

## Technological & Ethical Issues In Laboratory-Assisted Reproduction A Short History to Accompany the Lecture

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The treatment of infertility through laboratory-assisted techniques has made great strides during the last two decades. Three approaches have been taken to increase the likelihood of fertilization. These include superovulation with gonadotropins combined with intrauterine insemination, Gamete IntraFallopian Transfer (GIFT), and in vitro fertilization (IVF) and embryo transfer. The development of the assisted reproductive technologies has not been without controversy. I will review the historical development of these techniques, ethical issues created by their development, and the technique themselves.

Intrauterine insemination (IUI), in combination with the stimulus of follicular maturation through the use of exogenous gonadotropins, increases the likelihood that high quality sperm and eggs will meet, leading to fertilization. At the time of follicular maturation, ovulation is induced with the injection of human chorionic gonadotropin (hCG), which binds to LH receptors, and spermatozoa are recovered out of seminal fluid concentrated, and placed within the uterine cavity near the entrance of the Fallopian tubes. The processing of the sperm in the Andrology Lab serves several functions. It removes seminal plasma, which contains prostaglandins that may

lead to local uterine contractions or bronchospasm. It also removes components that play a role in preventing spermatozoa from undergoing capacitation and the subsequent acrosome reaction, processes essential for fertilization. The use of ISolate-densely gradient centrifugation enhances the in vitro capacitation of sperm that would normally take place within the cervical mucus. IUI is associated with the lowest chance of conception per cycle of the various laboratory-assisted techniques. From our own experience, conception rates have ranged from approximately 12 % to a high of 28% per treatment cycle (once a month), depending upon the patient's age and the clinical situation. However, this approach is associated with a significant risk of high order, multiple pregnancy and concurrent preterm delivery.

Both Gamete IntraFallopian Transfer (GIFT) and in vitro fertilization and embryo transfer (IVF/ET) guarantee the meeting of gametes and allow one to regulate the number of eggs or embryos placed within the reproductive tract. GIFT was devised by Dr. Ricardo Asch, at a time when IVF culture conditions were suboptimal and did not adequately mimic those that occur within the Fallopian tube. His concept was to recover eggs from follicular aspirates in a manner similar to that as performed for IVF, but then place them with spermatozoa directly into the distal ostium of the oviduct. However, this approach requires either laparoscopy or a mini-laparotomy to gain access to the Fallopian tubes. With the improved pregnancy rates following IVF and ET in the United States, GIFT is currently being performed with much less frequency.

The initial concept of IVF was to bypass damaged Fallopian tubes, achieving fertilization with in vitro culture, then returning the preimplantation embryo to the uterus thereafter. As is

well known, this was first accomplished by Robert Edwards, a scientist in Cambridge, England, who collaborated with Patrick Steptoe, a gynecologic laparoscopist. Louise Browne, the first IVF pregnancy, was born in 1978 in the United Kingdom. It is not as well appreciated that neither Edwards nor Steptoe were reproductive endocrinologists. On this basis, they attempted to achieve pregnancy utilizing unstimulated natural cycles and frequent serial monitoring of urinary LH, to attempt to define the initiation of the LH surge. Although they described the fertilization of a human egg in vitro in 1969, it took nearly 10 years of repeated attempts to achieve a birth. There was also much concern within the scientific community as to whether their work had scientific merit and was not an attempt “to be a doctor of human desire”. This term had been phrased by Paul Ramsey, a theologian at Princeton University, in 1971. Indeed, both he and Leon Kass, an ethicist at the University of Chicago now in charge of the President’s Council on Bioethics, published major articles in the Journal of American Medical Association and the New England Journal of Medicine, respectively, questioning the ethical merits of proceeding with IVF. Dr. Kass raised the concern that conception in vitro was an experimental procedure and that the child-to-be could not consent.

Alan Trounson and Alex Lopata initially played a major role in achieving the first Australian IVF pregnancy. The use of Clomiphene Citrate was introduced in an attempt to increase the number of eggs ovulated. In the United States, the Federal government initially placed a ban on IVF research in 1971, until it was determined that IVF was medically ethical. Although this was decided to be the case, no NIH funding was subsequently provided for its

development. Indeed, during this time, IVF did develop rapidly in Europe. However, it was not until Howard and Georgiana Jones, after reaching emeritus status at Johns Hopkins University (at this time, there was mandatory retirement age!), started a second medical career at the Eastern Virginia Medical Center and established of a program of *in vitro* fertilization in Norfolk, Virginia in 1979 financed through a private benefactor, that the first IVF birth in the United States occurred in 1981. Thereafter, through the initial efforts of the Jones to transfer the information they gained through a series of annual meetings, IVF programs developed throughout the United States. From this limited start and limited medical indications, IVF has spread world wide and become a treatment for all aspects of human infertility.

