Introduction

Assisted Reproductive Technology offers new hope and possibilities for couple who would otherwise be unable to have children. In Vitro Fertilization or IVF involves stimulating the woman's ovaries with fertility medication like gonadotropins to produce more number of eggs. These eggs when near to maturity are triggered with hCG injection, these eggs are then retrieved while the woman is under mild anesthesia. The eggs thus retrieved are fertilized with her husband's sperm in the IVF lab. The process of fertilization and embryo development are carefully monitored by an embryologist for 3-5 days. The resultant embryos are then thus transferred back to the woman's womb in anticipation of a successful pregnancy.

The decision to opt for an IVF procedure may be most challenging and difficult one a couple can make. We at Dr. Rama's Institute for Fertility strive hard to see that each couple have individualized plan as per their requirements in order to maximize their chances of a having a successful pregnancy.

Patience, individual attention, perseverance and emotional support is what we provide exclusively at our place because of which our hospital is ranked among the best institutes not only in India but perhaps across the globe.

Indications for IVF

IVF is generally recommended when simple treatments like IUI etc fail. When there is a tubal pathology or blockage, IVF remains the only way to help couple parent their own child.

Fallopian Tube Damage / Tubal factor

When there is a significant tubal damage the only options are surgical repair, or bypassing the role of fallopian tubes by opting for IVF. Sometimes even in cases where the fallopian tubes look healthy in the laparoscopy or hysterosalpingogram there can be mild infection which is not congenial for the fusion of spermatozoa and the eggs which can lead to infertility.

Anovulation

The majority of patients with an ovulation will conceive with simpler treatments. However patients requiring IVF are typically "high responders" to gonadotropin therapy and have good prognosis.

Male factor infertility

The advent of IVF process along with ICSI (Intracytoplasmic Sperm Injection pronounced as Iksee) the capacity to obtain fertilization in the IVF lab with severely abnormal sperm samples has revolutionized the options for couple having male infertility problems. It provides frontiers for men who were previously incapable of producing adequate sperm to father genetically related children.. ICSI is often recommended to couples having compromised semen parameters, either with motility, count, velocity, DNA, fragmentation, if spermatozoa are obtained surgically or if there has been a prior failure of fertilization.

Endometriosis.

Endometriosis may be effectively treated with a combination of surgical and medical therapy. IVF is very effective as a second line of treatment if the initial lines of treatment have been unsuccessful.

Unexplained infertility.

Approximately 20% of couples with infertility will have no identifiable cause of infertility after completing a comprehensive evaluation. It does not mean that there is no reason but implies that even in the modern scenario these causes cannot be diagnosed. IVF proves to be successful even in cases where the more conservative treatments have failed, especially because most of these couples actually have some block to fertilization, problems in fusion of egg and spermatozoa and further embryo development, movement of embryos from fallopian tube to its implantation site on the walls of the uterus.

Age Related Infertility

A women's ovarian function is diminished with age. Apart from the virtual age, there is something called as biological clock which keeps ticking away as the ovarian reserve of eggs keeps getting depleted. Insome this process is more rapid and can happen early on. Even in man with increasing age and exposure to pollutants or radiation there can be problems in the capacity of the spermatozoa to fertilize the egg on its own. In such cases these problems can be overcome through the use of IVF alone or in conjunction of this with techniques like ICSI, IMSI and Laser Assisted Hatching, PGD etc.

Preimplantation Genetic Diagnosis: - PGD

PGD or genetic testing may be indicated for patients who are at risk for genetic disorders like cystic fibrosis, thalassemia or breast cancer or for patients with infertility related chromosomal abnormalities such as recurrent IVF failure or recurrent pregnancy loss (RPL).

Couples requiring third party in order to parent a child.

In the female there may be severe compromise in the ovarian function like decreased ovarian function or fall in egg quality, in such cases egg donation from a donor who is capable of producing good quality eggs offers excellent chance of pregnancy. If male spouse is unable to produce spermatozoa capable the fertilizing an egg even with advanced techniques may also need to opt for sperm donation.

On the otherhand if the woman has problems in carrying the gestation to term either due to immune problems or medical problems like hypertension, abnormalities in the uterus or congenital problems where there is complete absence of the uterus, in such scenario the couple can still opt for a surrogate (host uterus) who will be a gestational mother for their child.

Investigations for couple prior to undergoing IVF treatment.

FEMALE

General investigations:

CBP

Hormonal analysis for AMH

Day 3 menstrual cycle

- Hormonal analysis for FSH and LH
- Transvaginal Ultrasound scan to see the number of follicles in each ovary their sizes, ovarian sizes, endometrial pattern.

