



Infertility National Public Awareness Campaign

## **Information Sheet**

### **Infertility Treatment**

#### **1: Intra Uterine Insemination (AIH and IUI)**

Artificial insemination of sperm is usually performed by placing a sample into the cervix (neck of the womb) or into the uterus (womb) itself.

##### ***Intra-cervical insemination of partner's sperm (AIH)***

This is a treatment option that has been used for many years. However, the indications for inseminating the unprepared (whole) sample into the cervix are few. In couples having normal intercourse there is no evidence that AIH increases the chances of conception. This treatment is best reserved for those couples in whom there is a physical problem getting the sperm to the right place. It is not helpful for couples in whom the male partner has a low sperm count or abnormal sperm function.

##### ***Indications include:***

1. Anatomical problems on the male side such as congenital abnormalities of the penis. An example of this is severe hypospadias where the ejaculate appears at the base of the penis instead of the tip. Injury to the penis can, on occasion, prevent normal penetration and ejaculation. Retrograde ejaculation is a condition where the spermatozoa and seminal fluid end up in the

bladder due to congenital or sometimes iatrogenic causes. This then requires the sperm to be separated from the urine and inseminated into the female partner or used on an IVF program.

2. Conditions preventing normal erection and ejaculation, whether psychological or organic, are best resolved by correct diagnosis and treatment of the underlying condition. However, where this is not possible and the male partner can produce a sample by masturbation, AIH has a place in the management.

3. Psychosexual problems on either side are best treated with appropriate counseling. An example of this is vaginismus in the female partner – muscles around the vagina can contract involuntarily making normal intercourse impossible. However AIH is available as a last resort.

4. In some situations it is necessary to freeze semen samples in liquid nitrogen and store them for AIH in the future. An example of this would be prior to chemotherapy or radiotherapy for a malignant condition on the male partner's side, as this treatment can render men sterile. If the male partner is away from home, perhaps due to work commitments, AIH using cryopreserved samples can help to expedite pregnancy if this is thought desirable.

In order to perform AIH the male partner must first produce a semen sample by means of masturbation. If this is not possible, samples can be collected into medical sheaths (containing no spermicide) during sexual intercourse but this method is not ideal. It is usual to advise at least three days sexual abstinence prior to collecting a semen sample as this ensures that the sperm density is optimal. The sample is collected into a clean plastic container either at the clinic or else produced at home and delivered within 1-2 hours.

Occasionally a patient may be asked to produce a split ejaculate that is to collect the first couple of drops in one container and the rest in a second pot. The reason for this is that the best quality sperm appear in the first part of the ejaculate, which can then be used whole for AIH. The simplest method is to perform a speculum examination and introduce the sample into the cervix with a syringe and plastic quill. This should be done on the day of ovulation as timed by cycle length, temperature chart, kits that test for a surge of luteinizing hormone or ultrasound scans. The placement of semen into the cervix needs to be done by a medical practitioner or nurse.

### ***Intra-Uterine Insemination of partner's sperm***

Intra-Uterine insemination (IUI) is the technique of placing partner's sperm directly into the uterus.

IUI is used in cases of poor cervical mucus, unexplained infertility and some cases of male factor infertility. There is little evidence that pregnancy rates are improved unless fertility drugs are

used prior to IUI. However, it is an option which may be considered before advanced procedures such as IVF and GIFT. Full semen assessment should be performed to determine whether the sperm are suitable for the IUI technique. The analysis documents the sperm count and motility, whether the sperm are normally formed, sperm survival over a period of time and the presence of anti-sperm antibodies. A minimum of 5 million motile sperm are necessary if IUI is to be successful. A semen culture may be performed to exclude the presence of infection prior to IUI.

Semen consists of two elements – the sperm cells themselves and the liquid (seminal plasma) which makes up most of the ejaculate volume. Seminal plasma contains prostaglandins which cause painful uterine cramps and must be ‘washed’ or removed before IUI. Washing is followed by an incubation step after which the motile sperm are carefully removed and used for the insemination.

The sperm are placed into the uterus with a thin plastic tube which is passed through the cervix. The procedure is usually painless and requires only a few minutes. About 10% of women will experience temporary menstrual like cramping. After the procedure all normal activities can be resumed. IUI is usually repeated for 3 cycles, after which the treatment strategy should be reevaluated. The cycles do not have to be consecutive to be effective.

