperm counts were expressed as million/ mL of suspension.

Effect on Sperm Motility Parameters and Total Epididymal Sperm Count: A sample drop from different epididymal areas was taken and percentage motile spermatozoa were estimated (approximately 200 cells) for each concentration and time points by a single observation under phase contrast optics Spermatozoa which represent various motility patterns including immotile sperm, slightly motile sperm, twitching sperm, slow moving sperm,

highly motile sperm were scored. Total sperm number was determined in 5 replicates.

Effect on Serum Testosterone Levels: Three groups of rats were taken and treated orally (test drug). The blood samples were collected from the tails and serum was separated. Subsequently serum testosterone level was measured using Enzyme Immunoassay Method (EIA).

Effects on Male Fertility: On day 31 of treatment, males were paired over night with a pro oestrous female (at 16:30–17:00 pm). The subsequent morning (08:00 a.m.–08:30 a.m.), successful mating was confirmed by the presence of sperm in the vaginal smear. If spermatozoa were present, their numbers were estimated (induplicate) using an improved Neubauer haemocytometer and gross morphology was noted by microscopic examination (×200). On day 14 of gestation, the female rats were laporatomized under mild ether anesthesia under aseptic conditions. Upon laparotomy, number of dead and live uterine implants and number of corpora lutea in both uterine horns were determined. Further, the width and the length of implants were recorded. At the end of the gestation period, number of live and dead pups was recorded.

Body and organ weights: Initial and final body weights of the animals were recorded. At the end of the one, two, four or twelve weeks, animals were sacrificed under ether anaesthesia 24 h after the last dosing of the respective treatment duration. The testes, hearts, liver and kidneys were removed and weighed.

Sperm motility, viability, counts and morphology: The caudal epididymis was dissected out; an incision (about 1 mm) was made in the caudal epididymis. Sperm fluid was then squeezed onto the microscope slide. Epididymal sperm motility was assessed by calculating motile spermatozoa per unit area and was expressed as percent motility. Epididymal sperm counts were made using the hemocytometer and were expressed as million/ml of suspension. The sperm viability was also determined using Eosin / Nigrosin stain as earlier described.

Fertility test: Male rats treated for 2 and 12 weeks were introduced to parous females in the ratio 1:2 for a period of seven days and the number of litters resulting from the cohabitation was recorded.

Serum biochemistry Blood was obtained by cardiac puncture from the rats in each study group after anaesthetized with ether. Each blood sample was spun at 2500 rpm for 10 minutes in a desktop centrifuge at 10-25˚C. Serum samples were assayed for testosterone using the enzyme linked immunoassay (EIA) technique.

Histological Processing: The organs were cut in slabs of about 0.5 cm thick

transversely and fixed in Bouin's fluid for a day after which it was transferred to 70% alcohol for dehydration. The tissues were passed through 90% alcohol and chloroform for different durations before they were transferred into two changes of molten paraffin wax for 20 minutes each in an oven at 57˚C. Serial sections were cut using rotary microtome at 5 microns. Slides were prepared from these tissues. The slides were de waxed and passed through absolute alcohol (2 Changes); 70 % alcohol and then to water for 5 minutes. The slides were then stained with haematoxylin.

Testosterone assay: At the end of the sixth week (Day 42), blood was obtained from each rat via the retro-orbital sinus. Each blood sample was spun at 2500 revolution per minute for 10 minutes in an angle – head desktop centrifuge at room temperature to obtain clear sera. Serum samples were assayed for testosterone in batches with the control sera at both physiological and pathological levels by standard quantitative ELISA technique. Analyses were carried out according to the manufacturer's instructions. Epididymal sperm count and motility. The animals were sacrificed and the male organs were removed. The cauda epididymis was excised; several incisions (1mm) were made in the cauda epididymis which was suspended in 1ml of Ham's F- 10 solution. After 10 minutes incubation at 37˚C, sperm concentration and motility were determined by haemocytometer method. The rest of the organs – (testes, epididymis, vas deferens and seminal vesicle) were excised and preserved in 10% formal saline and processed for histological studies. Gross and histopathological studies

The excised tissues were necropsied and cut up into smaller pieces (about 3mm thick) and fixed in 10% formal saline. Further histological processing was done using the Automatic Tissue Processor. The tissues were embedded and subsequently sectioned at 5µm using the Rotary Microtome. Sections were stained according to Haematoxlin and Eosin (H and E) technique. The sections were examined using Olympus Binocular microscope with in-built lighting system and their photomicrographs were taken using an eyepiece microscope-digital-camera.