A mock or trial embryo transfer will be done to measure the depth of uterine cavity so that embryos can be transferred to the pre determined location within the cavity during the actual IVF cycle.

Saline infusion sonohysterogram (SIS) can be done to evaluate the uterine cavity and to make sure that there are no abnormalities like uterine polyps, fibroids etc which may interfere or hinder implantation.

MALE

General investigations

-----**blanks to be inserted from our prescriptions**

- Semen analysis with 3-5 days abstinence
- Sperm function tests
- Semen for culture and sensitivity
- For indicated cases CASA, DFI

CASA (Computer Assisted Sperm Analysis)

DFI (DNA fragmentation Index)

These tests can be done with help of Sperm Class Analyser (SCA).

The IVF Process

The IVF cycle entails multiple steps, and each step takes place at a specific time during a six-week period.

The IVF procedure is based on the following steps:

(1) **Preparation for treatment**

(2) **Induction of ovulation**

(3) **Egg retrieval**

(4) **Embryo transfer**.

Cycle Preceding ART Cycle

1. Initiation of oral contraceptives
2. Initiation of Agonist Depot

IVF Cycle

1. Initiation of Oral Contraceptives
2. GnRH Analog (Lupron) Administration
3. Baseline Pelvic Ultrasound

4. Ovarian stimulation (e.g. Follistim, Gonal F, Menopur)
5. Monitoring of Follicle Development and Estradiol levels
6. Final Oocyte Maturation and HCG administration
7. Transvaginal oocyte retrieval
8. Insemination of oocytes
9. Embryo Transfer
10. Progesterone supplementation
11. Pregnancy test

Step 1- Initiation of Oral Contraceptives

Birth control pills are prescribed starting on day 5 of your cycle. There are two main reasons for taking birth control pills prior to your IVF cycle. First of all, taking birth control pills prior to a stimulation cycle may help the ovaries respond better to the stimulation medication. Secondly, taking birth control pills allows flexibility in coordinating your cycle. Please note that many patients experience “break through bleeding” when taking birth control pills. This is normal. Please continue taking the birth control pills daily regardless of the bleeding. Plan to be on birth control pills a minimum of two weeks. On day 3 of this cycle hormonal analysis for FSH & LH are done along with transvaginal ultrasound scan to know the ovarian reserve i.e the number of follicles in each ovary & their sizes. Based on these results the protocol regimen will be decided.

Step 2- GnRH Analog (Lupride) Administration

Lupride prevents the premature release of the eggs from the ovaries before the egg retrieval procedure. The Lupride injection is given on the 18th day in the cycle preceeding the actual IVF-ET cycle.

The dosage of lupride will depend on the investigations done on day 3 of the menstrual cycle.

Step 3-Baseline Pelvic Ultrasound

On day 3 of the menstrual cycle, a blood test is done to check the estradiol & LH level, an ultrasound scan to examine the ovaries & the baseline follicles in each ovary and the endometrial study. If your estrogen level is too high or if a cyst is detected, further a day or 2 are given to allow the cyst to resolve spontaneously or if the cyst is too big it is aspirated & ovarian stimulation medications are started.

Step 4-Ovarian Stimulation

If the baseline ultrasound shows no significant cysts, we start ovarian stimulation after menstrual bleeding begins. Ovarian stimulation medications are preparations of naturally occurring hormones, which are used to develop and mature multiple follicles by directly stimulating the ovaries.

Typically the injections are given daily for 8-12 days, depending on how your body responds to the medications. The average number of eggs retrieved at IVF is between 8 and 15. Please remember that it is the quality of the eggs, not the quantity that is important.

Step 5-Monitoring of Follicle Development and Estradiol & LH levels

Transvaginal ultrasound examination takes between 5-20 minutes to perform. It provides valuable feedback for monitoring follicular growth and determining when the follicles are mature and ready for retrieval. We correlate the estradiol levels in your blood with the ultrasound results frequently during the IVF cycle to ensure that you are taking the proper dosage of medication. We may adjust the dose of medication to improve follicular development. The amount of medication prescribed depends upon the results of the blood tests and ultrasound exams. Our goal is to make this process as easy and seamless as possible.

Step 6- Final Oocyte Maturation and HCG administration

Human chorionic gonadotropin (HCG) is a drug that stimulates the final maturation of the eggs. If it is given too early, few, if any, oocytes will be mature. If it is given too late, the eggs within the follicles may be post-mature and will not fertilize. HCG needs to be given 36 hours prior to the egg retrieval, so when we schedule your retrieval with the ARTS lab, we will notify you of the time that HCG is to be given. Since the oocyte maturation is an individual phenomenon apart from being a biological one our IVF team of doctors & embryologists work 365 days 24*7 round the clock not compromising on the time which is most crucial part of an IVF-ET regimen.