Sperm washing removes most of the bacteria from the ejaculate but neither the sperm nor the cervix can be truly sterilized. There is always the slight chance the IUI could produce an infection in the uterus called endometritis. Experience has shown this to be an uncommon occurrence with a risk less than 1%. Symptoms of an infection include lower abdominal pain, fever, vaginal discharge or continued vaginal bleeding or spotting. Mild cases can be treated with oral antibiotics but moderate or severe infections require hospitalization for treatment with intravenous antibiotics.

Occasionally even after washing, the sperm may irritate the pelvis and mimic an infection. This may cause discomfort and bloating but there will be no fever and no elevation of the white blood count.

### ***Super ovulation and IUI***

IUI can give better pregnancy rates than normal intercourse when combined with ovarian hyper stimulation even in normally ovulating women. These ‘fertility drugs’ (either tablet or injection) are advantageous because they can stimulate the ovaries to produce more than one egg each cycle and increase the estrogen and progesterone levels to maximize the chance of pregnancy.

However, multiple pregnancies may occur in up to 20% of cases. Ultrasound scans are often used to identify the number of follicles developing in the ovary and thereby to assess the number of

eggs likely to be released. Treatment cycles should be abandoned if more than 3 or 4 eggs are developing, in order to avoid the risk of a multiple pregnancy. Once the leading follicle is 18-20mm and the lining of the womb (endometrium) is suitably thickened, an injection of human chorionic gonadotropin (HCG) may be given to mimic the surge of luteinizing hormone and trigger ovulation.

IUI following ovarian stimulation is usually performed for 3 cycles after which time the treatment strategy is re-evaluated.

Pregnancy rates achieved by AIH will obviously vary according to the underlying cause of the infertility. In patients where the cause is anatomical, a pregnancy rate of 20% per treatment cycle could be expected. Where there is a problem with the quality of the sperm, the chance of pregnancy will drop to 10% per cycle even if IUI is performed following ovarian stimulation. Of course, in cases where the sperm count is very low, AIH may never achieve a pregnancy and in these cases in vitro fertilization (IVF) and embryo transfer may be suggested.

Normally these methods of assisted conception can be a source of stress for both partners. It is often difficult to estimate the chance of success and the treatment may need to continue over several cycles. Some couples find that a chat with an independent counselor or involvement with an infertility support group is helpful.

## **2: Gamete Intra Fallopian Transfer (GIFT)**

Louise Brown, the first baby conceived by in-vitro fertilization (IVF) was born in 1975. Since this time, IVF has become a widely accepted form of treatment for a number of different infertility problems and is practiced in infertility units throughout the World. The GIFT (Gamete Intrafallopian Transfer) technique has been a widely accepted alternative form of treatment for certain groups of infertile patients since the mid-1980's, but is now no longer practiced in most IVF Centers worldwide. GIFT can be considered to be both a sophisticated form of artificial insemination and a simplified form of IVF.

GIFT can only be used to treat patients who have one or more open and normal fallopian tubes. Patients with blocked fallopian tubes or pelvic adhesions are not suitable for this technique and must rely on IVF or tubal surgery. A group of patients who do particularly well with GIFT are those with idiopathic (unknown cause) infertility, who account for about 20% of all infertile patients. GIFT is also used in couples whose infertility is caused by sperm dysfunction, but it is

not suitable for the most severe forms of male factor infertility. Some patients are recommended to try IVF first, in order to prove the ability of the husband's sperm to fertilize the wife's eggs, and if this occurs, but pregnancy is not achieved, then in subsequent treatment cycles GIFT may be an alternative to repeat IVF.

### ***Preparation for a Treatment Cycle***

At a couple's initial consultation, all aspects of treatment will be discussed with couples and an indication as to whether they are suitable or not for the GIFT procedure will be reached. If GIFT is being considered, it may be necessary before proceeding to a treatment cycle to carry out an assessment laparoscopy in order to be certain that the fallopian tubes are open and functioning normally and that there are no other hitherto abnormalities in the pelvis. In some cases, it may be sufficient to carry out an hysterosalpingogram (HSG; an x-ray of the uterus and tubes) in order to show that the tubes are fully open. Once the preliminary investigations have been completed, then the patient will be ready to proceed to a GIFT cycle.

### ***Management of a GIFT treatment cycle***

The management of a patient in a GIFT treatment cycle is usually exactly the same as for an IVF cycle, up until the point at which the oocytes (eggs) have been recovered. There are a number of different drug regimes that are used to stimulate the ovaries to produce multiple oocytes, and these are always discussed with the couple at the time the treatment is started.

