ASSISTED REPRODUCTION FOR MALE INFERTILITY

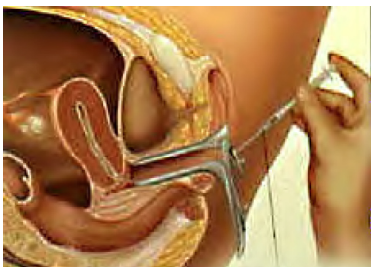
What is assisted reproduction?

Infertility affects 8 million couples in the U.S. In roughly 50% of couples, there is an associated male issue or "factor". Here we discuss how assisted reproduction can be used to treat (more accurately "bypass") the problem of male infertility. Since the birth of Louise Brown in 1978, assisted reproductive techniques (ART) are now widely used, with more than 1 million children conceived artificially. In the U.S., the number of babies born to infertile couples with ART has risen logarithmically: from 260 babies in 1985 to 48,756 in 2003.

The National Library of Medicine defines assisted reproductive techniques as any "clinical and laboratory techniques used to enhance fertility in humans and animals." By this definition, intrauterine insemination (IUI), ovulation induction (OI), in vitro fertilization (IVF) and other forms of egg or sperm manipulation are considered ART. The Society for Assisted Reproduction (SART) uses a more restrictive definition and uses the term to indicate any procedure that requires manipulation of eggs, sperm or embryos in vitro (i.e. outside of the body). By this definition, IUI is not considered ART, although it will be discussed here.

What is IUI and when is it used for male infertility?

Intrauterine insemination (IUI) involves the mechanical placement of a washed pellet of ejaculated sperm within the female uterus, beyond the cervix, at the time of ovulation. The placement of sperm with IUI is deeper into the female reproductive tract than sperm are normally "placed" with intercourse (i.e. the upper vagina). This proce-



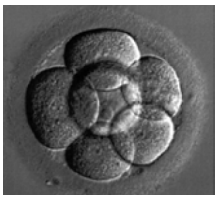
IUI involves placement of washed sperm beyond the cervix with a catheter.

dure is timed to the "fertile" or "ovulatory" period in the women's menstrual cycle. IUI is performed for female factor infertility due to cervical issues, either anatomical or physiological; if the cervix is bypassed, thenpregnancies may ensue. IUI is also used for male infertility due to low sperm counts or low motility, for immunologic infertility or the presence of antisperm antibodies, and in men with mechanical problems of sperm delivery, such as hypospadias, erectile dysfunction or the inability to ejaculate).

To be successful, there should be at least 5-40 million motile (moving) sperm in the ejaculate (volume x concentration x motility) and at least 5 million motile sperm should be inseminated after the ejaculate is processed. Success rates vary widely and are generally related to female reproductive potential, with pregnancy rates per cycle (month) ranging from 1-12%. Once sufficient numbers of washed sperm are obtained, IUI success rates are closely linked to female age and reproductive potential. While cases of male factor infertility can benefit from IUI alone, overall pregnancy rates with IUI are generally improved if the ovaries are stimulated with medication to make more than one egg available every month (also termed ovarian stimulation).

What is IVF and when is it used for male infertility?

IVF involves ovarian stimulation followed by egg retrieval, fertilization of the egg in the laboratory, and embryo transfer to the uterus. The goal of hormonal stimulation during IVF is to develop more than one mature follicle, so that enough eggs are retrieved later to counteract the inherent inefficiencies of fertilization, as well as the limitations of placing sperm and eggs together in the laboratory. For IVF, the female menstrual cycle is highly regulated in what is termed controlled ovarian stimulation.



An early embryo after fertilization with sperm.

The majority of the stimulation protocols currently used start by down-regulating the pituitary gland to prevent spontaneous activity. Subsequently, pituitary hormones are replaced with gonadotropins (FSH and LH) on a daily basis for 9-13 days and ovarian follicle development monitored with ultrasound. Each follicle contains an individual maturing egg or oocyte. Once adequate follicular size is achieved, another injection is administered to induce ovulation and eggs are retrieved surgically 36 hours later. This is performed transvaginally without an incision and guided by

ultrasound. All eggs that retrieved are then nurtured in the embryology laboratory.

A semen sample, generally obtained by masturbation on the same day as egg retrieval, is also needed for IVF. The semen is washed so that the sperm is separated from the semen fluid and the sperm are incubated with the eggs at a density of 100,000 sperm/egg. After 12-18 hours of co-incubation in an incubator, the eggs are observed too see if they have fertilized. With normal fertilization, 2 pronuclei are present and there is extrusion of the second polar body. The embryos are allowed to divide in the incubator and are transferred back to the women's uterus anywhere from 1 to 5 days after fertilization. Day 3 and day 5 embryo transfers are currently most commonly performed. The reason for in vitro culture of embryos beyond 3 days is that the hardier embryos will survive beyond the first several cell divisions. There may be an increased chance of implantation and pregnancy with these hardier embryos if they are cultured for 5 days after retrieval. Because they are more robust, fewer blastocysts (day 5) are transferred to the uterus than embryos (day 3) to obtain similar pregnancy rates, thereby also reducing the chance of twins or triplets. However, because the laboratory culture conditions do not replicate the optimal environment of the uterus, other experts believe that an earlier transfer (day 1-2) is warranted. A balance appears to be on day 3, when the embryo may have reached the 6- to 8-cell stage, and most IVF centers use day 3 embryo transfers in routine practice.