Step 7-Transvaginal oocyte retrieval

The couple are expected to report one hour early to the time of egg retrieval.During the retrieval, the anesthesiologist administers intravenous medications (pain relievers and sedatives) in order to minimize the discomfort that may occur. The egg retrieval is performed via vaginal ultrasound (similar to the ultrasound used for monitoring your follicles during your stimulation). Once you are comfortable and relaxed, a tip of a thin needle is passed through the top of the vagina and into the cul-de-sac (space behind the uterus). The ovaries are located near the bottom of the cul-de-sac allowing the tip of the aspirating needle to enter the ovarian follicles and aspirate the follicular fluid from them.

The egg retrieval takes approximately five to ten minutes. Sometimes there are ovarian cysts that contain no eggs but appear identical to follicles that do contain eggs. Also,

follicles of smaller size may not yield eggs. The number of follicles seen with ultrasound, therefore, may not correspond to the number of eggs retrieved. Ultrasound provides only an approximation of the number of oocytes that one can expect to recover.

Step 8-Insemination of Oocytes

Later, the sperm is prepared and placed with the eggs. In some cases, the embryologist will need to identify normal, motile sperm and inject them individually directly into each egg. This procedure is called Intracytoplasmic Sperm Injection (ICSI). Once the eggs are inseminated or placed with the sperm, they are placed into an incubator overnight. Semen samples for use in IVF procedures will be required on the day of egg retrieval and should be collected at the andrology lab at DRIFF. On rare occasions, the laboratory staff may request a second semen sample. The specimen should be obtained by masturbation after 3-5 days of abstinence. More than 5 days of abstinence is NOT recommended. It is important not to use any lotions or lubricants for sperm production as it may harm sperm motility. If you anticipate any collection difficulties, please notify your IVF coordinator prior to the procedure so we may have your husband cryopreserve semen before the day of the actual procedure. A normal 2-PN embryo on Day 1 Normal fertilization is characterized by a pronucleus of the egg and sperm that can be visualized under a microscope.

Step 9-Embryo Transfer

The embryo transfer (ET) is usually performed two, three days or five days after the oocyte retrieval. It is very important to have a full bladder before the embryo transfer. The procedure takes approximately 10-15 minutes and is very similar to the uterine measurement taken at your baseline appointment. You will not need anesthesia on this day, so there is no need to abstain from eating or drinking before your ET.

Once you, the embryologist and physician, have confirmed the plan for the embryo transfer, the physician will insert a speculum. An abdominal ultrasound will be used to visualize the uterine cavity. The embryologist will load the embryos into a small catheter which is then gently inserted through the cervical opening into the uterus, and the embryos are placed into the uterine cavity along with a very small amount of fluid. The catheter is then carefully removed.

Following the Embryo Transfer you will remain lying down for approximately one hour, then we would like for you to rest at home for at least 24 hours. You must have someone accompany you and drive you home.

Step 10-Progesterone Supplementation

You will take progesterone injections beginning the evening after the oocyte retrieval and continue daily until your serum pregnancy test. If pregnant, progesterone will be administered for a total of 10 weeks. Ordinarily, the granulosa cells in the follicle will produce progesterone following ovulation, but some of these cells are removed during

the oocyte retrieval. Therefore, supplemental progesterone is needed to help maintain the uterine lining for implantation.

Progesterone is a hormone normally produced by the ovarian corpus luteum during the last two weeks of the menstrual cycle and during early pregnancy. After the seventh or eighth week of pregnancy, the placenta takes over progesterone production. Natural progesterone is prescribed in many fertility treatments for luteal phase support of implantation and early pregnancy. Natural progesterone is also prescribed to support the luteal phase for patients in virtually every IVF program today. The progesterone prescribed is derived from natural sources and is identical to that produced in the body. Ocassionally estradiol may also be supplemented from day 7 of the egg pick-up if it is deficient.

Do NOT discontinue your Progesterone or estradiol until directed by us.

Step 11-Pregnancy Follow-up

After the embryo transfer pregnancy test is done 12 to14 days after the embryo transfer. The pregnancy test is obtained before 10:00 a.m. and the results will be called to you the same afternoon. Positive tests are repeated in one week, and a sonogram will be scheduled after 30 days from the day of embryo transfer. Once we document a heartbeat on the sonogram, the pregnancy is confirmed. If your pregnancy test is negative, we ask that you schedule a follow-up visit to review your cycle and discuss options.

