



## The “ART” of Making Babies

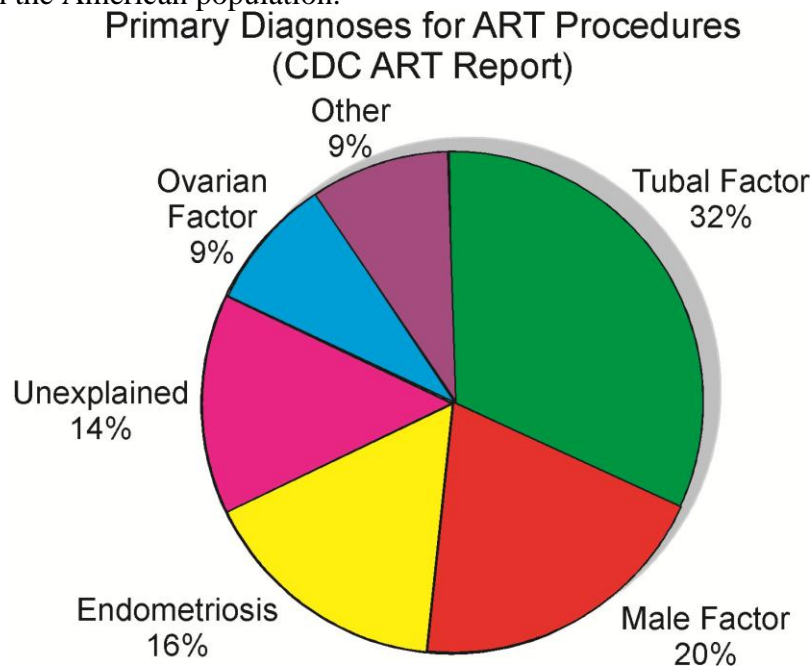
### Introduction

While many couples strive to prevent fertilization, others have the opposite problem. Try as they might, for whatever reasons, they are unable to conceive. As scientists began to unravel the mysteries of fertilization, they began to develop better methods for harvesting, handling and fertilizing gametes. The result is the development of the new reproductive technologies. Until around 20 years ago, the only way to conceive was by natural intercourse but things have changed, as they always do. Now at least 12% of American women have taken advantage of the available fertility services. We will first look at some of the terms related to in Assisted Reproductive Technologies and then at some of the information that has allowed these developments.

Much of this lecture is based upon data presented on the Center for Disease Control and Prevention website. The URL for the website is: <http://www.cdc.gov/ART/index.htm>. Each year the information is updated and new information is presented but the information covered in this lecture has been established over many years so any new information will only show minor differences from that presented here.

### Infertility Causes for ART Users

The reasons for human infertility are diverse and as expected couples decide to use ART for various reasons. The following pie chart shows the different reasons people used ART and the percentage of the ART population each category comprises. This chart thus gives a relatively accurate idea of how prevalent each cause of infertility is in the American population.



- Tubal Factor (blockage or damage)--32% of the couples used ART because the female suffered (for some reason) from blockage of her fallopian tubes.
- Male Infertility (low sperm count, motility problems, etc.)--20% of the couples used ART because the male was functionally sterile.

- Endometriosis (presence of uterine-like tissue in other areas)--16%
- Ovarian Causes (egg production problems)--9%
- Other (fibroids, DES exposure...)--9%
- Unknown--14%; the reason for the infertility was not detected

