CME CREDIT

New technology and new challenges for assisted reproduction

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ABSTRACT

As assisted reproduction technology advances, more types of procedures are becoming available, bringing more success at solving many types of infertility. In vitro fertilization has become simpler and less invasive, with success rates as high as 30% per cycle. Intracytoplasmic sperm injection has solved many types of male infertility. This article explains in vitro fertilization technology and discusses such ethical issues as embryo ownership, multiple births, and embryo genetic testing.

The strongest influence on fertility is the woman's age

LOUISE BROWN, the world's first "test-tube baby," is nearly 20 years old. As Ms. Brown has been growing up, so has the technology of assisted reproduction. Follicle-stimulating hormone, once derived from human urine, is now genetically engineered. The techniques used to harvest eggs are much less invasive. Embryos are now routinely frozen for later use. With the development of microsurgery techniques, genetic material from the male can be inserted directly into an egg, meaning that men with almost any form of male infertility can become fathers.

This article summarizes current in vitro fertilization methods available to help infertile patients become parents, advances expected in the near future, and the ethical questions raised by these technologies.

AGE-RELATED INFERTILITY: A GROWING PROBLEM

By 2002, we estimate that 6 million couples in the United States will be seeking help for infertility. The most common causes of female infertility—tubal disease, endometriosis, and anovulation—have not changed. But age-related infertility is becoming a bigger problem as more women postpone childbirth. The average age of the women in our infertility clinic is 35, and at that age, a woman's fertility is half of its peak at age 20. In contrast, male fertility remains constant until about age 65.

The age of the woman remains the strongest influence on fertility. In our center, women under 35 have a live birth rate of about 30% per cycle of assisted reproduction. In contrast, women between 35 and 39 have a live birth rate of 23% per cycle.

IN VITRO FERTILIZATION TECHNIQUES

Screening

Couples are considered infertile if they have not achieved pregnancy after a year of trying. However, we may begin treatment sooner if the woman is older than 37, or irregular menstruation suggests an ovulatory problem, or there are other medical circumstances such as a history of chemotherapy.

Younger women are expected to respond well to assisted reproduction. However, in older women, we first measure levels of follicle-stimulating hormone (FSH) at day 3 of a woman's menstrual cycle. An FSH level higher than 20 μM/mL this early in the menstrual cycle indicates that the ovarian reserve is diminished and the ovary will be unresponsive to natural or artificial stimulation. (A normal level would be less than 10 μM/mL.) In these cases, we advise the patient that her chances of conceiving are low (about 1% or 2%), and assisted reproductive techniques will not improve them. Elevated day 3 estradiol also predicts a poor ovarian response.

Ovarian stimulation

Once it has been determined that in vitro fertilization (IVF) is an option, the first step is ovarian stimulation. In humans, only one follicle develops to maturity per menstrual cycle. However, since our chances of a successful assisted pregnancy are only 4% to 6% per oocyte, we stimulate the maturation of additional follicles to allow us to harvest additional oocytes for fertilization.

At our institution, we usually follow a protocol known as the long regimen. We first suppress pituitary action with leuprolide acetate (a gonadotropin-releasing hormone agonist) for about 10 days, then administer FSH, a gonadotropin.[1]

At one time, gonadotropins were collected from postmenopausal women's urine, which was sometimes provided by convents. The resulting preparation contained a combination of FSH and luteinizing hormone. However, we now know that administering luteinizing hormone is not necessary for oocyte development. Recombinant gene technology has allowed us to produce pure FSH preparations that contain no luteinizing hormone and can be administered subcutaneously. Using recombinant FSH has improved assisted pregnancy success rates by about 5%.

Hormonal side effects

The ovarian stimulation regimen may cause unpleasant mood swings. If the drugs cause ovarian hyperstimulation syndrome, the patient may present with ascites, massive enlargement of the ovaries, and decreased intravascular volume, renal perfusion, and urine output. Thromboses and adnexal torsion are very rare complications. Some researchers believe that hormonal stimulation may raise the risk of ovarian cancer over the long term, but this is difficult to confirm because the women who undergo ovarian stimulation are typically older and have no children, and thus are already at higher risk for ovarian cancer. Most of the studies of ovarian stimulation and ovarian cancer have focused on patients who take clomiphene citrate, which may pose a higher risk because it persists in the body for up to a month and is an oral medication that may be taken by outpatients for months. We use injected gonadotropins, which are administered in the clinic for only a few cycles and are metabolized within 8 hours, which probably reduces the risk of long-term effects.