During embryo transfer the embryo is transferred back to the uterus in a small procedure without anesthesia. Embryos are loaded into the catheter with a small volume of culture medium and then expelled into the uterus with a syringe. The number of embryos transferred back depends on the age of the patient. In general, more than one embryo is transferred because the "take" rate or implantation rate is not 100% for every embryo returned. Younger women generally have better quality embryos that are more likely to implant and therefore require the transfer of fewer embryos. Although different clinics use different guidelines for the number of embryos to transfer, national guidelines have been developed for this procedure. These guidelines are important because multiple embryo transfers increase the likelihood of having twins or triplets. In fact, roughly 30%-40% of all IVF cycles result in "multiples" simply because of multiple embryo transfers. The indications for the use of IVF for male infertility are listed in the Table.

Please see Table on following page.

Male Factor Issue	IVF	IVF-ICSI
Retrieved sperm (testis and epididymis)		Yes
Low motile sperm count (< 5 million sperm)	Yes	Maybe
Poor sperm morphology (<4% strict or Kruger)		Yes
Failed trial of IUI	Yes	Maybe
Immunologic Infertility (antisperm antibodies) and failed IUI		Yes
Correctable male factor with limited maternal reproductive potential	Yes	Maybe

Table. *Indications for IVF and ICSI with Male Factor Infertility*

What is ICSI and when is it used for male infertility?

In 1992, a laboratory addition to IVF was described, termed intracytoplasmic sperm injection (ICSI). With this technique, individual sperm are pulled with suction into a microscopic pipette after crushing the sperm tail to immobolize it. The sperm are injected directly through the egg shell (zona pellucida) covering the unfertilized egg through the (perivitelline) space that separates the egg from the shell, and directly into the egg itself.

The ICSI technique: a single sperm about to be injected into a human egg.

With this technique, the numerical sperm requirement for egg fertilization dropped from the need for hundreds of thousands of moving sperm for each egg with IVF to a single live sperm for ICSI. This approach allows "immature" sperm obtained surgically or by aspiration from men with no sperm in the ejaculate to be used for conception. Indeed, ICSI has become so popular that over 56% of IVF cases performed in U.S. clinics now routinely use this technique to boost fertilization rates during IVF. In fact, the ability of ICSI to improve egg fertilization with live sperm from any source in the man has encouraged Dr. Turek and others to look beyond the ejaculate and into the male reproductive tract to find sperm for biologic pregnancies. At present, sources of sperm that are amendable for use with ICSI include sperm obtained from the vas deferens, epididymis, and testicle. Based on a meta-analysis of what has been published on the use of sperm from these three reproductive tract organs, there are insufficient data to say that any one source of

sperm is preferred to any other for ICSI.

The male factor indications for IVF and ICSI are listed in the Table and explained here. One indication for IVF-ICSI is the use of surgically retrieved sperm. Except for very rare cases of pregnancies with motile sperm aspirated from the vas deferens and used with IUI, sperm aspirated from the epididymis and testis routinely require IVF and ICSI for success. Other indications to pursue ART include immunologic infertility (IVF-ICSI), failure to conceive with a trial of IUI, low numbers of motile ejaculated sperm (<5 million/ejaculate) and poor sperm morphology (IVF-ICSI). Male genetic infertility (i.e. Y chromosome microdeletions, karyotype abnormalities or other definable syndromes) falls under the ART indication of low motile sperm count, as this condition is thought to cause infertility mainly by impacting sperm numbers or motility. Other reasons for a couple to pursue ART instead of alternatives revolve around issues of convenience, cost or time. One example of time-based consideration is a couple with mild male factor due to varicocele along with advanced maternal age (say >40 years old). Since Dr Turek first reported that the average time to conceive after fixing a varicocele is 8 months, the benefits of waiting for this treatment option to mature can "use up" valuable, declining female fertility potential. Remarkably, a uniform, accepted set of criteria for clinicians to counsel their patients regarding the use of ART does not exist. Often, ART is thought of as a "big hammer" than can handle many different types of "nails," or infertility problems and is applied liberally to treat infertility without much evidence that it is needed. This is especially true of ICSI, which was used with just over half of IVF cases with male factor infertility in the U.S. in 2003, but also in 47% of cases without male factor infertility diagnoses.