### Types of ART procedures

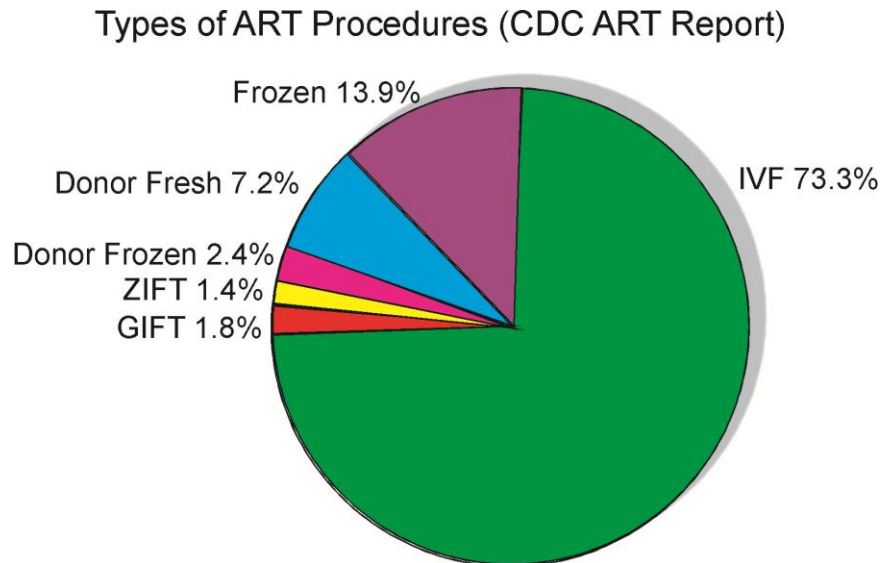
IVF (In Vitro Fertilization) involves extracting a woman's eggs, fertilizing the eggs in the laboratory, and then transferring the resulting embryo(s) into the woman's uterus through the cervix.

GIFT (Gamete IntraFallopian Transfer) involves using a fiber-optic instrument called a laparoscope to guide the transfer of unfertilized eggs and sperm (gametes) into the woman's fallopian tubes through small incisions in her abdomen.

ZIFT (Zygote IntraFallopian Transfer) involves fertilizing a woman's eggs in the laboratory and then using a laparoscope to guide the transfer of the fertilized eggs (zygotes) into her fallopian tubes. -Most IVF, GIFT, and ZIFT cycles used fresh, non-donor eggs or embryos.

### ART procedures used in the US

It is important to note that information is reported as "cycles". ART cycles are not a single event but a series of steps taken over weeks. In addition, the most recent data is often a 2 or more years old because the follow up analysis of birth success requires the time required for pregnancy, live birth success and data acquisition. The following pie chart shows the breakdown for the types of ART that are used by couples using the procedures.



- IVF = 73.3% of couples used standard IVF with their own (nondonor) eggs
- GIFT = 1.4% of couples used GIFT with their own (nondonor) eggs
- ZIFT = 1.8% of couples used ZIFT with their own (nondonor) eggs
- 13.9% of couples used their own eggs which had been frozen
- 9.6% used fresh or frozen eggs from another female (donor) eggs

### Harvesting Eggs

A typical female ovulates at most a few eggs each month during her reproductive years. Because larger numbers of eggs are required for the various ART, hormonal priming is necessary to induce the maturation and ovulation of larger numbers of eggs. Usually combinations of drugs are used to enhance ovulation while

simultaneously inhibiting any other events that will affect the process. Two of the commonly used drugs are Humegon™ and Lupron™.

### Steps in procedure:

- Treat patient with hormone regimen
- Ovaries stimulated and many follicles mature
- Use ultrasound to detect mature follicle
- Use laparoscopy to remove oocytes from ripe follicles

### Assessing Sperm/Semen Quality

- Visual: Phase contrast microscope--assess sperm density (numbers/mL; historically counted in a blood cell counter or haemocytometer), normality (normal vs. abnormal cells), motility (Sperm Motility Index; SMI), abnormalities (headless, two heads, etc.).
- SMI is best indicator probably because the sperm need all of the necessary attributes to swim well.
- Other tests: e.g., Sperm Quality Analyzer (SQA); Hamster Test

### Causes of Sperm Loss

- Temperature affects spermatogenesis: high temp., low sperm count
- Used as contraceptive method (poor)
- Males encouraged to wear loose underwear if trying to conceive
- Many other causes beyond scope of this course

### Problems in Studies on Sperm Loss in Males

- Evidence exists that there has been a decrease in quality of semen (# sperm/mL, motility of sperm) in last 50 years in males of industrialized countries--due to pollution?
- But it is very possible that methods of assessing sperm numbers vary from lab to lab and with time and that many of reported changes in sperm counts due to various factors are not valid because of this.
- Swan & Elkin (1999, BioEssays 21: 614-621) have reviewed the literature and conclude that although the older methods (i.e., sperm counting in a hemocytometer which was originally designed for counting blood cells) are "surprisingly reliable" in spite of a large random error.
- Thus new more precise methods such as the use of flow cytometry should be used to find out if there is an actual decrease in the quality of human semen (e.g., Hacker-Klom et al, 1999. Human Reproduction 14: 2506-2512.)

