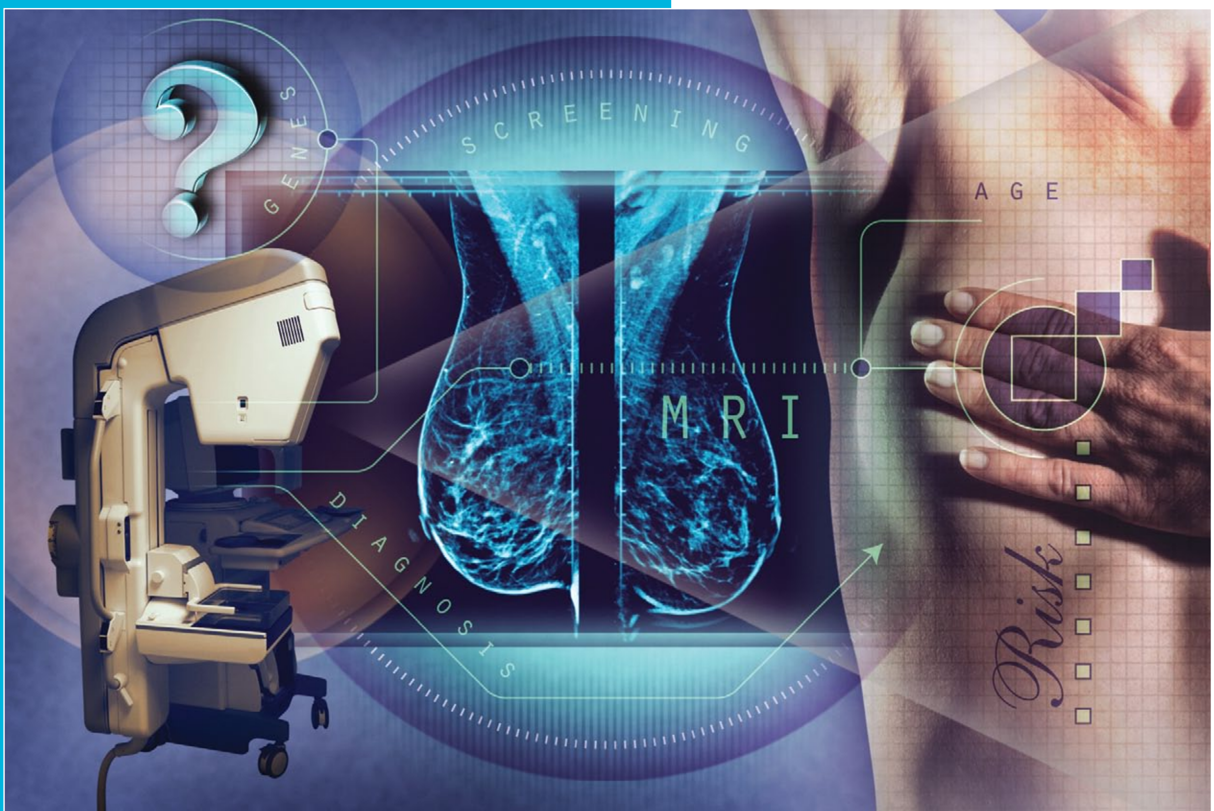
The Female Patient®

MAY 2010 | VOL. 35, NO. 5 www.femalepatient.com



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IVF Then and Now, 30 Years Later: Best Practices in Reproductive Medicine, Part 2

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The first of this series presented the milestones of in vitro fertilization (IVF) from its standard technique to intracytoplasmic sperm injection and blastocyst culture. Part 2 examines third-party reproduction and preimplantation genetic diagnosis and discusses the new technologies of egg freezing, comparative genome hybridization, and metabolomics.

This review article continues the discussion of IVF history and its place in today's practice. It will allow the ObGyn clinician to understand the evolving science of advanced reproductive technology to appropriately counsel patients and provide treatment options.