Possible reasons for cycle cancellation are

- The follicles are not developing properly.
- An inadequate blood estrogen level.
- Excessive estradiol level, indicating an increased risk for ovarian hyperstimulation.
- Less than 5 maturing follicles seen on ultrasound.
- If a cycle is cancelled, medication may be modified in subsequent cycles in an attempt to improve your response.

Intracytoplasmic Sperm Injection (ICSI)

Men normally produce millions of sperm in each ejaculate. These sperm “swim” through the cervical opening and into the tubes to the site of fertilization. Some men have sperm defects such as a reduced sperm count, deformed sperm, or sperm that cannot swim effectively. When any one of these or a combination of these abnormalities are present, it can prevent normal fertilization. ICSI bypasses sperm defects because a single sperm is “selected” and placed inside the egg. ICSI is performed as a part of the IVF cycle. **ICSI** involves the placement of a single sperm directly into the egg using a microscopic pipette, thus sperm and egg interactions involved with normal fertilization are by-passed. During IVF, the eggs are retrieved from the ovaries the surrounding cells are

stripped off from the egg, best spermatozoa with morphology & motility is selected & injected directly into the egg held held in position with a holding pipette. All the micromanipulations are done on an inverted microscope & micromanipulator. ICSI procedure ensures fertilization of the eggs upto 90%. ICSI has been successfully applied for the treatment of severe male infertility including suboptimal ejaculate samples, ejaculatory failure, obstructive and nonobstructive causes of azoospermia (complete absence of sperm).

Indications for ICSI:

1. Couples who have had unexplained fertilization failure in a previous **IVF cycle**.

2. Decreased sperm concentration

3. Decreased sperm motility, including totally immotile sperm (e.g. Kartagener's syndrome), provided they are viable.

4. Unusually high percentage of morphologically abnormal sperm, including round-headed sperm (globozoospermia).

5. Complete absence of sperm in the ejaculate due to an obstruction (obstructive azoospermia) caused by conditions such as congenital absence of the vas deferens (CAVD), post-inflammatory obstruction of the epididymis or vas and failed vasectomy reversal. MESA Testicular sperm extraction (TESE) is used to retrieve sperm for ICSI.

6. Complete absence of sperm in the ejaculate due to defective sperm production (non-obstructive azoospermia). Patients who have normal sperm formation in at least some areas of the testis (identified by testicular biopsy), providing enough viable sperm can be retrieved with TESE.

7. Ejaculatory dysfunction caused by retrograde ejaculation (enough sperm are usually recovered from the urine) or paraplegia. Electroejaculation or TESE can be usede in these cases to get spermatozoa.

8. Immunological factors; antisperm antibodies in female sera, follicular fluid or on sperm caused by vasectomy or genital tract infection.

9. Testicular cancer patients with semen samples frozen prior to treatment.

ICSI Success Rates and Risks

The ICSI procedure involves stripping cells from around the egg and injecting a spermatozoon into the egg. A small percentage of eggs, often the less healthy eggs, may be damaged by these procedures and degenerate (5-10%).Sometimes eggs fail to fertilize normally or arrest at an early stage of development. ICSI procedure can thus be

used to overcome these hurdles ICSI pregnancy rates and live birth rates are similar to those achieved with IVF.

Because some causes of male infertility are familial, and are related to genetic defects (e.g. Y-chromosome deletions) male offspring may inherit fertility problems. Therefore, patients with non-obstructive azoospermia or severe oligospermia (low sperm counts), who are likely candidates for genetic causes of infertility, should consider genetic counseling and karyotyping prior to ICSI.

In some cases, after ICSI embryos are co-cultured with follicular cells collected from the follicles during the egg aspiration to provide extra nourishment and remove any embryo-toxic factors that may be present.

Prior Vasectomy

Couples have the option of a vasectomy reversal or **IVF-ICSI** with epididymal or testicular sperm extraction. Age of the female partner and length of time since prior vasectomy are important factors in decision-making. It can sometimes take 6-9 months to recover adequate sperm counts following vasectomy reversal. Also, the greater the length of time between the vasectomy and the reversal, the greater the chances are that the surgery will be unsuccessful or that anti-sperm antibodies will form, preventing the recovered sperm from penetrating the eggs without IVF-ICSI. Spermatozoa for IVF-ICSI can be obtained with either Microsurgical Epididymal Sperm Aspiration (MESA) or Testicular Sperm Extraction (TESE).