The growth of the ovarian follicles in which the eggs are developing is always monitored with a combination of serial ultrasound scans and often blood or urine tests. When the follicles are considered to be mature enough, arrangements are made for the patient to receive an injection of Human Chorionic Gonadotropin (HCG) and the egg recovery procedure is planned some 34-36 hours later.

Most clinics now will recover the oocytes by the transvaginal ultrasound directed technique, as for IVF. Some clinics will, however, recover the eggs laparoscopically. Once the oocytes have been collected and identified, the best two or three are selected. A preparation of the husband's sperm is taken into a fine catheter, together with the eggs, and the tube is gently inserted under direct vision through the laparoscope into the outer ends of one or both fallopian tubes and the egg/sperm mixture is injected into the tube(s). At the end of this procedure, the patient is returned to the ward, where she will recover and will go home the same day. Most patients are given either injections or pessaries of the hormone progesterone and some 15 days later a pregnancy test is carried out to determine whether there is early evidence of a pregnancy.

### ***The Success Rate of GIFT***

GIFT is generally found to be slightly more successful than IVF in most clinics. This is probably because the fallopian tube is a more physiological environment for fertilization to occur in than in a laboratory culture dish. However, the procedure does require a laparoscopy, and most clinics now do not generally believe that the extra inconvenience of and potential discomfort of a laparoscopy makes the slightly increased pregnancy rates worthwhile. If a clinic is achieving good results with IVF, then they will generally not do GIFT. Clinics achieving poorer success rates with IVF may find that GIFT is considerably more successful and therefore recommend it.

Pregnancy rates for IVF are commonly quoted as being in the range of 15-30% and for GIFT, between 25-30%. The success rates for both procedures vary widely from unit to unit and depend, to a large extent, on the types of patient who are accepted onto the program. Couples considering GIFT should always discuss the pros and cons of this procedure against other treatment options with the medical and nursing staff of the unit they are attending.

## **3: In Vitro Fertilization and Embryo Transfer (IVF-ET)**

### ***Introduction***

Conception depends on a woman releasing an egg each month. The egg enters the fallopian tube where it meets the sperm. A sperm cell penetrates the egg, a process known as fertilization. The resulting embryo is transported down the tube to the uterus where it implants into the uterine lining (endometrium) a few days thereafter.

In general 75% of couples will achieve a spontaneous pregnancy within six months of exposure, 90% by a year and 95% by two years. Three major factors determine the chances of a natural conception; female age, sperm quality and duration of exposure.

IVF involves four basic steps; ovarian stimulation, egg recovery, insemination and finally embryo replacement.

Couples with less than two years exposure, need only consider IVF with proven tubal disease, significant semen abnormalities and moderate to severe endometriosis. Couples with unexplained infertility of less than two years, in women less than 35, need only consider IVF after two or more years of trying and after a year in women older than 35. Minimal endometriosis is unlikely to be the cause of a delay in conception.

Although IVF was originally devised for women with tubal damage or dysfunction, in combination with intracytoplasmic sperm injection (ICSI) is a very effective method to treat male factor infertility. With ICSI a single sperm is selected by the biologist and injected into the egg. Surgical sperm retrieval may also be necessary with IVF/ICSI in men with absent or obstructed ducts. IVF treatment may be used in selected instances with donated eggs, sperm or embryos.

Selection of an IVF clinic, the published live birth rate per cycle started, at various clinics, should be treated with great caution.

Patient selection ultimately determines the live birth rate for IVF. Couples need to be aware of the population treated by a center as those able to select younger women with a short duration of infertility having their first treatment cycle, are more likely to report better results

It is important to establish the cause of infertility before proceeding to IVF. Investigations would usually include checks of ovulation, tubal patency and an ultrasound for the female partner. A semen analysis is required for the male partner.

## **Management of an IVF/ET cycle**

### ***Ovarian stimulation***

During a natural unstimulated cycle, a follicle containing a single egg develops to maturity and it is therefore necessary to stimulate the ovaries with a group of drugs known as gonadotropins. The clinic should give you written information about these drugs and their side effects. Your Doctor can also explain how each drug works and their side effect.