Harvesting oocytes from follicles

Oocytes were once collected through laparoscopic procedures, but ultrasound-guided needle aspiration has allowed us to do it as a 10-minute office procedure. We simply image the ovary with a transvaginal ultrasound probe, locate mature ovarian follicles (those 16 mm or more in diameter), insert a needle, and aspirate the fluid from each follicle. The fluid is then examined microscopically for oocytes. We collect an average of 11 oocytes from each patient.

Sperm preparation

When possible, semen is donated by the partner on-site and prepared with special washing media, which concentrates sperm and prepares (or capacitates) them to bind with the zona pellucida.

Fertilization

Each oocyte is incubated overnight with 50,000 to 100,000 sperm. In about 70% of cases, in vitro fertilization is sufficient to fertilize the egg (although 5% of the time, the egg will be fertilized by more than 1 sperm, preventing development).

Embryo transfer

Once the oocytes are fertilized, they are cultured for 3 to 5 days, during which 85% of them will undergo cleavage. By 5 days, the embryo forms a blastocyst. Problems in cell division and development are often evident at this stage, helping us to identify morphologically normal embryos to transfer to the patient's uterus.

The process of transferring an embryo into a woman's uterus is a simple office procedure that takes no more than a few minutes. Unfortunately, the implantation rate is low. When 10 embryos are transferred to 5 women, on average about 2 pregnancies will result. Current research shows that the most important factor is the health of the embryo itself, not the uterine environment. Chromosomal abnormalities are common among embryos, and embryos that contain abnormalities often

Transferring several embryos increases the chance of pregnancy, but also increases the chance of multiple births

will not implant. Also, implantation rates are higher among younger women and for embryos formed from eggs donated by younger women. This latter fact indicates that the key factor in the success of embryo implantation is the age of the egg donor, not the age of the uterus.

Transferring more than 1 embryo at a time increases the chances of a pregnancy, but it also increases the chances of multiple births. We strike a balance in most cases by transferring 2 embryos, although when the woman is older than 35, we often recommend 3. Our low transfer numbers make our program one of the most conservative centers in the country.

SOLVING MALE INFERTILITY

We have nearly solved the problem of male infertility. As long as we have access to sperm (or its gene-carrying progenitor "round cells") in the testicle, there does not even have to be any sperm in the ejaculate. Sperm can be collected by microsurgery directly from the epididymis or testicle. An oocyte can then be fertilized by a microsurgical technique known as intracytoplasmic sperm injection (ICSI).

We have nearly solved the problem of male infertility

Intracytoplasmic sperm injection

Using micromanipulators, we hold the egg in place, immobilize a single spermatozoon, and inject it into the egg. Through the microscope, we can see immediately whether the sperm was placed correctly and whether the procedure damaged the egg. A successfully fertilized egg is allowed to grow in culture for 3 to 5 days and then is transferred to the uterus using the standard embryo transfer technique.

Genetic abnormalities in male infertility

Although most forms of male infertility can be overcome, men with severe infertility are more likely to have chromosomal abnormalities, so we recommend genetic testing and counseling. For example, the prevalence of Klinefelter syndrome (the XXY genotype) and the cystic fibrosis gene is higher among azoospermic men than among men with normal fertility. Oligospermic men have a higher frequency of autosomal translocations, particularly balanced translocations. Microdeletions in the Y chromosome are found in about 7% of men, apparently with no other effect but to impair fertility. These men may pass along their infertility to their male offspring.

In all of these cases, it is important that couples learn as much as possible about potential problems and discuss them with a qualified genetic counselor before choosing ICSI.[2,3]

Are intracytoplasmic sperm injection babies healthy?

Most children conceived through assisted reproductive techniques are no different from any other children, but there are some exceptions. Whereas spontaneous X-chromosome abnormalities occur in about 0.2% of the general population, the rate among ICSI children is about 0.8%. Amniocentesis may be recommended during the pregnancy.[3,4]

Researchers at the center with the longest experience with ICSI babies, the Brussels Free University in Belgium, have found no health or developmental differences between ICSI children and other in vitro fertilization children, or between ICSI children born from cryopreserved embryos and those born from fresh ones.[5,6] However, a much smaller and controversial Australian study suggested that male ICSI children may have an increased risk of slight developmental delays at age 1.[7]

NEW FIELDS OF RESEARCH

With a success rate of roughly 30% per cycle, assisted reproductive technology has plenty of room for improvement. Here are some of the new fields of research.