THIRD-PARTY REPRODUCTION: UP TO 5 "PARENTS"

Following the first successful IVF egg/embryo donation pregnancy in the mid-1980s, third-party reproduction was embraced by patients requiring a donor egg, gestational carrier, and donor embryo.[1,2] The most challenging aspect of egg donation involves synchronizing the endometrium of the embryo recipient with the egg donor. This is typically realized by prescribing the embryo recipient a gonadotropin-releasing hormone (GnRH) agonist to suppress ovulation then achieve endometrial proliferation with a stepwise addition of estradiol, until adequate endometrial thickness of at least 7 to 8 mm is visualized by ultrasound while the recipient awaits follicle growth by the donor. Embryo transfer is determined by the stage of embryo development matched with the day of progesterone supplementation of the recipient. Many clinics perform an endometrial proliferation mock cycle to ensure the recipient will achieve adequate endometrial thickness in a defined amount of time for appropriate preparedness during the "fresh cycle."

Recent advances in egg freezing have allowed the creation of "donor egg banks."[3] While egg freezing is currently designated "experimental" by the American Society for Reproductive Medicine (ASRM), the prediction is for rapid acceptance and utilization of this method, thereby avoiding the inconvenience of a synchronized fresh cycle between 2 women.

Because of the increasing number of supernumerary embryos frozen from prior IVF cycles and remaining in cryopreserved storage, infertile couples also have the option of embryo donation. All of the methods described above can also use an IVF surrogate, resulting in the theoretical "5-parent" fertility treatment cycle: intended parents + egg donor + sperm donor + surrogate. Because a woman's uterus does not age biologically as do oocytes, pregnancy rates are dramatically higher age-per-age with egg donation (Figure 1).

> **FOCUSPOINT**
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TWO FOR THE PRICE OF ONE: THE ENDURING DILEMMA OF MULTIPLE GESTATION

The recent exploitation in the media over Octomom has led to a heightened awareness of the risk for multiple gestations from IVF. ASRM has

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released several guidelines in order to reduce this significant maternal and fetal problem (Figure 2).[4] Risks of a multiple gestation pregnancy include miscarriage, congenital anomalies, low birth weight, cognitive deficiency, and maternal complications such as diabetes and hypertension.[5] Given poor insurance coverage for IVF, patients are usually reluctant to accept elective single-embryo transfer, citing an inability to undergo repetitive cycles. In years past, physicians were inclined to transfer high numbers of embryos to maintain a competitive edge in the marketplace, particularly since the CDC annually publishes IVF-clinic pregnancy statistics. Despite the ASRM disclaimer not to compare clinics, patients use this information as one way to distinguish the quality of an IVF program. This is far from a litmus test for the merit of an IVF program, particularly since some centers have restrictive criteria to qualify a patient for IVF and/or to cancel during the stimulation due to a poor follicular response.

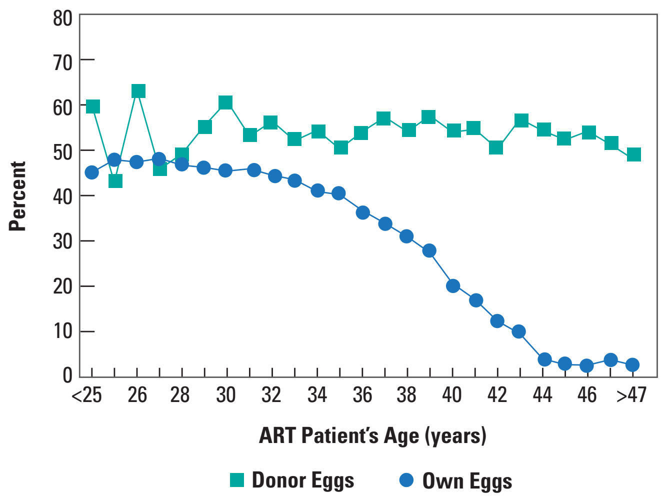

FIGURE 1. Percentages of transfers that resulted in live births for assisted reproductive technology (ART) cycles using fresh embryos from own and donor eggs, by ART patient's age, 2006.
Source: www.cdc.gov/ART/ART2006/sect4_fig44-48.htm#f45.