INVITRO METHOD FOR MALE:

Semen Samples Collection:

Semen samples were collected in sterile container after 3 days of moderation and kept at 37˚C immediately prior to the examination for liquefying. The non liquefied samples were checked at 20-minute intervals until they were liquefied. All the routine semen parameters were consistent with the normal ranges.

Sperm Processing:

Semen specimens were pre processed by swim-up optimization technique to obtain

highly motile spermatozoa. They were then diluted with the Ham's F-10 medium (Sigma) to obtain a sperm suspension with a concentration of 106/mL. The percentage of progressively motile spermatozoa and sperm motility should be higher than 45% and 80%, respectively.

In Vitro Incubation of Spermatozoa With the test drug:

The sperms of each sample were divided into 4 groups. For group 1 (control), Ham's F-10 medium (0 drug concentration) was added. For groups 2, 3, and 4, Ham's F-10 medium with test drug were added, respectively. The parameters recorded for each sample were measured after 15, 30, 60, and 120 minutes of incubation at 37C, 5% CO_2, and humidity 90%.

Sperm Concentration and Motility Analysis:

Semen quality analysis was performed using the computer-assisted semen analysis (CASA) version 12 IVOS (Hamilton Thorne Biosciences). For automatic analysis, 5 mL semen samples were dropped on the sperm analysis chamber. Using CASA, at least 10 fields were evaluated regarding sperm concentration, sperm motility, and different sperm motion variables, including percentage of progressive motility (percentage of A and B level of spermatozoa) and movement characteristics such as curvilinear velocity (VCL) and straight line velocity (VSL). After 0 seconds and once in every 15, 30, 60, and 120 minutes of incubation, the sperm motility parameters were estimated in 10 randomly chosen fields using the CASA system.

Sperm Viability Analysis:

Eosin B staining was carried out to assess sperm viability. Four drops of semen were mixed thoroughly with one drop of 1% eosin B and mixed well. Immediately, a drop of the mixture was placed on a clean glass slide and allowed to be air dried. The prepared slide was examined that 100 cells per sample were determined. Pink-stained dead sperms were differentiated from unstained live sperm, and their numbers were recorded. The percentage of the live spermatozoa was calculated triplicately.

Determination of DNA Fragmentation:

For all the experimental and control groups 30 mL of samples were mixed with70 mL of 1% low-melting point aqueous agarose (to obtain a 0.7% final agarose concentration) at 37˚C. Aliquots of 50 mL of the mixture were pipetted onto a precoated glass slide with 0.65% standard agarose dried at 80˚C and covered with a coverslip. Then, slides were left to be solidified at 4˚C for 4 minutes. The coverslips were removed slowly, then the slides were immediately immersed horizontally in fresh acid denaturation solution (0.08 N HCl) at 22˚C for 7 minutes in a dark space. The slides were transferred

to a tray with neutralizing and lysing solution No. 1 (0.4 M Tris, 0.8 M dithiothretol [DTT], 1% sodium dodecyl sulfate [SDS], and 50 mM ethylenediamine tetra-acetic acid [EDTA] with pH of 7.5) at room temperature for 10 minutes. All the slides were incubated in neutralizing and lysing solution No. 2 (0.4 M Tris, 2 M NaCl, and 1% SDS with pH of 7.5) at room temperature for 5 minutes. This stage was followed by washing in Tris-borate-EDTA buffer (0.09 M Tris-borate and 0.002 M EDTA with pH of 7.5) for 2 minutes, dehydrating in sequential 70%, 90%, and 100% ethanol baths (each for 2 minutes), and air drying. The cells were stained with Wright stain (1:1 in phosphate buffered saline) (Merck) for 10 minutes. Then the slides were studied with light microscopy under 100magnification.