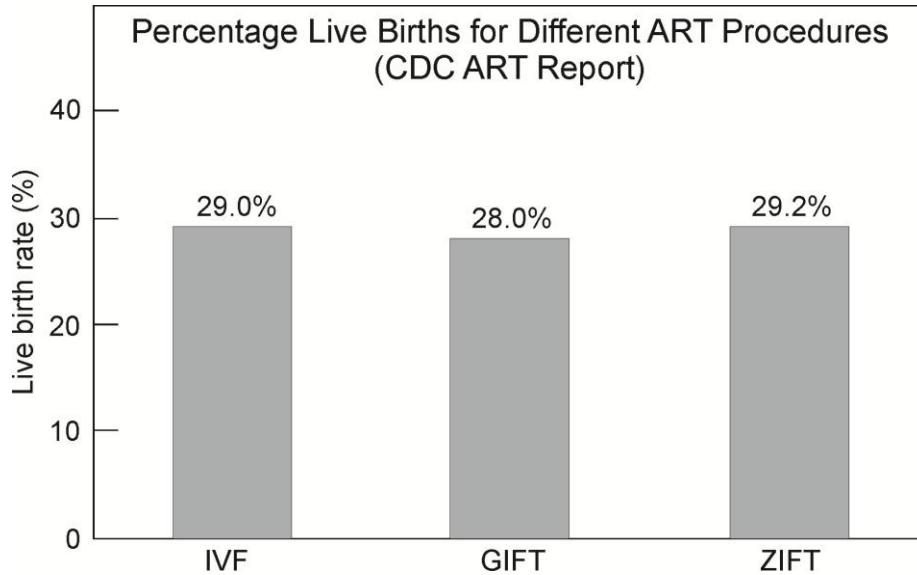
### Successful ART - Sequence of Events

There are various steps in ART procedures and at each stage an assessment of success for that stage can be determined.

1. **Assessment of Egg Production.** An ART cycle is started when a woman begins taking medication to stimulate the ovaries to develop eggs or, if no drugs are given, when the woman begins having her ovaries monitored (using ultrasound or blood tests) for natural egg production.
2. **Egg Retrieval.** If eggs are produced, the cycle then progresses to egg retrieval, a surgical procedure in which eggs are collected from a woman's ovaries. Once retrieved, eggs are combined with sperm in the laboratory.
3. **Egg Transfer.** If fertilization is successful, one or more of the resulting embryos are selected for transfer, most often into a woman's uterus through the cervix (IVF).
4. **Implantation Signals Clinical Pregnancy.** If one or more of the transferred embryos implants within the woman's uterus, the cycle then progresses to clinical pregnancy.

5. **Live Birth.** Finally, the pregnancy may progress to a live birth, the delivery of one or more live-born infants. (The birth of twins, triplets, or more is counted as one live birth.)

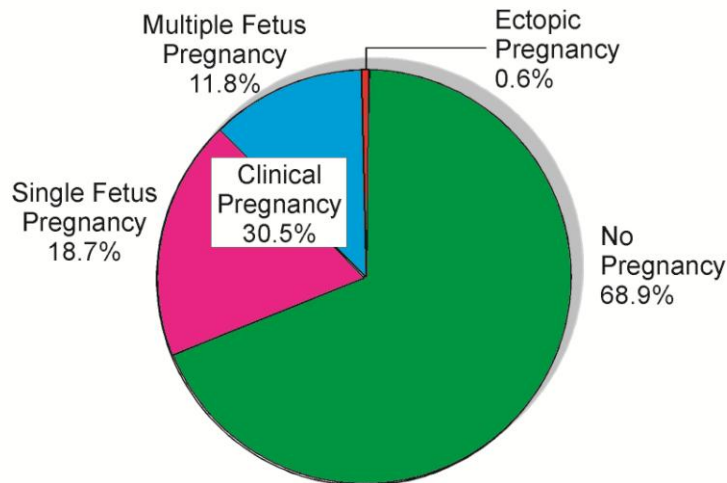
A cycle may be discontinued at any step for specific medical reasons (e.g., no eggs are produced or the embryo transfer was not successful) or by patient choice. Success rate for all ART procedures (IVF, GIFT, ZIFT) are equal as shown in the following figure:



**Pregnancy & ART**

The following figure shows the results of the 1998 ART cycles that used fresh, non-donor eggs or embryos.