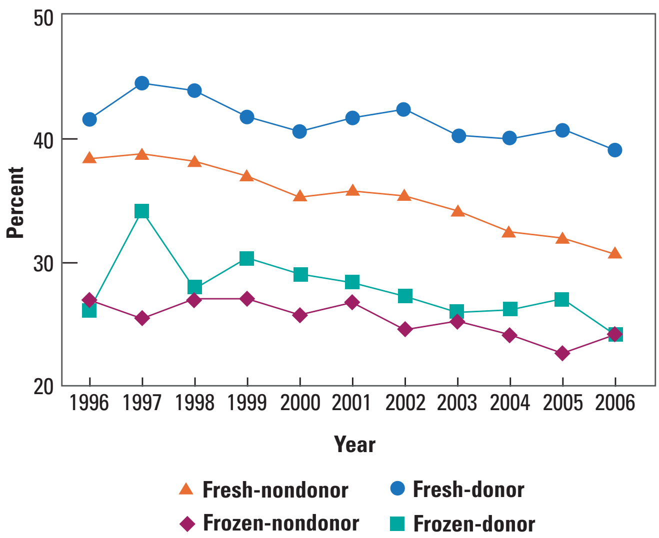

FIGURE 2. Percentages of cycles that resulted in multiple-infant live births, by type of assisted reproductive technology cycle, 1996-2006.
Source: www.cdc.gov/ART/ART2006/section5.htm.

CRYOPRESERVATION AND FERTILITY PRESERVATION: STOCKING UP ON EGGS

Since the first successful frozen embryo pregnancy, cryopreservation has allowed many couples the opportunity of a less invasive, less costly, but often less successful alternative to a stimulated fresh egg retrieval.[6] Advances in freezing technology using an ultrarapid freezing process called vitrification has enabled improvements in egg freezing with reduced damage to the egg's cytoplasmic mitotic spindle. As a result, fertility preservation options for cancer patients and young women electing to defer their fertility now include the experimentally designated egg freezing. Pregnancy rates using egg freezing with thawing, fertilization, and embryo transfer are approaching traditional frozen embryo replacement cycles.[7] Ovarian tissue freezing with subsequent reimplantation into the pelvis (orthotopic) or heterotopic sites (abdomen, forearm) has resulted in one successful pregnancy to date using the former method.[8] Until an adequate number of live births have been achieved, the safety and efficiency of egg freezing remains under close surveillance.

ADVENT OF REPRODUCTIVE GENETICS

In 1990, blastomere cells from a cleavage-staged embryo were removed and analyzed using DNA amplification for the Y chromosome, and preimplantation genetic diagnosis (PGD) was born.[9] Now cells can be analyzed by the polar body of an oocyte, the cleaving embryo blastomeres, or trophectoderm of the blastocyst, using polymerase chain reaction to avoid transmitting lethal genetic mutations in carriers of single gene defects, eg, cystic fibrosis and sickle cell anemia. Following removal of a blastomere cell on day 3 of embryo development, results occur within 2 days, allowing for a day 5 blastocyst embryo transfer. Estimations for misdiagnosis with PGD range from 2% to 11%, making parental testing imperative.[10] Analysis of a consortium of PGD patients appears to demonstrate a slightly lower success rate than standard non-PGD IVF cycles. This may be due to multiple theories, most of which stem from genetic testing that actually screens out embryos that would otherwise have been implanted, albeit with the undesired genetic disease. Consequently, the number of suitable embryos for transfer is decreased.[11]

Despite initial studies suggesting improvements in pregnancy rates using preimplantation genetic screening (PGS) through fluorescence in situ hybridization technology to examine the 9 to 11 most common chromosomes implicated in miscarriages, PGS has not been shown to improve the live-birth rate in women of advanced maternal age and/or with repeated implantation failure/pregnancy loss.[11]

While PGD has avoided the birth of children with disease, the technology has also been applied to elective gender selection. As the Human Genome Project's knowledge is applied to PGD, the fear of genetic engineering for other superficial traits such as hair and eye color remains the single most polarizing argument against advances in assisted reproductive technology since its inception in 1978.

WHAT'S NEXT?

Using no to minimal stimulation to mature oocytes in the laboratory, in vitro maturation has been evolving since 1994. This method is of particular utility in patients with polycystic ovary syndrome, given their proclivity for multiple antral follicles.[12]

The first analysis of every chromosome in the embryo by DNA amplification through comparative genome hybridization (CGH) in 2000 holds the promise of surpassing the expectations of PGS, allowing for elective single transfer of a chromosomally normal embryo, thereby reducing multiple gestation, improving implantation rates, and reducing miscarriage.[13,14] Because the results may take up to 3 weeks, the embryos from the fresh egg retrieval must be cryopreserved, then transferred in a subsequent frozen embryo replacement cycle. Despite the high pregnancy rate potential with CGH, the holy grail of embryo selection remains evasive, because single cell analysis of an embryo does not ensure a chromosomally euploid embryo, due to mosaicism in the other cells of the same embryo.