Sperm processing, motility, vitality and membrane integrity:

Fresh semen samples were obtained from a single animal to avoid inter-donor variability. The semen was washed three times after analysis and layered on top of a discontinuous two-layer colloid gradient of Pure Sperm in a 13.5 ml centrifuge tube. The bottom layer consisted of 1.0 ml of 80.0% Pure Sperm in Ham's F10, whilst the next layer contained 1.0 ml of 40.0% Pure Sperm in Ham's F10 and 1.0 ml of semen on top. The gradients were centrifuged at 300 × g for 20 min. The supernatant was removed from each tube and the pellet re suspended in 3.0 ml sperm wash medium. The tubes were then centrifuged at the same speed for 10 min. The supernatant was removed and washed again with 3.0 ml HSA supplemented Ham's F10 at 300 × g for 10 min. The final sperm-pellet was re suspended in 1.0 ml Ham's F10 (control) supplemented with HSA, or 1.0 ml of medium supplemented with the herbal extract to final concentrations of 1.0, 2.5, 6.5 and 10.0 mg/ml. The sperm was then incubated at 37 °C, under 6.0% CO2, 95% humidity in air with test tube tops loosened (for gaseous exchange) for 48 h. Sperm motility analysis was performed after 0, 4, 24, and 48 h of incubation. Motility was noted as rapid progressive motility or slow or sluggish progressive motility or non-progressive motility. The eosin–nigrosin and the Hypo Osmotic Swelling (HOS) tests were performed after 24 and 48 h of incubation to determine sperm vitality and membrane integrity. All counts (motility, eosin–nigrosin and HOS) were performed in triplicate.

INVIVO METHOD FOR FEMALE:

Mating procedure:

On the 16th day, all the rats were housed two per male animal in a cage. Vaginal smears were examined every morning for detection of spermatozoa. The day on which spermatozoa was detected in vaginal smear was considered day 1 of pregnancy.

Evaluation of animals:

All the animals were inspected daily for signs of abortion, illness and prolong duration of pregnancy.

Caesarean section for implantation sites:

On the 10^{th} day of pregnancy corresponding to mid gestation period, half of the females in all the groups were sacrificed by cervical dislocation. The implantation sites were checked and the embryos counted and weighed.

Reproductive indices:

All the remaining pregnant females were allowed to give birth to their offspring. From pregnancy day 19 the animals' cages were inspected for births. As soon as possible after birth the numbers of viable and death newborns were recorded, the pups were weighed and generally inspected for any deformity up to day 7 after birth. The following reproductive indices were calculated.

Mating index is defined as the number of sperm positive females/number of mated females × 100.

Pregnancy index is defined as the number of pregnant females/number of sperm positive females × 100.

Delivery index is defined as the number of females delivering/number of pregnant females × 100.

Birth live index is defined as the number of live offspring/number of offspring delivered × 100.

Post implantation loss index is defined as the number of implantation sites - number of live fetuses/number of implantation sites × 100.

At the end of the experiment, the number of delivered female rats, the number of live births (and death if any), and the infants weight were recorded. Moreover, the gender of infants was detected by determining anogenital distance, and their gender ratio (male/female) was recorded thirty days after birth.

CONCLUSION

Childbearing and family are considered as a right of every human being. Infertility is a health disorder that requires proper treatment approach. Modern medical science has developed advanced therapies to assist reproduction. The main causes of infertility are the problems of fallopian tubes, hormonal imbalance, ovarian failure and impotence. Many reasons are sorted out for female infertility, but through proper diagnosis and counselling for treatment of infertility can be only a beam of hope. Review reveals extensively all the major causes, diagnosis, infertility treatments and biological screening methods of in fertility. This review will be helpful to all the scientific, medical researchers to study the infertility.

REFERENCES

[1] Benjamin. F. Miller M.D., Claire Brackman Keane R.N B.S.M.Ed., Encyclopedia & Dictionary of Medicine, Nursing & Allied Health. 3rd Edition W.B. Saunders company 1983; 971.

[2] Douglas. M. Considine Van nostrand's Scientific Encyclopedia. 8th Edition International Thomson Publishing Inc 1994, 1698.

[3] Benjamin.F. Miller M.D., Claire Brackman Keane R.N B.S.M.Ed., Encyclopedia & Dictionary of Medicine, Nursing & Allied Health. 3rd Edition W.B. Saunders company 1983; 417.

[4] Chaudhary. S, Chaudhary. A, Human Anatomy & Physiology, S. Vikas & Co, 2010, 259.

[5] http://www.biosci.uga.edu/almanac/bio_103/notes/apr_1 5.html.

[6] www.wikipedia.com.

[7] Mohammad Kanedi, Sutyarso, Hendri Busman, Risky Amellia Mandasari and Gina Dania Pratami. International Journal of Biomedical Research. 2019; 10(02): 1-4.