Ovarian hyperstimulation (an excessive response) is the major and potentially life threatening complication associated with gonadotropins. Couples should consider treatment in centers with low rates of OHSS. It is safest to monitor the response of the ovaries by daily estrogen measurements and ultrasound. Ultrasound scans are used to see the number and size of the follicles and to judge when to do the egg recovery. The estrogens are necessary to determine the ovarian response to stimulation. By interpreting the results of ultrasound and estrogen, the specialist will determine the best time to perform the egg collection. About 36 hours before the egg collection is due, an injection of human chorionic gonadotropin (hCG) is given to initiate the final process of egg maturation. Precise timing is necessary as the eggs will be suitable for recovery 34 to 36 hours after the hCG injection.

### ***Egg collection***

This is done under sedation or general anesthetic using a vaginal ultrasound probe. A needle is guided through the top of the vagina into the ovary. Each follicle is aspirated through the needle using a suction device.

### ***Insemination and fertilization***

The eggs are identified in the laboratory and placed in culture medium. They are then placed in dishes in an incubator. The male partner produces a semen sample by masturbation and this is prepared in the laboratory. A number of motile sperm are extracted and used to inseminate the eggs some hours later. It takes about 18 hours for fertilization to be completed and about 12 hours later the embryo starts to divide. Two or three days after egg collection, when the embryos have reached the 2 - 6 cell stage, they are ready to be replaced into the woman's uterus.

### ***Embryo transfer***

This is a most important step and is best performed under ultrasound guidance. The procedure is virtually painless. A maximum of two embryos may be replaced. Couples should consider the replacement of a single embryo to prevent a twin pregnancy. There is no evidence that bed rest makes a difference to the outcome and most units' recommend resuming normal activities.

### ***Luteal support***

Hormone supplementations in the form of hCG injections, progesterone pessaries or injections are usually recommended after embryo transfer to support the uterine lining.

### ***Embryo freezing***

Most IVF clinics offer embryo freezing and storage for spare embryos. However, not all surplus embryos are suitable for freezing, not all survive the procedure and the implantation rate after transfer is lower than with fresh embryo transfer.

### ***Abandoned cycles***

The abandoned cycle rate varies considerably between different units. Cycles may be abandoned before egg recovery as the ovarian response is either inadequate or excessive, and before embryo replacement if no eggs are recovered or the eggs fail to fertilize or the embryos don't divide. In many instances it will be possible to try again using alternative drugs or methods e.g. ICSI for failed fertilization.

Occasionally IVF cycles are abandoned because of a high risk of ovarian hyper-stimulation. The risk of ovarian hyper-stimulation is increased in women with polycystic ovaries). In these circumstances the cycle may be cancelled during ovarian stimulation and restarted with a lower dose of drugs or allowed to proceed but all the embryos are frozen and replaced when the ovaries have returned to normal. The ovarian hyper-stimulation syndrome is potentially life threatening. This syndrome occurs in about 2 to 3% of cases and consists of severe nausea and vomiting, a rapid gain in weight, abdominal swelling and shortness of breath. The fluid and electrolyte imbalance needs careful management in experienced centers. It starts a week after the hCG injection and is made worse by pregnancy.

It is well recognized that undergoing assisted conception treatment, particularly IVF, is stressful both emotionally and physically. It is essential that patients fully understand the proposed treatment program and the commitment in time required for monitoring the cycle. Most clinics have information sheets and some have support groups which help in times of stress. All licensed centers are obliged to offer independent counseling to patients considering IVF treatment. This may prove helpful as it gives the opportunity for you to discuss your infertility and treatment confidentially with an impartial person.

#### **4: Drugs used in treatment**

Do you know enough about the drugs used to stimulate your ovaries when you go through a cycle of assisted reproduction? Do you know which drugs are currently available, and what are the differences are between them?

Patients should have access to up to date information about every aspect of their treatment, and that includes any drugs which may be prescribed. Some centers will give one drug or another without any discussion on the subject, whilst others may discuss treatment options with you and offer a choice in the drugs prescribed. But do you feel you know enough to make a decision?

The drugs used to stimulate the ovaries during IVF are mainly female hormones. Human eggs are contained within follicles which grow from microscopic size to large cystic structures as they mature. The principle stimulant is Follicle Stimulating Hormone (FSH) which makes follicles grow, and it is usually administered daily to create raised concentrations (high / normal) in the blood. High concentrations of FSH in the blood lead to the growth of all follicles that are available to grow (sensitive to FSH), with the aim of producing a number of eggs in just one cycle. There are occasions where the other main drug of this type (luteinizing hormone, LH) is

also required – and this can be supplied in two formats: directly in a pure form or in a form combined with FSH.

### ***Drugs used in fertility treatment***

MENOPUR - is a highly purified urinary product comprising both FSH and LH activity. It is injected subcutaneously (just under the skin) using a small needle. Patient injection kits are made available by the manufacturers that help with administration.