Perhaps the most exciting development stems from a new method of potentially determining the healthiest embryos for transfer called metabolomics (eg, ViaMetrics-E, developed by Molecular Biometrics). By analyzing culture media surrounding the embryos, metabolite by-products of the embryo are profiled from oxidative stress biomarkers to assess embryo viability, hence enabling single embryo transfer.[15]

FOCUSPOINT

Fear of genetic engineering for superficial traits such as hair and eye color remains the single most polarizing argument against advances in assisted reproductive technology.

LINGERING CONCERNS

As many studies have examined an association between IVF and birth defects, data have been difficult to dissect and stratify the effect of multiple gestation, preterm delivery, maternal age, and infertility. To illustrate the ambiguous association, an initial high-order embryo implantation can spontaneously reduce to a singleton but still carry the obstetric complications of a multiple gestation, due to the phenomenon of uterine "memory."[16] As a result, this pseudo-singleton pregnancy is falsely categorized as a complication from IVF rather than the initial multiple implantation. Most recently, research has demonstrated IVF offspring (as well as those from intrauterine insemination) had a marginally higher increased risk for congenital anomalies diagnosed prenatally or at birth compared with infants naturally conceived.[17,18] Nevertheless, the debate continues without a clear cause and effect.

Conflicting reports have also plagued the application of intracytoplasmic sperm injection and the risk for birth defects in offspring, but the largest study to date appears to support an increased risk for specific anomalies. However, it is still unclear whether the risk is due to the procedure itself or inherent abnormalities in the sperm.[19] Males with severe oligozoospermia or azoospermia are more likely carriers of cystic fibrosis, Y-chromosome microdeletions, and chromosomal aneuploidy.

Concern had been raised regarding risk for ovarian and breast cancer from ovulation-inducing medications, but this has been refuted. Although infertile patients may have a higher rate of ovarian cancer, there is no evidence this increase is to due to ovulation induction medications.[20]

For several years, evidence has been accumulating over the association between abnormal genomic imprinting via DNA methylation (or epigenetics) and IVF.[21] Though 9 imprinting syndromes exist, IVF is only linked to the following rarities: Beckwith-Wiedemann syndrome, Angelman syndrome, and maternal hypomethylation syndrome. Nevertheless, knowledge is still limited on the exact etiology, syndrome, and degree of risk, if any.

POLITICAL/SOCIAL/RELIGIOUS FACTORS

IVF has always been at the forefront of technology-stirring debate in many areas including mandatory insurance coverage, same-sex parenting, stem cell research on unused cryopreserved embryos to conquer devastating diseases, high-order multiple births, appropriate parental screening, gender selection, frozen embryo custody battles, and wrong patient embryo transfers. Additional ethical/moral dilemmas involve a couple desiring PGD for human leukocyte antigen–matching to bring into the world a sibling for use in a lifesaving bone marrow transplant on their existing child with leukemia or Fanconi anemia, as well as a patient who desires PGD for *BRCA1* testing of her embryos, though neither she nor her embryos may ever acquire breast cancer. As the field of IVF continues to evolve rapidly, the responsibility of reproductive physicians continues to be *primum non nocere*.

The author reports no actual or potential conflict of interest in relation to this article.

Conclusion of clinical scenario from *The Female Patient*, April 2010, page 27: What fertility treatment options are available to this couple to use the male's sperm? As mentioned, microsurgical epididymal or testicular sperm aspiration, particularly from a patient with obstructive azoospermia, is a simple and exceedingly successful method of obtaining sperm, but it necessitates the use of IVF-ICSI due to immature function of sperm retrieved from the epididymis or testis.

How can they avoid the potential transmission of cystic fibrosis to their offspring? Clearly, PGD is the treatment of choice if the couple desires the use of their eggs and sperm, as opposed to a donor. The caveat and slippery slope are whether the couple would transfer an embryo with carrier status or request discarding all carriers of otherwise healthy embryos for the purpose of ridding it from their family gene pool.

Are there any fertility-sparing options available today? Now more than ever before, the standard procedures of embryo and sperm cryopreservation have been joined by experimentally designated oocyte and ovarian tissue freezing. Only the former new technology has demonstrated reasonably proven efficacy.