Results of ART Procedures Using Fresh Non-Donor Eggs (CDC ART Report)

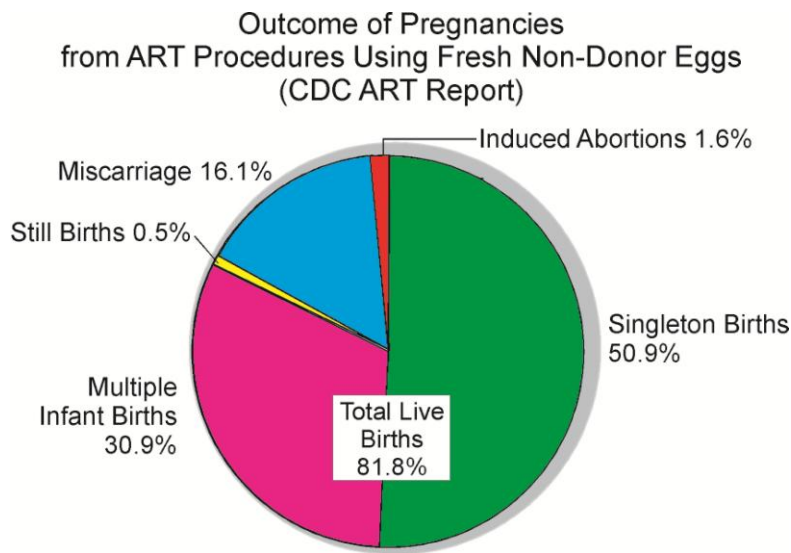


Most of these cycles (68.9%) did not produce a pregnancy, while a very small proportion (0.6%) resulted in an ectopic pregnancy (the embryo implanted outside the uterus). Clinical pregnancy was achieved in 30.5% of these ART cycles. More specifically,

- 18.7% resulted in a single-fetus pregnancy.
- 11.8% resulted in a multiple-fetus pregnancy.

**What percentage of pregnancies results in live births?**

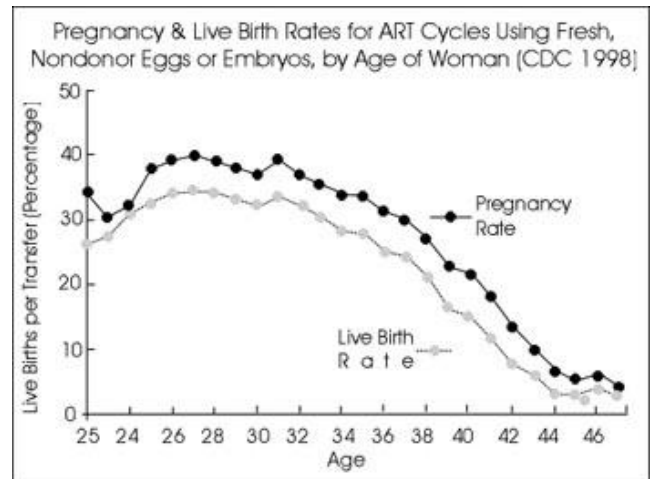
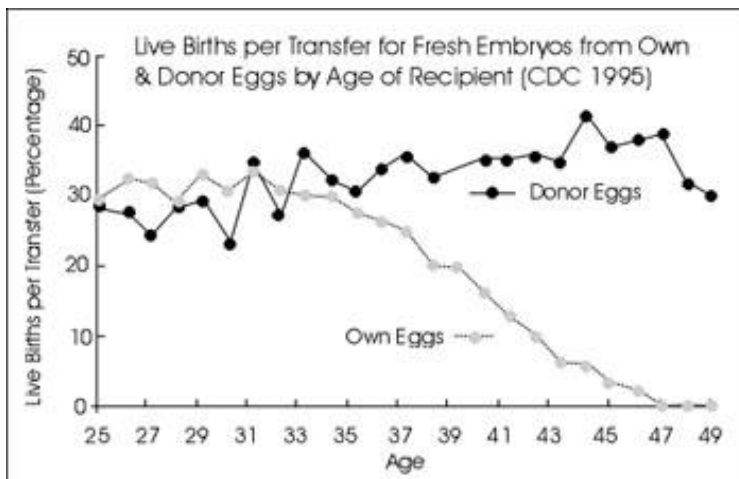
The following figure shows the outcomes of pregnancies resulting from ART cycles in 1998 discussed above. Approximately 82% of the pregnancies resulted in a live birth (50.9% in a singleton birth and 30.9% in multiple-infant births). Approximately 18% of pregnancies resulted in an adverse outcome (miscarriage, induced abortion, or stillbirth). Although the birth of more than one baby is counted as one live birth, multiple-infant births are presented here as a separate category because they often are associated with problems for both mothers and infants. Infant deaths and birth defects are not included as adverse outcomes because the available information for these outcomes is incomplete.



