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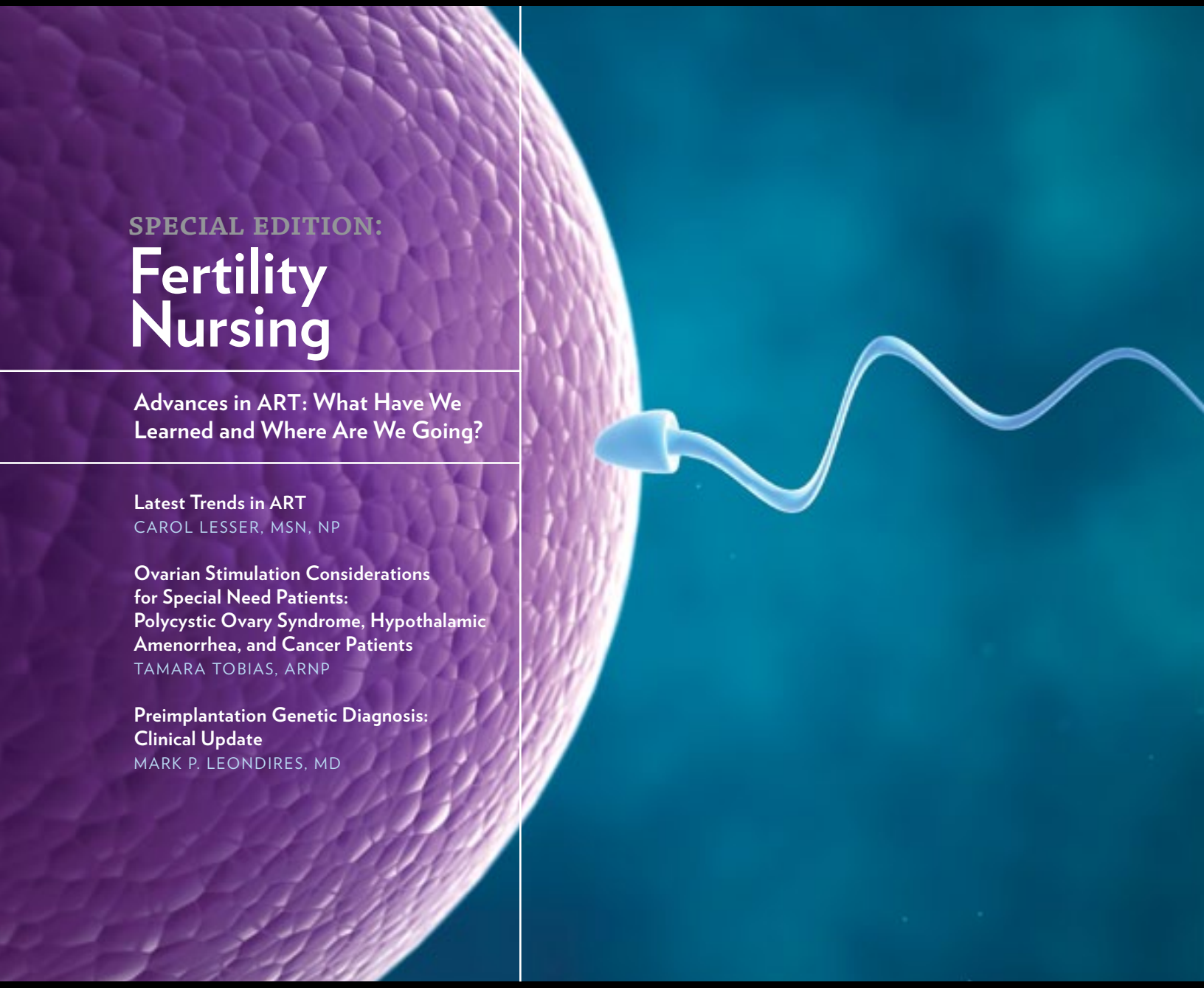

SPECIAL EDITION:

Fertility Nursing

Advances in ART: What Have We Learned and Where Are We Going?

Latest Trends in ART
CAROL LESSER, MSN, NP

Ovarian Stimulation Considerations for Special Need Patients: Polycystic Ovary Syndrome, Hypothalamic Amenorrhea, and Cancer Patients
TAMARA TOBIAS, ARNP

Preimplantation Genetic Diagnosis: Clinical Update
MARK P. LEONDIRES, MD

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Course Description
Fertility nurses play a vital role in the care team as patients navigate the complex journey of infertility. This CE activity is designed to provide fertility nurse professionals with education and enabling skill sets applicable to their critical patient-centered activities, as well as the fertility care team they support. In this issue, the second in a series of four, faculty address the latest trends in ART, stimulation considerations for special needs patients and genetic and chromosomal screening in IVF.