MERIONAL HP - A similar product to Menopur

GONAL F – A recombinant FSH which is injected subcutaneously, and comes ready prepared, in a multi-dose administrator by use of an injection pen. It is administered by mass (weight) rather than as ‘international units’.

PUREGON - A recombinant FSH, injected subcutaneously. Puregon comes in a ready-for-use solution. A multidose cartridge is also available and both are administered via an injection pen. The product is marketed in multiples of 50 IU.

All these hormone drugs are administered once per day, as they are quite efficiently cleared in the urine.

ELONVAR - Elonvar is a new recombinant (created) form of FSH which lasts much longer in the blood than the other forms. One injection lasts for 5 to 7 days. The initial concentrations are high, and it tends to recruit the maximum number of follicles available. It is very convenient and effective, but care should be taken in women with a high ovarian reserve.

It is important to note that the concentrations of these hormones in your blood are generally similar to normal levels found in women with normal menstrual rhythm, even when higher doses are given, and much lower than in a menopausal woman. Administration of the drugs over a number of days does however, lead to high estrogen concentrations for a few days, and so the responses do need to be monitored. However, as the drug concentrations are normal, the process has generally a low risk potential, when monitored sensibly.

### ***Other drugs commonly used***

Unfortunately, FSH is not the only drug needed, as most programs use other hormones called GnRH analogues, which are required to limit the fluctuations of LH, and they can be used in two different ways. They are used to stop the process of ovulation (release of the eggs) happening before the eggs can be collected. The traditional, well established method is using GnRH agonists in multi-dose or in depo formulations. It was found that when given in a high frequency

(say 4 or 5 times per day, or in a depo under the skin) these drugs block the release of LH, which is the hormone that causes release of the egg from mature follicles (ovulation). The 'ovulation' process must be timed precisely so that eggs can be collected at the right time directly from the ovary.

The ovulation blocking effect of GnRH-agonists needs about 2 weeks continued treatment to work effectively, so these drugs are started either a week before a menstrual bleed (in the cycle prior to treatment), or soon after a menstrual bleed, and the FSH injections are delayed for 10 to 15 days later. The GnRH agonists can be administered by daily injection (eg Suprecur s.c.), multiple nasal spray applications each day (Suprecur n/s, Nafarelin), or by a single depo under the skin that lasts for approximately one month (Prostap SR, Gonapeptyl s.c.).

The newer versions of these drugs are the GnRH antagonists, which require no pre-treatment as their blocking action is immediate, and they can suppress LH very rapidly. They are needed only when there is a threat of increased LH in the blood, which generally starts around 5 days after starting FSH injections. They generally require daily administration until it is time to stop the FSH treatment and prepare for the egg pickup. The two main products of this type are Cetrotide (0.25mg daily, but also available in a 3.0mg preparation, which lasts for 3 days) or Ganirelix (0.25mg).

***When discussing these drug packages with your consultant, what would you want to take into consideration?***

Most analyses of effectiveness of the different products in routine IVF programs, show that, in general, no individual drug or method is significantly more effective than another. However, there are circumstances where some combinations would not be recommended, eg using the high dose (3mg) GnRH Antagonist with 'pure' FSH.

If you want to know more about the drugs you've been prescribed, you shouldn't feel nervous about asking the medical team who are treating you, talk to them about which drugs they recommend, and why. The majority of patients may be perfectly happy to follow their consultant's suggestions, but just knowing more about what you are taking and why may help you to feel more in control of your treatment.

For advice on fertility drugs being used in their treatment cycle or the administration of these drugs, patients should always contact the clinic where they are being treated.

# INTRA-CYTOPLASMIC SPERM INJECTION (ICSI) and SPERM RETRIEVAL TECHNIQUES

## Background

For people relatively new to the field of In Vitro Fertilization (IVF) the term “Micro-Assisted Fertilization” may seem a little strange. This term is still used by some centers, but the technique it now refers to has the acronym ICSI, which stands for Intra-Cytoplasmic Sperm Injection. The substance of the egg is called cytoplasm, and intra-cytoplasmic sperm injection as the name implies, is the injection of a single sperm directly into the cytoplasm of the egg. This procedure bypasses all the natural barriers that the sperm has to encounter. It has been used by animal embryologists since the late 1950s as a tool to understand the mechanisms of fertilization. In recent times it was developed to procure conception and pregnancy, which resulted in live offspring in domestic and laboratory animal species. However, scientists were reluctant to use the ICSI approach for the treatment of infertility because it is so invasive. Instead, a range of other procedures which involve by-passing the outer shell of the egg, but not penetrating the egg itself, had been developed - these have been given acronyms such as “PZD” and “SUZI”, but ICSI has now replaced them all.