Microsurgical Epididymal Sperm Aspiration

In MESA procedure, under local anesthesia and general sedation, an incision is made in the scrotum, exposing the epididymis, the tubules immediately adjacent to the testicles that collect the sperm. Using an operating microscope, an incision is made into these tubules and sperm is aspirated. Although millions of motile sperm can often be collected, this sperm has not acquired the ability to penetrate an egg and must be injected into eggs via the IVF-ICSI technique. The advantage of MESA over TESE for men with obstructive azoospermia is that sperm collected in this manner can usually be frozen, and even if his partner has to undergo more than one IVF procedure, the MESA should provide adequate sperm for all subsequent IVF procedures. A **TESE or testicular sperm extraction** is a procedure that involves directly aspirating the sperm from the testes or obtaining sperm from a testicular biopsy. It is usually performed under local anesthesia block and can be done as an office surgical procedure. The disadvantage is that in many cases, testicular sperm is much more scarce and therefore difficult to freeze. Usually, there is only enough sperm recovered for one IVF procedure, and if further IVF attempts are needed, the TESE procedure will need to be repeated.

Non-obstructive Azoospermia

Men with very poor sperm production in the testicles and no sperm in the ejaculate often demonstrate high blood FSH levels and sometimes low testosterone levels. The testicular size may be small. These men are usually considered to have relative testicular failure. TESE or testicular biopsy is usually the only option for them as there are no sperm in the epididymis and even testicular sperm production can be “patchy” and scarce within the testes. Men with this diagnosis who have been told they have no sperm on routine testicular biopsy frequently can be found, on further investigation, to have sperm present in a scattered distribution within the testicle. If so, these areas can be re-aspirated for IVF-ICSI with some degree of success, depending on the amount of sperm obtained.

Sertoli Cell Only Syndrome

Complete absence of sperm progenitor cells and absence of spermatogenesis is a rare condition. Sperm donation or adoption are the only options in these cases.

IMSI Technique - Intracytoplasmic Morphologically Selected Sperm Injection

For the creation of a competent embryo two ‘partners’ are needed: a mature oocyte and a ‘good/healthy’ spermatozoon.

In the area of assisted reproduction, when we decide to do the classical in vitro fertilization we leave nature to choose the spermatozoa that will fertilize the available oocytes; on the contrary when we decide to do ICSI (in cases with male factor infertility) we have to choose the spermatozoa that we inject into the oocytes. For ICSI, after sperm preparation an optical magnification of 200x to 400x is used to examine the sample. The best 'normal looking' motile spermatozoa are selected based on their major morphology and then injected into oocytes retrieved after ovarian stimulation. With ICSI, even men with severe male factor infertility could possibly achieve pregnancy. However, despite 20 years of technological improvements, both clinical pregnancy and live birth rates remain relatively low at approximately 35% and 25% per started cycle, respectively.

Therefore there is a kind of subjective selection, which is not adequately feasible due to the microscopic size of the spermatozoa. For the improvement of this selection a new innovative technique has been developed. The innovative technique is called **IMSI** (Intracytoplasmic Morphologically Selected sperm Injection) ‘motile sperm organelle morphology examination' (MSOME). This technique requires the analysis of minor morphological criteria using ultra-high magnification ≥ 6000 times higher magnification when compared to the normal ICSI microscopy and it implies a powerful microscopy system which allows for the morphologic selection of the ‘good’ spermatozoon with strict criteria; morphology is associated with genetic quality of the spermatozoon and consequently with the creation of a good embryo. When using this technique, the motile sperm fraction is examined based on six subcellular organelles: acrosome, postacrosomal lamina, neck, mitochondria, tail, and nucleus. Even the

minute defects in the spermatozoa become visibl under this powerful magnification like the size & shape of the head, vacuoles in the spermatozoa head the midpiece etc.

Studies comparing the IMSI and ICSI techniques indicate that IMSI does not improve fertilization rates or the morphologic quality of the embryos, but increases the pregnancy rates and reduces the miscarriage rates.

Couples with indication for IMSI are those with

Sperm demonstrating high degree of DNA fragmentation and/or **increased abnormal spermatozoa (teratozoospermia)**, conditions which often accompany oligoasthenospermia.

Additionally, IMSI may be beneficial for couples experiencing recurrent IVF failures and/or **recurrent biochemical**|**pregnancies/miscarriages**.

The promising results of IMSI lead us to adopt the technique in order to strengthen our goal which is to help our couples to have healthy children.

PICSI

In the ICSI procedure, an individual sperm is selected and injected into an oocyte. Until now, the only technique available to embryologists to select the sperm has been visual observation. Using PICSI procedure we are able to determine sperm selection in much the same way it happens in human biology.

Sperms are placed in PICSI dish containing samples of hyaluronan hydrogel. Hyaluronan is a naturally occurring biopolymer found in all human cells, including the gel layer surrounding the oocyte.