**ART & Multiple Births**

The ultimate goal of ART is to allow couples to produce a single healthy child. Currently about 1 in three ART pregnancies leads to multiple births. The key is to be able to select a single, viable embryo that has a high chance of implanting in the uterus. This goal is slowly being attained but to ensure pregnancy occurs more than one embryo needs to be implanted at present.

**Female Age vs Success Rate**



- The age of the female donor is the most important factor when own eggs used

- Female fertility, as evidenced by the ability to give birth, decreases with age
- ~25% success 22-33
- Steady decrease in success after age 34
- Age is also a predisposing factor for autosomal trisomy in the offspring (Hassold and Jacobs, 1984. *Ann. Rev. Genet.* 18: 69-97).
- Women older than 47-48 are physiologically sterile
- But are eggs from older females less viable?

### ART Success vs Recipient Age

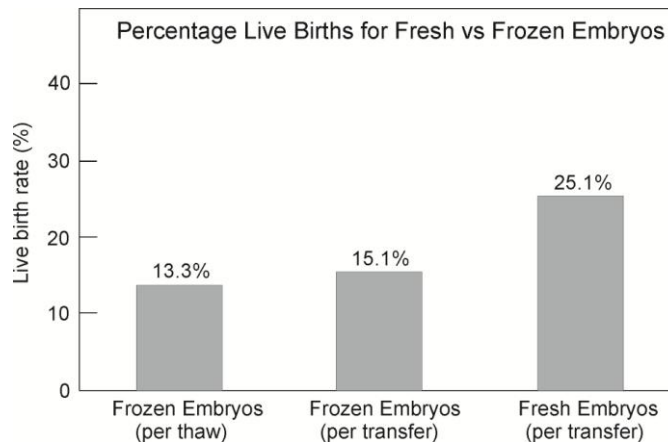
The results of this study are a bit unexpected, considering that the older a woman is, the less likely she is to give birth. Women were either implanted with their own fertilized eggs or with fertilized donor eggs from a young woman.

- The results with females implanted with their own eggs mirrors the previous figure showing that with age there is a drop off in live births (i.e., the eggs are less viable in older females).
- However, when eggs from young females were transferred into recipient females, the age of the female was not a negative factor.
- The age of the female recipient has no negative effect on success. latest available stats for ART just being posted
- In fact, success appears to increase with the age of the recipient.
- Thus, eggs are less viable with age but the uterine environment of older females remains good and is able to support embryonic development

### Sperm Storage: Bad & Good News

First the bad news. The storage and use of frozen sperm has been well established. It has been shown that clinical pregnancy rates using donor insemination with previously frozen sperm approaches natural pregnancy rates (Clarke et al., 1997. *Fertil. Steril.* 68: 112-117). One issue that has recently come to light is problems of microbial contamination with sperm samples stored in "straws" in liquid nitrogen tanks and is discussed in a short opinion article by Clarke (1999. *Human Reproduction* 14: 2941-2943). Often the samples are not sterilized on the outside and there is evidence that the straws can crack during storage. Certain viruses (e.g., HIV, hepatitis B and C) and bacteria (e.g., syphilis) can then travel from one frozen sample to another in liquid nitrogen. The goal now is to test all semen samples for any known harmful microbes prior to storage and to develop better storage procedures.