The first clinical success of ICSI, in 1992, led to worldwide adoption of this procedure as the ultimate micro-assisted fertilization approach. ICSI as it is now recognized as a treatment of choice for at least 30% of all couples requiring IVF technology.

Patients attending a center for ICSI specifically may wish to enquire of the experience of the ICSI practitioners; they may wish to obtain evidence of the clinic/practitioners particular experience.

## ***Who requires ICSI?***

There are two main groups of patients that may require ICSI:

- (i) Patients who have a severe sperm problem, which prevents them even attempting conventional or a modified form of IVF.
- (ii) Patients who have previously attempted IVF but have failed to achieve fertilization (generally on more than one occasion).

In group (i) patients can be further subdivided into routine, complicated and surgical.

### ***Routine***

In this classification we have patients with a very low sperm count, very poor motility or a high percentage (greater than 95%) of sperm with “abnormal” shape (morphology). However, the majority of men have a combination of these problems often associating all three.

### ***Complicated***

Amongst this group of patients there is a range of difficult conditions which include men who do not appear to ejaculate any sperm (cryptozoospermia). In this situation we are able to use modified methods of sperm preparation where we can concentrate the seminal plasma into a volume the fraction of a teardrop. Often we find just a few sperm, literally just as many (or sometimes less) as there are eggs. However, we only need one sperm per egg for ICSI. Other conditions include men who have a range of different abnormalities in all their sperm; for example, a condition called globozoospermia, where the top half of the sperm head (the acrosome) is missing; or a condition in which all the sperm are immobile. In this latter condition, many of the sperm may be living, but the tail is unable to function. We now have methods of selecting the living sperm from the truly dead sperm. By this procedure we can individually select the living sperms and use them for ICSI.

There is also a large group of men who, for various reasons, have problems obtaining an erection and ejaculating. This includes men with spinal cord injuries, Hodgkin’s disease, diabetes and numerous others. There are a number of ways to approach this problem and one of which is a procedure using electrostimulation. In this procedure a probe is inserted through the rectum which provides gentle stimulation to the nerves which bring about ejaculation. Often millions of sperms can be recovered by this procedure, but their quality is too poor for anything other than ICSI.

However, by utilizing the ICSI procedure the technology is extremely successful. Depending on the individual’s situation, anesthesia may or may not be required. Should it be difficult to obtain sperm by electrostimulation, a surgical procedure could be utilized.

### ***Surgical***

A number of conditions fall into this category and these include obstructive azoospermia - where sperm is manufactured in the testicles but there is an obstruction in the reproductive tract preventing the release of sperm - congenital absence of the vas-deferens (the tube that carries the sperm from the testes to the penis) - in which some men were born without either vas-deferens - and failed vasectomy reversal. In these cases sperm can be recovered from the network of tubing called the epididymis.

The epididymis are attached to the testicle and act as the immediate reservoir for sperm before they are transferred into the vas and ejaculated. Sperm can be recovered from the epididymis either by inserting a needle across the skin, which does not require surgery, this procedure is called Percutaneous Epididymal Sperm Aspiration (PESA); or, but now less common, a procedure called MESA (Micro-surgical Epididymal Sperm Aspiration) can be used. There are a number of cases where it is impossible to recover sperm from the epididymis, and occasionally this arises even though doctors have attempted the PESA or MESA approach in the first instance. It is now possible to recover sperm directly from the testicle either by using a needle to aspirate the sperm (TESA - Testicular Sperm Aspiration), or by doing a biopsy of the tubes (Seminiferous Tubules) that actually manufacture the sperm (TESE - Testicular Sperm Extraction).

Therefore, providing sperm is being manufactured in the testes, it can be obtained either from the ejaculate, the tubes leading from the testes or from the testes themselves, depending on the individual condition. If a mature sperm is obtained by any of these procedures, using ICSI means there is a chance to have a baby, even in the so called “impossible” situations.