Mature, biochemically competent sperm bind to the hyaluronan where they can be isolated by the embryologist and used for ICSI. This procedure mimics a key step in the natural fertilization process, the binding of mature sperm to the oocyte complex. As a result, the selected sperm is essentially the same as one that would be successful in the natural reproductive process.

The research proved that hyaluronan-bound PICSI-selected sperm are, in the vast majority of cases, more mature, exhibit less DNA damage, and have fewer chromosomal aneuploidies.

We consider the PICSI method to be a biologically more natural and effective form of fertilization in comparison to ICSI, because only those sperm are chosen for fertilization that are able to form a bond with the oocyte cumulus complex, i.e. only mature sperm are selected

This method is suitable for everyone but we highly recommend it especially in the following cases:

- previous total failure or low fertilization even after ICSI
- low embryo quality or their failure to develop
- repeated abortions

Laser Assisted Hatching

The human embryo has an outer shell called as the zone pellucida. This layer sometimes is either thickened or hardened due to various reasons, like advanced maternal age, elevated FSH levels, PCOS or sometimes due to suboptimal cultural conditions hereditary etc. In such cases the embryo cannot hatch on its own to implant on the walls of the uterus and thus could lead to implantation failure, thereby causing infertility.

This scenario can be compared to the seed. When we need the seed to germinate we soak it in water such that when the water seeps in the force is sufficient to break the outer seed coat and germination occurs, same is with human embryo too.

Assisted Hatching is generally performed on a cleavage stage embryos either on day 2, day 3 or day 5 of invitro culture, prior to transferring them back to the patient's womb.

We at Dr.Rama's Institute For Fertility use the latest cutting edge laser technology available today to provide our patients with safest, fastest and uniform way of Laser Assisted Hatching.

In this technique the outer shall of the embryo is breached with few laser pulses of very low intensity to create holes in the zona pellucida thus making it thin. As the embryo develops further to the blastocyst having >150 cells the pressure from with in will be sufficient to facilitate hatching of the zona pellucida giving way for the embryo to implant on the walls of the uterus successfully.

This procedure just takes a few seconds per embryo to be completed. It thus helps to overcome failure due to failed hatching.

Laser Assisted Hatching is beneficial for patients:-

- With PCOS
- Elevated maternal age > 35 years of age
- Patients with previous IVF failure
- Thick or abnormal egg shell.
- Poor quality or slow developing embryos
- Embryos exhibiting excessive fragmentation.

- Patients having elevated day 3 follicle stimulating hormone (FSH)

Since this procedure is done on the outer shall it is in no way harmful to the embryos.

Preimplantation Diagnosis

Preimplantation genetic diagnosis (PGD) or PGS Preimplantation Genetic Screening is a screening test used to determine if genetic or chromosomal disorders are present in embryos produced through in vitro fertilization (IVF). Preimplantation genetic diagnosis screens embryos before they are transferred to the uterus so couples can make informed decisions about their next steps in the IVF process. Embryos unaffected by the genetic or chromosomal disorder can be selected for transfer to the uterus.

For couples undergoing IVF, preimplantation genetic diagnosis may be recommended when:

- One or both partners has a history of heritable genetic disorders
- One or both partners is a carrier of a chromosomal abnormality
- The mother is of advanced maternal age
- The mother has a history of recurrent miscarriages

For couples with heritable genetic disorders

Preimplantation genetic diagnosis can be used to determine if embryos produced through in vitro fertilization carry a gene mutation associated with a specific genetic disorder, such as cystic fibrosis or muscular dystrophy.

The benefit of preimplantation genetic diagnosis is that the diagnosis can be made before the embryos are transferred to the uterus and a pregnancy is established. Embryos unaffected by the genetic disorder can be selected for transfer to the uterus, therefore greatly reducing the risk that a couple will pass a genetic disorder onto their child.

Couples who are at high risk of having a child with a severe genetic disorder may choose preimplantation genetic diagnosis for many reasons, including:

- Previous loss of a child from the genetic disorder
- Previous pregnancy adversely affected by the disorder
- Objection to terminating a pregnancy affected by the condition
- Couple has a relative with the disorder

For couples with chromosomal disorders

Preimplantation genetic diagnosis is also offered to couples when one partner has a chromosomal abnormality, such as an unbalanced translocation or aneuploidy. If the abnormality is present in the embryo, the condition could ultimately prevent embryo implantation, leading to pregnancy loss, or result in the birth of a child with congenital malformations (physical problems) or mental retardation.

The benefit of preimplantation genetic diagnosis is that the diagnosis can be made before the embryos are transferred to the uterus and a pregnancy is established. Embryos unaffected by the chromosomal abnormality can be selected for transfer to the uterus, therefore greatly reducing the risk that the pregnancy will be adversely affected by the chromosomal abnormality.