The concept of IVF is straightforward, but its achievement requires excellence at many levels. A major contribution of Howard and Georgiana Jones was the use of gonadotropins to stimulate the production of multiple follicle maturation and control the time of predicted ovulation. Initially, preparations of gonadotropins that stimulated growth of ovarian follicles were obtained from protein precipitates of menopausal urine. Bruno Lunenfeld collaborated with an Italian chemist, Piero Donini, to develop a product, *Pergonal*, which was initially field tested in Israel. *Pergonal* needed to be administered intramuscularly, due to its high percentage of extraneous proteins other than gonadotropins that could lead to local skin reactions if it were administered subcutaneously. An average course of gonadotropins is given daily for approximately 10 - 12 days beginning on the third day of the menstrual cycle. Frequent monitoring of serum estradiol and ultrasonic follicular diameter is required. Superovulation has

become more sophisticated since its initial concept. Currently, preparations of Follicle Stimulating Hormone (FSH) obtained through recombinant DNA technology are self-administered subcutaneously, usually in conjunction with a gonadotropin release hormone (GnRH) agonist. The latter may be utilized to either suppress endogenous gonadotropin stimulus of the ovaries, in an attempt to synchronize development of multiple follicles and block the LH surge, or administered concurrently with gonadotropins, to promote follicular maturation through release of endogenous gonadotropins from the pituitary.

Follicles grow from a diameter measuring approximately 6-8 mm during menses to diameters of approximately of 17.5 to 21 mm at their maturation. Human chorionic gonadotropin (hCG) is administered when at least 2 follicles are present measuring approximately 18.5 mm in diameter, in lieu of LH, to promote initiation of meiosis. HCG was initially obtained from the urine of pregnant women, although pure preparations through recombinant DNA technology are now available that can also be administered subcutaneously. This hormone binds to LH receptors and will mimic, when given in high dose, the preovulatory LH surge. This initiates the first meiotic division that is necessary for successful fertilization, as well as release of the egg from the follicle, approximately 36-40 hours after its administration. IVF programs time follicular aspiration at approximately 34.5 hours following the administration of hCG. On this basis, the egg has undergone its maturation process in situ and is recovered just before its release from the ovarian follicle. Initially, follicular aspiration was performed laparoscopically, with direct visualization of the ovaries. A major breakthrough occurred when Dellenbach, in

Strasbourg, France described the ultrasound-guided transvaginal puncture of follicles and their aspiration. Subsequently, devices were established that act as needle guides attached to vaginal transducers to simplify the recovery of eggs, which now usually takes place during a period of 15-20 minutes utilizing IV sedation.

Follicular fluid is collected by serial follicular puncture and handed off to an embryologist to document the presence of eggs. While initially follicles were flushed multiple times to recover eggs, this technique is utilized only infrequently. It often resulted in multiple follicular flushings that needed to be scanned by the embryologist, increasing the time for egg recovery. Subsequently, it was learned that if the needle tip is centered with the follicle and then twisted during aspiration of follicular fluid, the follicle collapses around its point. This ensured a high yield of eggs (80-10%).

Sperm and eggs are then placed in the same culture droplet. Fertilization requires that spermatozoa be able to undergo an acrosome reaction, penetrate the cumulus oophorus and zona pellucida, and adhere to the oolema, where it becomes incorporated by the oocyte. Fertilization rates in vitro should run approximately 70-80% in the face of normal semen parameters. As an old rule of thumb, the fertilization rate dropped approximately 20% for each abnormal sperm parameter; that is, concentration, motility, morphology. More recently, sperm function tests have been utilized, in the face of abnormal semen quality, in an attempt to predict the likelihood of successful fertilization in-vitro, and which couple would benefit from intra-cytoplasmic sperm injection (ICSI).

Initially, preimplantation embryos were transferred back to the uterus on the third day following fertilization. More recently, embryos have been cultured in vitro for an additional 2 days through development of the blastocyst. This approach has been taken to cull out those aneuploid embryos of poor quality that are destined not to continue to grow during a longer period of culture. This allows the transfer of one or two high quality embryos to the uterus, achieving high rates of pregnancy while lowering the risk of multiple pregnancy. There is evidence that approximately one third of embryos created by in vitro fertilization are aneuploid, and this incidence rises with maternal age. It should be noted that there was initial concern by basic scientists regarding the safety of longer-term in vitro culture. The human embryonic genome is activated during this time, and it was unclear whether there might be developmental problems in the offspring of these pregnancies, due to altered genomic imprinting, an epigenetic phenomenon in which a gene derived either from the mother or father is turned on or off. The details of this process are not well known and altered imprinting of genes could have the potential of causing harm. However, this does not appear to be the case, and the vast majority of children born from transfer of blastocysts appear to be healthy.