Some couples have been classified as “unexplained” infertility because of the tests available have not found a significant problem on the female side or with the sperm. However, a considerable number of these patients (somewhere in the region of 20%) will have a fertilization problem. This can be overcome by using ICSI. There are also a range of rare conditions with the male, which produce unusual sub-microscopic defects in sperm preventing fertilization. It is possible that these also can be overcome using ICSI. In some couples there may be a problem with the outer shell of the egg, which prevents sperms either attaching to or penetrating the outer shell. Sometimes it is difficult to evaluate this condition, and it is paramount to ascertain whether the failed fertilization is related to an egg or sperm problem. Patients should possibly consider a crossover donor sperm insemination for diagnostic purposes only. The use of donor sperm with the unfertilized eggs would give us an indication as whether or not it was an egg or sperm problem. This provides maximum information and does not waste opportunities for gaining knowledge. This is very important as doctors need to assess whether ICSI or egg donation should be the considered route in the future.

### ***The Procedure***

The woman is stimulated for follicle (the tiny sack in the ovary which carries the egg) production, as in conventional IVF. Egg recovery is identical to that for routine IVF, but sperm treatment differs according to individual patient circumstances and, even if obtained from the ejaculate, often requires a modification of the procedures used for IVF. This modification uses a high centrifugation method to concentrate the contents of the seminal plasma into literally a tiny fraction of a teardrop from which just a few sperm can be obtained and extracted. In certain circumstances we might use our micro-injection needles (approximately 12 times thinner than a

single strand of human hair) to isolate single sperms from the seminal plasma. Whichever method is used, centrifugation or single sperm isolation, it is essential that the sperm is washed free of the seminal plasma.

The human egg aspirated from the follicle is surrounded by thousands of specialized cells - the cumulus cells (these perform a “nursing” role while the egg is in the follicle). These cumulus cells are removed by treatment with an enzyme (hyaluronidase) - a natural enzyme that is produced by the sperm during its passage through the cumulus cells whilst in the fallopian tube, in less than a couple of minutes the enzyme digests away the cells leaving the egg encased in a few layers in another type of specialized cells. These are mechanically removed by the embryologist using gentle suction into a very finely-pulled glass tube. The egg denuded of almost all its surrounding cells is then accessible for ICSI.

The ICSI procedure per se begins by first immobilizing the sperm. This is often performed by transferring the sperm into a viscous solution, which dramatically slows down its motility. In its sluggish state the single sperm has its tail permanently immobilized - this has been shown to be an extremely important part of the process.

Sperm is then aspirated into the tiny micro-needle and carefully maintained at its tip. The microinjection needle is manipulated using a micro-manipulator which has extremely fine control capabilities. The egg itself is held onto another micro-tool by gentle suction to keep it firmly positioned. The micro-needle containing the sperm is pushed gently up against the outer shell (zona pellucida) and carefully pushed through the shell, through the outer membrane of the egg and directly into the center of the egg itself, i.e. the egg’s cytoplasm.

Once the needle is inside the egg, a tiny amount of cytoplasm is aspirated into the micro-needle to mix with the sperm and ensure that the egg has been properly penetrated. Despite the tiny size of the egg (approximately 7 times smaller than the average full stop), the membrane is a very elastic structure and can be extensively stretched without actually being ruptured (I often liken this to poking a finger into a balloon - you can touch the other side but not rupture the membrane). Once the embryologist is certain that the egg has been penetrated, the sperm and cytoplasmic mixture is injected back into the egg. This procedure rarely causes residual damage to the egg, and has no lasting effects on further development. The whole procedure is performed under a high powered microscope.

In some cases, immotile but living sperm is being used. It is therefore important for the embryologist to be able to distinguish between dead and living immobile sperm. In this situation a solution is used which causes the tail of a living but immobile sperm to curl. The curled tail indicates that the sperm is actually living. It is these that the embryologists are able to select with their micro-needle. Once isolated, they can then be transferred to the viscous solution and treated in the same way for the ICSI procedure.

At the end of the injection procedure the micro-injection needle is carefully withdrawn and suction on the egg is released. The egg is washed through a few changes of normal culture medium, and left overnight in an incubator at 37°C in conditions similar to routine IVF culture. The subsequent culture procedures, checking for fertilization, cleavage of the fertilized egg and transfer of any embryos to the womb occurs in the same routine manner as that for conventional IVF.

### ***Results and Outcome***

As mentioned above, ICSI can be used for all types of sperm problems - even those with the most extreme condition. Taking into account even the most difficult cases, recent data indicates that 98% of all patients should achieve fertilization and the transfer of at least one embryo with the ICSI technique, and 30% of patients having embryo transfer should achieve clinical pregnancy (fetal heart on ultrasound scan). Analysis of the international data indicates that, overall, couples have the same incidence of implantation in pregnancy as those undergoing conventional IVF (i.e. about 25% per treatment cycle started).