Couples who are at high risk of having a child with a chromosomal disorder may choose preimplantation genetic diagnosis for many reasons, including:

- Previous pregnancy loss due to a chromosomal abnormality
- One partner carries a known chromosomal abnormality
- Multiple pregnancy losses after spontaneous conception.

Genetic counseling is an important step to determine if preimplantation genetic diagnosis is an appropriate option for a patient. For couples undergoing IVF who are concerned that their child may inherit a genetic disorder or chromosomal abnormality, genetic counselors are available to discuss options and can advise patients on how raising a handicapped child may affect a family.

Preimplantation genetic diagnosis is available for couples undergoing IVF.

The steps of the IVF process include:

- Medications are used to suppress a woman's natural menstrual cycle.
- Her ovaries are then stimulated with medications to produce multiple follicles, each of which may contain an oocyte (egg).
- The eggs are retrieved from the woman's ovary by a needle placed in the vagina.
- In the lab, the eggs are combined with the male partner's sperm in a special culture medium that allows fertilization and the growth of high–quality embryos.

Embryo biopsy may be performed after 3 days of culture in the laboratory. The embryos are typically 8-cell embryos on Day-3 and the process involves the removal of one to two cells.

After the biopsy and following receipt of the results from the genetic/chromosomal testing, embryo(s) of the best quality that are not affected by the genetic disorder or chromosomal abnormality) are selected for transfer to the uterus. For day 3 embryo biopsies, the embryo is usually transferred "fresh" following two additional days of culture in the laboratory (Day-5 embryo transfer).

In some cases, the biopsy will be done on either Day-5 or -6 (trophectoderm biopsy). At this stage, the embryo consists of many cells and is called a blastocyst. Cells are removed from the outer layer of cells called the trophectoderm.

Following the biopsy of a good quality blastocyst, the blastocyst is then frozen. When the patient receives the results from the genetic testing, the non-affected or chromosomally normal blastocyst(s) are thawed and transferred in a subsequent frozen embryo cycle.

Embryos are analyzed by one of the techniques described below:

Testing for heritable genetic disorders

Polymerase Chain Reaction (PCR) is performed on the biopsied cell(s) to determine the presence of a single gene. This is done when a couple has a significantly increased risk of conceiving a child with a severe genetic disorder. When PCR is to be performed, the cell(s) obtained at biopsy is loaded into a tiny tube of medium and sent to for analysis. The specific area of DNA of interest is amplified by making thousands of copies of the DNA through repeated cycles of DNA strand separation and replication. The sample can be analyzed for the presence of a specific sequence of DNA or gene and also for linkage markers near the gene. The biopsied cell(s) are destroyed during this process. Therefore, they cannot be used for another purpose or returned to the embryo.

Testing for chromosomal abnormalities

The genetic material (DNA) within the biopsied cell(s) is amplified using a technique called the polymerase chain reaction (PCR). This amplification produces enough DNA to use a second technique, known as array comparative genomic hybridization (aCGH). Array CGH assesses the amount of DNA derived from each chromosome, revealing whether or not there are both a normal amount and correct number of chromosomes. The biopsied cell(s) are destroyed during this process. Therefore, they cannot be used for another purpose or returned to the embryo. aCGH can be used to screen for numeric abnormalities in all chromosomes and/or known rearrangements of chromosomes (translocations). Array CGH does not detect all types of chromosome aberrations or genetic mutations and cannot distinguish between no translocation present and balanced translocation present.

The results of preimplantation genetic diagnosis are reported to the couple no later than the morning of their scheduled day for embryo transfer. Typically this is five days after oocyte retrieval and in vitro fertilization are performed. Of the embryo(s) that are not affected by the genetic disorder or chromosomal abnormality, the best quality embryo(s) are selected for transfer to the uterus. If additional unaffected and good-quality embryos are available, they may be cryopreserved for a future embryo transfer.

Preimplantation genetic diagnosis does not replace prenatal testing, such as chorionic villus sampling or amniocentesis. Preimplantation genetic diagnosis provides diagnostic

information based on the analysis of a *single* cell. Therefore, prenatal testing is still recommended and currently remains the standard of care.

Blastocyst Transfer

A blastocyst is an embryo that has been developed in the laboratory for five or six days after insemination, in contrast to conventional IVF which involves transferring embryos to the womb two to three days after egg collection and insemination (this is referred to as a Day 2 or 3 transfer).