[8] Mohammadi F., Nikzad H., Taherian A., Mahabadi J.A. and Salehi M. Effects of Herbal Medicine on Male Infertility. Anatomical Sciences, 2013; 10(4): 316.

[9] Venkatesh Palaniyappan, Ektha Prasanthi Bommireddy, hephzibah Gudipudi, Ratna Deepika Chitturi, Neraja Yandamal. In vivo fertility enhancing activity (aphrodisiac) of *ficus carica* fruit on male wistar rats. International journal of pharmacy and pharmaceutical sciences. 2013; 5(2).

[10] Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, regional, and global trends in infertility prevalence since 1990: A systematic analysis of 277 health surveys. PLoS Med. 2012; 9(12): e1001356.

[11] World Health Organization. Infertility. 2013.

[12] WHO. Sexual and reproductive health. 2013.

[13] Taylor A. Extent of the problem. ABC of subfertility. 2003; 327(7412): 434-436.

[14] Kakarla N, Bradshaw K. Evaluation and Management of the Infertile. Glowm. 2008.

[15] Kamel RM. Management of the infertile couple: an evidence based protocol. Reproductive Biology and Endocrinology. 2010; 8: 21.

[16] Tayebi N, Ardakani SMY. Incidence and prevalence of the sexual dysfunctions in infertile women. European Journal of General Medicine. 2009; 6(2):74-77.

[17] Fraser DM, Cooper MA. Myles text book for midwives, 15th Ed. Churchill Livingstone Elsevier. 2009.

[18] Centers for Disease Control and Prevention. Infertility FAQs. 2013.

[19] Bosteels, Weyers S, Puttemans P, et al. The effectiveness of hysteroscopy in improving pregnancy rates in subfertile women without other gynecological symptoms: a systematic review. Human Reproductive Update. 2010; 16(1): 1-11.

[20] Jungwirth A, Diemer T, Dohle GR. Guidelines for the investigation and treatment of male infertility. European Urology. 2012; 61(1): 159-163.

[21] Razak RNHA, Isa MLM, Wahab AYA, Yusuf AM, Muhammad H & Ramli R. Animal Studies on Male Fertility Enhancing Properties of Plants in Malaysia: A Review of the Past 16 Years. Journal of Biotechnology and Strategic Health Research. 2017; 1(1): 17 – 23.

[22] Sahni SK, Mark BS. Guidelines for care and use of animals in scientific research. Indian National Science Academy: New Delhi. 2000.

[23] World Health Organization. WHO Laboratory Manual for Examination of Human Semen and Sperm-Cervical Mucus Interaction, 4th edition New York: Cambridge University Press. 1999.

[24] Shalaby, M.A. and S.M. Mouneir, 2010. Effect of *Zingiber officinale* roots and *Cinnamon zeylanicum* bark on fertility of male diabetic rats. Global Vet., 5: 341-347.

[25] Hafez, D.A., 2010. Effect of extracts of ginger goots and cinnamon bark on fertility of male diabetic rats. Journel of American Science, 6: 940-947.

[26] Al-Khamas, A.J.H., 2018. Effect of *cinnamon zeylanicum* bark water extract on male diabetic albino rats fertility. Bas. J. Vet. Res., 17: 123-135.

[27] Jahromi, V.H., K. Parivar and M. Forozanfar, 2011. The effect of cinnamon extract on spermatogenesis hormonal axis of pituitary gonad in mice. Iran. J. Applied Anim. Sci., 1: 99-103.

[28] Shrilatha B, Muralidhara. Early oxidative stress in testis and epididymis sperm in streptozotocin-induced diabetic mice: Its progession and genotoxic consequences, Repro Toxicol, 2007; 23:578-587.

[29] Keskes-Ammar L, Feki-Chanroun N, Rebai T, Sahnoun Z, Ghozzi H, Hammami S, Zghal K, Fki H, DAmak J, Bahloul A. Sperm

oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men, Arch Androl, 2003; 49: 83-94.

[30] Kefer JC, Agarwal A, Sabanegh F. Role of antioxidants in the treatment of male infertity, International Journal of Urology. 2009; 16:447457.

[31] Mohammadi F., Nikzad H., Taherian A., Mahabadi J.A. and Salehi M. Effects of Herbal Medicine on Male Infertility. Anatomical Sciences, 2013; 10(4): 316.