There appears to be a slight reduction in the incidence of clinical pregnancy in cases where (1) sperm manufacture - as opposed to sperm release (a condition known as non-obstructive azoospermia) - is severely compromised, (2) where living but immobile sperms are used, and (3) there have only been two reports of a successful pregnancy with the condition called globozoospermia. In certain areas other specific conditions, such as the cause of immobile sperm due to Kartagener Syndrome, there is a reduction in the incidence of fertilization and pregnancy.

However, in these extreme groups the numbers treated are obviously few and statistical information is currently unreliable.

In patients who have congenital absence of the vas-deferenes, and in a few other situations, it is necessary for couples to be assessed for cystic fibrosis carrier status. It has been shown that in certain parts of the world as many as 70% of men with congenital absence of the vas-deferenes are carriers of cystic fibrosis.

### ***Safety***

There has been some concern as to the safety of the ICSI procedure. This concern has revolved around the incidence of congenital abnormalities at birth and the findings of the chromosome studies of the fetuses assessed in utero. The major studies that have been reported on nearly two thousand pregnancies, indicate that the overall incidence of congenital abnormality at birth is not significantly higher than after IVF or the normal population. However, there has been some

concern as to a slightly higher risk of abnormal sex chromosomes (the X and Y chromosomes) arising after ICSI. More information has to be obtained to truly assess the risk.

It is very important for patients to appreciate that, in most of the abnormal conditions, we are unable to explain the cause of the problem. Men should be assessed for their chromosome status, but it is not possible in all circumstances to study their genes (the coded information carried by the chromosomes which determine every aspect of our make-up). It is highly probable that in a number of men their sperm problems are caused by underlying genetic problems. We now know that there is a certain gene on the Y chromosome which, if affected, causes very low sperm counts.

It is therefore probable that sons resulting from ICSI will carry the same genetic problem as their fathers and, therefore, might themselves require ICSI (or some other technique) for them to conceive. Hence, some men should have blood taken to screen their chromosomes, assess their genetic condition and their status regarding cystic fibrosis. Many centers counsel their patients with regard to amniocentesis (testing cells around the baby during pregnancy) after ICSI, especially if ICSI has been used successfully in a particularly rare situation. This is a sensible approach and one that needs to be discussed with all the appropriate professional parties.

Therefore, despite the staggering success with ICSI, we must not lose sight of the fact that this procedure is still new and as such our excitement must be tempered with caution. At the moment, more than 5,000 babies have been born worldwide. Although the low incidence of congenital abnormalities is encouraging, we must adhere to screening programs, not become complacent, but continue to gather all the data on outcome of treatment and make available all information to patients. Currently we believe that a many as one quarter of patients may carry a genetic condition which could be passed on to their children.

### ***The Future***

Until recently, it was assumed that the sperm used to procure successful fertilization and birth had to be a mature spermatozoon. The only men we could not treat, therefore, were those who did not manufacture any form of sperm or only immature sperm. Recently, scientists have achieved the world's first publication of a pregnancy using a spermatid (the immature sperm) using ICSI technology<sup>1</sup>. This technology has been continued in our associated centres internationally to try to obtain more information on the viability of this procedure<sup>2</sup>.

Worldwide there have been approximately 20 babies born using immature sperm, but these are case reports and it is not known how efficient this procedure is. Too few patients have been treated to provide reliable data on the incidence of pregnancy. All appropriate screening of patients will also be essential for those seeking this technology in the future.

ICSI technology has revolutionized the treatment of male factor infertility. This is considerable, because we now appreciate that male factor infertility is probably the largest single cause of infertility amongst couples. Although this is exciting technology, we are also aware that it requires considerable skill and expertise to provide a successful treatment. In centers that offer this level of skill, more than 90% of couples treated will have the same chance of a healthy pregnancy and delivery as in conventional IVF. Patients should embark on an ICSI program with the same enthusiasm as those requiring conventional IVF, but detailed discussions should be undertaken with the professionals with regard to the couple's individual circumstances and their risks and opportunities.

*References:*

1. *Pregnancy after Intracytoplasmic Injection of a Spermatozoon. The Lancet*, 345 pp1641– 1642 (1995)
2. *Human Fertilisation with Round and Elongated Spermatozoa. Human Reproduction*, 12 pp336-340 (1997)