- With a blastocyst transfer, the embryo has advanced to the five or 6-day stage. This means the embryo has divided many more times into many more cells over this period. Blastocysts have a very thin outer shell thus potentially increasing the chances of implantation into the uterine cavity. Most of the blastocyst contains a fluid cavity and it is possible to see the cells which will become the baby and those which will make up the placenta.
- The cells in a blastocyst have just started to differentiate
- The surface cells that surround the cavity (just under the outer shell) are called the trophectoderm and will later develop into the placenta
- A more centrally located group of cells - the inner cell mass, will become the fetus

While the majority of fertilised eggs will develop into a three-day old embryo, only perhaps 40% of these embryos will develop into a blastocyst. Therefore, blastocysts are considered to be a more "select" group of embryos with a higher chance of pregnancy.

The 5 or 6 day blastocyst is a much more advanced structure than the 3 day old embryo and the real advantage of transferring blastocysts is the high live birth rate associated with blastocyst transfer.

Why Blastocyst Transfer

Selection of embryos

We know that at least 50% (or higher in women over 40) of embryos are not viable, and many of these arrest their development before the blastocyst stage. A large proportion of these embryos have a chromosome or genetic defect and it is believed that those embryos that failed to develop to the blastocyst would not, in any event, have established a pregnancy. Where there are large numbers of good quality embryos available at the blastocyst stage these can be frozen. DRIFF has excellent post thaw survival and pregnancy rates with frozen blastocysts.

Extended in-vitro culture systems

- In the past, it was difficult to get high quality blastocysts with in vitro culture systems - unless "feeder" cells were utilized - called coculture.

- However, since 1998 more advanced culture media have been commercially available that (if used properly) can yield high blastocyst formation rates.
- Now blastocyst embryo transfer is a viable IVF treatment option for many couples.

Embryos are transferred to the right place at the right time

Some researchers believe that the conditions in the womb may be more optimal for a blastocyst than a day 2/3 embryo as there are slightly differing conditions in the fallopian tube and the womb on day 2/3.

Higher pregnancy rate in women having Blastocyst Transfer

Data suggests that blastocyst transfer can increase the chances of a live birth but it needs to be remembered that each couple must be considered independently.

Confirmation of development to the blastocyst stage

Our specialists believe that for those patients who have recurrent failure of implantation, extended culture gives an opportunity to examine the embryo quality over a longer period. If the embryos arrest or become fragmented this may help to clarify a potential problem.

Specific situations where blastocyst transfer is applicable

Where single embryo transfer is specifically indicated (eg. previous history of multiple pregnancy, patient preference, uterine anomaly etc) blastocyst transfer may be a particularly useful option.

Extended culture and Blastocyst Transfer with Frozen Embryos

Some patients have large numbers of frozen embryos and it can difficult to know which have the best potential for pregnancy. An option in these cases is to thaw all embryos and culture through to the blastocyst stage to allow the best 1 or 2 embryos to be replaced based on development.

The main benefit of the blastocyst transfer approach is in the ability to discriminate between different embryos in terms of their quality and implantation potential. It is essential to understand that the extended culture process doesn't enhance an embryos quality per se; it is principally a method for choosing the 'front runner' or 'runners' from a group of embryos.

It is important that we carefully choose the patients who are most likely to benefit.

Our general guideline is to proceed to blastocyst transfer only if there are 4 or more good quality embryos available on day 3.

EMBRYOSCOPE, INNOVATIVE TECHNOLOGY FOR YOUR DREAM

The EmbryoScope™ is an IVF incubator with a built-in camera for automated time-lapse imaging of fertilized oocytes in a safe incubation environment from conception until the time of transfer. Preliminary studies showed that it can increase up to 20% the chance of success of assisted reproductive technology. Definitely one of the key factors in the success of a medical assisted procedure is the opportunity to continuously follow the stages of embryo development and, therefore, to choose the most appropriate time for its implantation in the uterus, with the best guarantees for a peaceful pregnancy.

Embryoscope ™ is one of the most technologically advanced and innovative devices available to the assisted reproduction centers. It is a system that integrates a multi-gas incubator, a microscope with an integrated camera shooting continuous image and an advanced software for the acquisition and subsequent analysis of all data relating to the development of embryos. It's the only instrument in the world documenting with this flexibility all stages of the process, automatically, without opening the incubator: it therefore operates in a completely safe and non-invasive manner. The characteristics of this device allow the embryologist constantly monitoring the conditions of the embryos and to act in the best way to ensure the highest chance of success. The Embryoscope also opens new and important perspectives from the point of view of clinical research. Its ability to store in a continuous and unique manner of the morphological characteristics of the embryo during every stage development, allows developing more precise and effective criteria for evaluating the potential implantation of individual embryos.The next evolution of the instrument will be able to create a predictive algorithm, which will provide the embryologist with further and useful indications to choose the embryo to implant.