The relationship between poor sperm morphology and ART deserves further comment. The rigorous determination of sperm shape after staining is termed sperm "morphology." It is done in addition to the semen analysis but on the same sample. Within these classification systems, sperm are considered normal or abnormal based on a list of specific features and measurements of the head, midpiece and sperm tail. An increased percentage of abnormal sperm within a semen sample is termed teratozoospermia. Sperm morphology may correlate with fertility potential in that it may affect: 1) the ability of human sperm to successfully penetrate the cervix; 2) the ability of sperm to bind to the egg "shell" or zona pellucida; and 3) the embryo implantation rate. Although the relationship between successful conception and IUI based on poor morphology is controversial, that between sperm morphology by strict or Kruger criteria and egg fertilization rates with IVF is well

accepted. In general, in an otherwise normal semen analysis (i.e. normal volume, concentration and motility), if >14% of sperm are "normal" by strict morphology criteria, then egg fertilization rates are high (>80%). However if strict morphology is <4% normal, then egg fertilization rates with IVF drop to 7%. Thus, in cases of low sperm morphology, ICSI is done with IVF and appears to "rescue" many cases of that may have resulted in failed fertilization with IVF alone.

How are success rates for ART procedures measured?

How fertility centers report pregnancy outcomes is a complex matter. The proportion of eggs that fertilize in a dish with 2 pronuclei within 18 hours of sperm insemination, termed the normal fertilization rate, is one commonly used, but indirect, measure of ART success. More importantly, however, the following are accepted definitions of pregnancy outcomes with ART.

Live birth/cycle:
the percentage of ART cycles started that result in a live birth. This rate is the most meaningful outcome measure from ART procedures.

Pregnancy/cycle:
the percentage of ART cycles started that produce a pregnancy. This rate is higher than the live birth per cycle rate because some pregnancies end in miscarriage, induced abortion, or stillbirth.

Live birth/egg retrieval:
the percentage of ART cycles in which eggs are retrieved that result in a live birth. It is generally higher than the live birth/cycle rate because it excludes cycles that were canceled before eggs were retrieved.

Live birth/transfer rate:
includes only those ART cycles in which an embryo or egg and sperm were transferred back to the woman. This rate is the highest of the measures of ART success.

Singleton live birth/cycle:
the percentage of ART cycles started that result in a singleton live birth. This has become a very important ART outcome measure, as singleton births have a much lower risk than multiple-infant births for adverse infant health outcomes, including prematurity, low birth weight, disability, and death.

What are the risks and complications of ART procedures?

As a medical procedure, IVF can have complications that are medication-related, procedure-related, or procedure-associated. These types of complications are outlined below.

Medication-related complications

These include ovarian hyperstimulation syndrome (OHSS) and ovarian torsion. Thought to result from excessive hormone stimulation, severe OHSS occurs in <1% of IVF cycles, but can be life threatening. It is associated with very high estrogen levels and involves increased leakage of fluid from blood vessels. It can cause electrolyte imbalances, dehydration, fluid in the abdomen and total body swelling. There is also a predisposition to forming blood clots and pulmonary emboli. Most cases of OHSS resolve on their own with time, but they may require careful monitoring, generally in the hospital and occasionally in the intensive care unit. Ovarian torsion is also rare and involves the twisting of the ovary on its blood supply. It is due to the large increase in the size of the ovary with hormone stimulation is treated surgically by untwisting the ovary.

Procedure-related complications

The process of egg retrieval and embryo transfer to the uterus have low complication rates. However, in < 1% of cases, there can be injury to nearby bowel or bladder, pelvic infection or bleeding requiring transfusion.

Procedure-associated complications

By far the most common type of complication, these include multiple births, possibly higher birth defect rates and concerns about an increase in imprinting disorders. The high incidence of multiple gestations is the major concern with ART procedures. Multiple pregnancies occur up to ~35% of ART pregnancies, compared to 1-2 % of naturally conceived pregnancies. Multiple gestations are associated with lower birth weights, higher overall complication rates and increased learning disabilities relative to singletons. In addition, the delivery costs associated with managing multiple, often premature, gestations are at least 10-20 times higher than that associated with singleton deliveries. There is strong evidence to suggest that the incidence of low birth weight singletons is also increased after ICSI. Because of these issues, the goal of most respectable IVF programs is to reduce the multiple gestation rate by transferring fewer embryos to the uterus with each IVF cycle.

There are several concerns regarding the health of offspring obtained after IVF-

ICSI. Although the overall rates of major birth defects associated with IVF and ICSI (3.3%) are similar to birth defect rates observed for children conceived after intercourse or IVF alone, there is concern about risks of sex chromosome anomalies and other specific malformations, including hypospadias, in IVF-ICSI offspring. Two other concerns with IVF-ICSI are: (1) Since IVF and ICSI may eliminate many natural selection barriers that exist during natural fertilization, genetic defects that caused the infertility are expected to be passed on to offspring unabated. This is simply because there is no reason to assume that criteria used to chose a sperm by an embryologist with ICSI is the same as that which occurs naturally. This has large ethical implications, especially with respect to diseases like Klinefelter syndrome that might be expected to resurface again in grandchildren of the affected, but treatable infertile male. (2) Recent data show that offspring born to infertile couples with this technique have a 4-fold higher rate (0.8%) of sex chromosomal anomalies than do children who are naturally conceived. Even more recently, concerns have arisen regarding whether rare imprinting diseases such as Beckwith-Weideman Syndrome and Anglemann Syndrome are increased in children conceived with ICSI. It is also controversial whether subtle development defects and delays exist in ART children.

How does ART compare with other treatments for male infertility?

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