Implantation rates have improved dramatically over the past 10 years. It is not unusual to achieve total pregnancy rates of 50-60% per treatment cycle in women under the age of 35. However, this includes biochemical pregnancies, in which a gestational sac is never imaged, as well as those clinical pregnancies that subsequently miscarry, occurring approximately 20 % of the time. Hence, the national live birth rate in the United States averages approximately 40 %

per cycle in this age group. The chance of pregnancy diminishes between the age of 38 - 40 (30%) and considerably beyond the age of 41 (10 - 15%). This parallels the known decrease in fecundity of women as they approach the end of their reproductive years, due to the rising incidence of oocyte aneuploidy. The women in this age group can be offered use of ovum donation from young women, to achieve pregnancy.

Embryo transfer is usually performed awake by passing a catheter retrograde through the cervix. It is important to perform a mock trial transfer prior to the actual event. This allows one to determine the depth and shape of the uterine cavity, minimizing the chance of trauma at the actual transfer and the associated likelihood of provoking uterine contractions. Many programs also utilize ultrasound guidance of the catheter, for exact placement of the tip approximately 1.5 cm from the uterine fundus.

When IVF was initially proposed, there were strong arguments suggesting that the development of this technology would both destroy human relationships and create the opening wedge into a slippery slope that would lead to genetic engineering as described in Aldous Huxley's *Brave New World*. During the last three decades we have seen the opposite, the creation of families that would not have taken place without these techniques. We have not seen the appearance of rogue scientists, Dr. Strangelove types. A number of subsequent techniques have developed that have promoted the success of IVF and improved reproductive outcomes. These have included embryo cryopreservation, intracytoplasmic sperm injection, and preimplantation genetic diagnosis.

Cryopreservation allows one to store embryos created in an initial IVF cycle that would not be transferred back to the uterus. The couple can save those frozen embryos for future use in a subsequent cycle, if pregnancy does not occur following the initial transfer of fresh embryos. Freezing of embryos has given couples a greater choice as regards number of embryos they wish to transfer back to the uterus initially. With the increasing awareness of the incidence of high order pregnancy created by these techniques, there has also been a trend to establish criteria to transfer fewer embryos to the uterus at any time. Currently, in the United States, it is not uncommon to transfer a single embryo, in women who are 37 years old or younger, if these are high quality and there are additional embryos that could be frozen for future use. In women in their late-thirties, most programs would recommend the transfer of 2 embryos, and 2 or 3 embryos are suggested in women who are 40 or older, as there is a greater likelihood of the creation of non-viable aneuploid embryos at this age. The overall likelihood of a triplet pregnancy following the transfer of 3 embryos to the uterus is approximately 3 - 10%, depending upon a woman's age. The main concern, should this occur, is the considerable risk of preterm labor and premature birth, with its associated risks to the newborn and need for long term neonatal ICU care.

The injection of sperm directly into the ooplasm (ICSI), leading to fertilization of these eggs, is a major breakthrough that has increased the ability of men with severe oligospermia to become fathers. While the majority of children born from this procedure are apparently healthy, they are only now reaching their own reproductive age. There are some remaining concerns of

basic scientists that male pronucleus formation following ICSI does not occur in a manner similar to that following normal fertilization, and it has been theorized that this may increase the risk of sex chromosome aneuploidies. Indeed, two centers in Europe have documented a three-fold increase in the incidence of Klinefelter's and Turner's syndrome in children conceived by ICSI, although this has not been noted in the majority of programs. Given the increasing evidence that smoking may lead to damage of DNA within spermatozoa, as well as alterations in sperm motility and shape, there is concern regarding the utilization of such sperm for ICSI, unless these men undergo a period of smoking cessation. There is also concern that severe oligospermia may be of possible genetic etiology secondary to new mutations arising in these men that lead to disorders in spermatogenesis. Although it is possible to confirm the normalcy of a leukocyte karyotype, as well as the absence of a small number of mutations in the Y chromosome that are associated with severe oligospermia, many other mutations discovered through mutagenesis experiments in mice that lead to disordered spermatogenesis cannot be tested in humans. If present, these mutations may lead to sterility in the male offspring conceived by ICSI in such cases.

Laboratory-assisted reproduction has also lead to the possibility of what has been termed "bifurcated motherhood"; that is, a woman may be the gestational mother of child following ovum donation, but not the genetic parent. Conversely, a woman born with Mullerian agenesis and her partner could contract with another woman to be a gestational mother for their genetic child. For these reasons, law and medicine must interact, to confirm or extinguish the rights of

the genetic gamete donors and the parents of the child. In considering ovum donation, another concern is the maximum age that “would be reasonable” for a woman to become pregnant. We know from recent experience that women in their late 50’s carry small but present increased risks associated with pregnancy. One must also consider the ability of such older women and their partners to be parents of young children as they age.

Thousands of embryos are currently in storage in liquid nitrogen in IVF centers throughout the United States. The majority of these will be utilized to established pregnancies. However, some frozen embryos will remain following completion of families. These embryos could potentially be utilized for scientific investigation or could be donated to infertile couples to establish their own family. Most states do not have laws that address the issue of embryo donation; hence, this is currently addressed through contracts and family courts.