[32] M. A. Akbarsha, P. N. L. Latha, and P. Murugaian, "Retention of cytoplasmic droplet by rat cauda epididymal spermatozoa after treatment with cytotoxic and xenobiotic agents," Journal of Reproduction & Fertility, 2000; 120 (2):385–390.

[33] B. Robaine and L. H. Hemo.Efferent ducks, Epididymis and vas deferens: structure, function and their regulation," in The Physiology of Reproduction, E. Knobiland J.D. Neill, Eds., Vol. 2, pp. 1988–1999.

[34] L.C. Saalu, G.G. Akunna, and J.O. Ajayi, "Modulating role of bitter leaf on spermatogenic and steroidogenesis functions of therattestis," American Journal of Biochemistry and Molecular Biology.2013: 3(3), 314–321.

[35] S. C. Sikka, M. Rajasekaran, and W. J. G. Hellstrom, "Role of oxidative stress and antioxidants in male infertility," Journal of Andrology.1995; 16(6), 464–468.

[36] Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: a review of literature. Journal of Human Reproduction Science. 2015; 8:191-196.

[37] Elahi RK, Asl S, Shahian F. Study on the effects of various doses of Tribulus terrestris extract on epididymal sperm morphology and count in rat. Glob Veterin. 2013; 10: 13-17.

[38] Fekrazad E, Keyhan H, Fekrazad R, Tajik A. Effect of diode lasers on human sperm motility. Acad Res Int. 2014; 5: 21-25.

[39] Remya M, Sharma RC, Shoaib H, et al. In vitro effect of Aegle marmelos on human sperm motility. J Med Plants Res. 2009; 3: 1137-1139.

[40] Mukhopadhyay AK, Kumar A. Follicular Growth Ovulation and Fertilization: Molecular and

Clinical Basis. Boca Raton, FL: CRC Press; 2002.

[41] Lo´pez G, Lafuente R, Checa MA, Carreras R, Brassesco M. Diagnostic value of sperm DNA fragmentation and sperm high magnification for predicting outcome of assisted reproduction treatment. Asian Journal of Andrology. 2013; 15: 790-794.

[42] Figueiroa MS, Cesar Vieira jS, Leite DS, Filho RC, Ferreira F, et al. Green tea polyphenols inhibit testosterone production in rat Leydig cells. Asian Journal of andrology. 2009. 11(3):362-370.

[43] Khayatnouri M, Safavi SE, Safarmashaei S, Babazadeh D, Mikailpourardabili B. The effect of Saffron Orally Administration on Spermatogenesis Index in Rat. Advances in Environmental Biology, 2011. 5(7):1514-1521.

[44] El-Shahat A, Gabr A, Meki A, Mehana E. Altered testicular morphology and oxidative stress induced by cadmium in experimental rats and protective effect of simultaneous green tea extract. International Journal of Morphology, 2009. 27:757-764.

[45] Fadhel ZA and Amran S. Effects of black tea extract on carbon tetrachlorideâ induced lipid peroxidation in liver, kidneys, and testes of rats. Phytotherapy Research. 2002. 16(S1): 28-32.

[46] Awoniyi D, Aboua G, Marnewick JL, Plesis SS du, Brooks NL. Protective effects of rooibos (*Aspalathus linearis*), green tea (*Camellia sinensis*) and commercial supplements on testicular tissue of oxidative stress-induced rats. African Journal of Biotechnology. 2011. 10(75): 17317-17322.

[47] Khan MR, Ahmed D. Protective effects of *Digera muricata* (L.) Mart. on testis against oxidative stress of carbon tetrachloride in rat. Food and Chemical Toxicology, 2009.

[48] Awoniyi D, Aboua G, Marnewick JL, Plesis SS du, Brooks NL. Protective effects of rooibos (*Aspalathus linearis*), green tea (*Camellia sinensis*) and commercial supplements on testicular tissue of oxidative stress-induced rats. African Journal of Biotechnology. 2011; 10(75): 17317-17322.

[49] Abdeen SM, Mathew TC, Dashti HM, Asfar S. Protective effects of green tea on intestinal ischemia-reperfusion injury. Nutrition. 2011; 27(5): 598-603.

[50] Zegers-Hochschild F, Adamson D, Dyer S. The international glossary on infertility and fertility care. Human Reproduction. 2017; 32: 1786–1801.