Assisted hatching. To facilitate fertilization of eggs from older women, we can dissolve or remove a part of the oocyte's outer membrane, the zona pellucida. This procedure appears to increase fertilization rates a little.

Preimplantation genetic testing. When the embryo reaches the blastocyst stage at 5 days in culture, a single blastomere can be removed and tested for the genes for Tay-Sachs disease or cystic fibrosis, as well as for chromosomal abnormalities.

Egg cryopreservation. Although freezing does little harm to sperm and embryos, it may kill oocytes, lower their fertilization rates, or cause chromosome loss. When embryos do form from frozen eggs, they have lower cleav-

age rates. Thus, a man who is about to begin cancer treatments that might impair his fertility can preserve gametes for use later, but women do not really have the same option. It is also much easier for a man to donate sperm to other would-be parents than it is for a woman to donate an oocyte. Research into freezing oocytes is ongoing, particularly in some European countries, where laws based on ethical concerns prohibit freezing embryos.

Egg maturation. Immature spermatozoa can be used to fertilize oocytes, but immature oocytes are not viable, and we have no method of maturing them in vitro. Thus, a woman donating oocytes must go through at least a month of preparation so that the follicles will mature. If oocytes could be artificially induced to mature, we could remove and preserve a piece of ovary containing hundreds of immature eggs, which, for example, could give a cancer patient a chance of fertility after her treatment.[8]

Cloning. A detailed discussion of cloning is beyond the scope of this paper, but clearly there has been an explosion of recent research in this area.

CURRENT AND FUTURE ETHICAL ISSUES IN ASSISTED REPRODUCTION

Who owns frozen embryos?

Embryos that are not transferred are usually frozen for future attempts. Frozen embryos not used by the parents pose ethical and legal problems. There is no consensus about who owns them or how long they can be kept frozen, although some countries and some states in the United States have tried to legislate answers to these questions. The problem of ownership becomes especially difficult if the parents separate, divorce, or die, or when they are not married in the first place. We try to prevent problems by requiring all assisted reproduction patients to sign consent forms stipulating what will happen to unused embryos, but it is clear that this is an evolving ethical and legal issue.

Incentives for egg donation

Because it is older women who most often seek assistance in becoming pregnant, but it is often the age of the oocyte which determines the success of the procedure, oocyte donation poses unique ethical problems. For example, desperate women might be willing to offer thousands of dollars for oocytes from a younger woman, a proposal that might be tempting to impoverished teens or to college students at tuition time. This is considered unethical because it can be coercive. In addition, body parts and tissues are routinely donated, but we are uncomfortable with the concept of "buying" them. Unused frozen embryos formed from donated eggs may also be problematic, because it is not clear whether they would belong to the would-be parents or to the genetic parents. Right now, the only oocyte donation we permit at the Cleveland Clinic is non-anonymous donation in which the potential parent recruits a woman willing to donate an egg.

Multiple births

Excessive multiple births increase the risk of premature births and certain birth defects, and can also result in high medical costs. Although the cost of the in vitro fertilization procedure and medication is about $6,000, the cost of premature birth can be $100,000 or more. It is important to note that in vitro fertilization does not carry a high risk of multiple births because we transfer only a small number of embryos to the uterus. In contrast, superovulation-insemination procedures (such as the one that resulted in an octuplet birth last December in Houston, Texas) stimulate the maturation of multiple eggs. If the woman is inseminated in vivo, any or all of these eggs can be fertilized simultaneously to produce a multiple pregnancy.

Men with severe male factor infertility are more likely to have chromosomal abnormalities

Sex selection and genetic testing

With in vitro fertilization techniques, it is possible to determine embryos' sex and transfer only those of the desired sex. It may also be possible to test embryos for certain desirable or undesirable genetic traits. Both of these possibilities, obviously, carry troubling ethical questions. We do not offer sex selection except when the patient carries a severe X-linked genetic disease, and we test only for the most serious genetic disorders such as cystic fibrosis and Tay-Sachs disease.

Human cloning

Although the technology and the ethical issues surrounding human cloning are beyond the scope of this brief paper, we feel it is necessary to note that human cloning will be done somewhere. Not in the United States, but somewhere. The greatest challenge in the future of assisted reproduction is the resolution of the new ethical questions that inevitably accompany changes in human reproduction.

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