### ART: Fresh or Frozen?

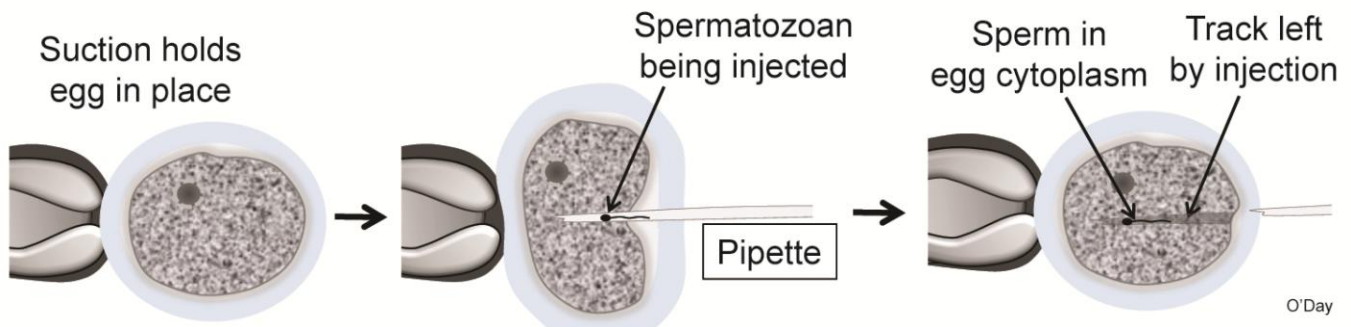


- Not all eggs survive freeze/thaw cycle so per thaw rate is lower (13.3%) than per transfer rate (15.1%)
- Fresh embryo success rate is much higher (25.1%)

Freezing and thawing of human cells is not a simple task and is done under very specific conditions. Clearly more needs to be learned about how to store gametes for ART. The issues surrounding egg and embryo freezing and the current state of the art is discussed in an opinion paper by Gook & Edgar (1999. Human Reproduction 14: 2938-2940).

### IVF: ICSI

#### Intracytoplasmic Sperm Injection (ICSI)



Here's the URL for a site that has many videos on aspects of IVF: <http://www.ivfindia.com/ivf-lab-videos.htm>

The human egg is about the size of a period at the end of a sentence in a book (~1mm egg diameter; ~1.5-2 mm zona pellucida diameter). As you can see, this means that attempts to inject things into it can be a major technical feat. Also the egg has to be held in place and this is done by using a vacuum to suck it tightly against a pipette (left hand side of picture above) so the needle (right) can pierce the zona pellucida and then the egg cell membrane. Material can then be injected into the egg cytoplasm. This formed the basis for ICSI which has been reviewed recently by Tesarik & Mendoza (1999. BioEssays 21: 791-801).

- Intra-Cytoplasmic Sperm Injection (ICSI) involves the injection of a sperm cell into an egg; done since 1991 (Palermo et al., 1992. *Reprod. Fertil. Dev.* 7: 211-218).
- By 1999, more than 20,000 children have been born worldwide due to this technique
- 1 Sperm cell is injected directly into the egg cytoplasm via a fine needle
- Success rate 50-70% of eggs injected begin development
- Allow for fertilization with defective spermatozoa that would otherwise not be able to penetrate the egg naturally
- Typically used when low sperm motility (e.g., Kartagener's syndrome), abnormal shape, etc.
- Question: Do congenital abnormalities result from this method?
- Answer: no increase in major abnormalities detected; only small increase in minor congenital anomalies except for hypospadias which may be due to hypofertility of male donors (Wennerholm et al, 2000. *Human Reprod.* 15:944-948).

#### What Else Could We Inject?

- Nucleus of Other Cell Type--Cloning can be done by this approach; injection of other stages of spermatogenesis (e.g., spermatid nuclei) is now being done for ICSI.
- DNA--Gene Therapy

- DNA--Genetic Engineering of new Genotypes
- Recent research: injecting nucleus alone is not sufficient; need cytoplasmic components (e.g. Centrioles)

### **Selecting Sperm: Choosing the Sex of Your Baby**

- Sperm are motile: some selection methods are based on swimming rates (e.g., fastest sperm are male); not very successful
- DNA content: Y chromosome is smaller than X therefore sperm have less DNA
- Density gradient: based on weight (poor)
- Fluorescence automated cell sorter (FACS): It is possible to label cells with fluorescent dye; sort out on the basis of brightness (fairly successful; new)

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