REFERENCES

1. Lutjen P, Trounson A, Leeton J, Findlay J, Wood C, Renou P. The establishment and maintenance of pregnancy using in vitro fertilization and embryo donation in a patient with primary ovarian failure. *Nature.* 1984;307(5947):174-175.
2. Trounson A, Leeton J, Besanko M, Wood C, Conti A. Pregnancy established in an infertile patient after transfer of a donated embryo fertilised in vitro. *Br Med J (Clin Res Ed).* 1983;286(6368):835-838.
3. Nagy ZP, Chang CC, Shapiro DB, et al. Clinical evaluation of the efficiency of an oocyte donation program using egg cryo-banking. *Fertil Steril.* 2009;92(2):520-526.
4. Practice Committee of the American Society for Reproductive Medicine; Practice Committee of the Society for Assisted Reproductive Technology. Guidelines on number of embryos transferred. *Fertil Steril.* 2009;92(5):1518-1519.
5. Elster N. Less is more: the risks of multiple births. Institute for Science, Law, and Technology Working Group on Reproductive Technology. *Fertil Steril.* 2000;74(4):617-623.
6. Trounson A, Mohr L. Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo. *Nature.* 1983;305(5936):707-709.
7. Oktay K, Cil AP, Bang H. Efficiency of oocyte cryopreservation: a meta-analysis. *Fertil Steril.* 2006;86(1):70-80.
8. Donnez J, Dolmans MM, Demylle D, et al. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. *Lancet.* 2004;364(9443):1405-1410.

9. Handyside AH, Kontogianni EH, Hardy K, Winston RM. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. *Nature.* 1990;344(6268):768-770.
10. Lewis CM, Pinêl T, Whittaker JC, Handyside AH. Controlling misdiagnosis errors in preimplantation genetic diagnosis: a comprehensive model encompassing extrinsic and intrinsic sources of error. *Hum Reprod.* 2001;16(1):43-50.
11. Practice Committee of Society for Assisted Reproductive Technology; Practice Committee of American Society for Reproductive Medicine. Preimplantation genetic testing: a Practice Committee opinion. *Fertil Steril.* 2008;90(5 Suppl):S136-S143.
12. Trounson A, Wood C, Kausche A. In vitro maturation and the fertilization and developmental competence of oocytes recovered from untreated polycystic ovarian patients. *Fertil Steril.* 1994;62(2):353-362.
13. Wells D, Delhanty JD. Comprehensive chromosomal analysis of human preimplantation embryos using whole genome amplification and single cell comparative genomic hybridization. *Mol Hum Reprod.* 2000;6(11):1055-1062.
14. Sher G, Keskintepe L, Keskintepe M, Maassarani G, Tortoriello D, Brody S. Genetic analysis of human embryos by metaphase comparative genomic hybridization (mCGH) improves efficiency of IVF by increasing embryo implantation rate and reducing multiple pregnancies and spontaneous miscarriages. *Fertil Steril.* 2009;92(6):1886-1894.
15. Seli E, Botros L, Sakkas D, Burns DH. Noninvasive metabolomic profiling of embryo culture media using proton nuclear magnetic resonance correlates with reproductive potential of embryos in women undergoing in vitro fertilization. *Fertil Steril.* 2008;90(6):2183-2189.
16. Pinborg A, Lidegaard O, Freiesleben NC, Andersen AN. Vanishing twins: a predictor of small-for-gestational age in IVF singletons. *Hum Reprod.* 2007;22(10):2707-2714.
17. Hansen M, Bower C, Milne E, de Klerk N, Kurinczuk JJ. Assisted reproductive technologies and the risk of birth defects—a systematic review. *Hum Reprod.* 2005;20(2):328-338.
18. El-Chaar D, Yang Q, Gao J, et al. Risk of birth defects increased in pregnancies conceived by assisted human reproduction. *Fertil Steril.* 2009;92(5):1557-1561.
19. Bonduelle M, Wennerholm UB, Loft A, et al. A multi-centre cohort study of the physical health of 5-year-old children conceived after intracytoplasmic sperm injection, in vitro fertilization and natural conception. *Hum Reprod.* 2005;20(2):413-419.
20. Brinton LA, Moghissi KS, Scoccia B, Westhoff CL, Lamb EJ. Ovulation induction and cancer risk. *Fertil Steril.* 2005;83(2):261-274.
21. Amor DJ, Halliday J. A review of known imprinting syndromes and their association with assisted reproduction technologies. *Hum Reprod.* 2008;23(12):2826-2834.