Learning Objectives
At the conclusion of this activity, participants should be able to:
- Select the appropriate ovarian stimulation options for special needs patients
- Evaluate the basic technologies behind embryo testing
- Evaluate how the latest trends and improvements in ART technology can optimize fertility outcomes

Target Audience
These articles are designed to meet the Continuing Medical Education needs of the IVF and fertility nursing professional.

Accreditations and Credit Designations
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This activity is designated by the Pennsylvania State Nurses Association for a maximum of 1 Contact Hour.

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Latest Trends in ART

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The validity of in vitro fertilization (IVF) was proven with the birth of Louise Brown in 1978. Amid heated controversy marked by strong protest from the Catholic Church and public concern for the safety and health of offspring created this way, IVF emerged in a setting of adverse publicity and little support.[1]

Fortunately, the creators of IVF believed in its value and worked painstakingly to overcome the countless obstacles confronting them. As a testament to their perseverance, assisted reproductive technology (ART) is now responsible for more than 1% of the babies born in the U.S.[2] and more than 5 million babies worldwide and is an accepted form of procreation.[3]

Most of us are unaware of the immense challenges the early teams of scientists faced at its inception. While we celebrate the far-reaching accomplishments of our field (as well as the new technologies that hold further promise), we must pay homage to the extraordinary vision and dedication of these innovators who, despite criticism and condemnation, made IVF a reality. Those of us fortunate enough to work in this field stand on the shoulders of these giants.

April 2013 marked the passing of one of these giants: Robert Edwards, who, along with Patrick Steptoe and Jean Purdy comprised the famous dream team that brought the first IVF baby into the world. These 3 individuals set up the first IVF clinic in Bourn Hall, England in 1980, paving the way for the exponential expansion and acceptance of IVF that we have today. Largely due to his genius, after 102 failed IVF cycles, success was finally realized. The death of this extraordinary man came just a few years after he was awarded the Nobel Prize for medicine in 2010, followed by his being knighted in 2011.[4-6]

In the words of Peter Braude, Emeritus Professor of Obstetrics and Gynecology at King's College in London, when describing Robert Edwards, "Few biologists have so positively and practically impacted human kind. Bob's boundless energy, innovative ideas, and resilience, despite the relentless criticism of naysayers, changed the lives of millions of ordinary people who now rejoice in the gift of their own child."[5,6]

We have witnessed the dramatic expansion of IVF since these early days. In the U.S. alone there are more than 400 clinics.[7] Success rates have steadily improved and adjunct treatments that rely on IVF technologies now treat problems that in decades past had no cure. There have been marked improvements in our laboratories, culture media, pharmacologic agents, transfer catheters, and tools that help us select the most competent embryos.

In the words of Robert Edward's chief collaborator, Patrick Steptoe, after Louise Brown's birth, "We are at the end of the beginning, not the beginning of the end."[8] These words have proved to be prophetic as we come to learn that as early as the 1960s the early IVF fathers predicted the therapeutic use of stem cells as well as the possibility of preimplantation genetic diagnosis.

With this forward progress come clinical issues that require our attention. In this article, we will examine the current focus of thought leaders and what we can expect to see in the coming years as we strive to refine and improve the services we offer.

First, we will discuss the problem of multiple pregnancy and the trend towards single embryo transfer (SET) as a way to control multiple pregnancies. We will also review a newer method of oocyte freezing called vitrification and the emergence of commercial egg banks (CEB) as the latest trend in third party reproduction and fertility preservation. We will provide an overview of improvements in comprehensive chromosomal screening (CCS) along with additional methods to improve success rates so that single embryo transfer may be more attractive to our patients.

Over the past few decades IVF has been blamed for the rise in twin and high order multiple births, with concern for the associated increased morbidity and mortality. As a result of this costly personal and public health concern, there has been a shift towards changing protocols and procedures in a direction that favors a healthy singleton birth. In fact, there are several European nations that mandate single embryo transfer in certain patients to deal with this global problem.[9]

Past and current practice evolved from the recognition that not all embryos are created equal. Even as our ability to describe embryos morphologically keeps advancing, we are not yet to the point that we can guarantee that a single embryo will implant and result in a healthy baby. We are very familiar with the limitations of morphological appearance of embryos as a predictor of a healthy implantation.

When it comes to embryos, looks can be deceiving. For this reason it has been customary to transfer more than 1 embryo in a majority of cycles so that success rates remain in the range that the public has come to expect.

While transferring more embryos improves success rates, this comes with the high price of multiples. Also, given the cost of IVF— still prohibitive for many—couples often feel compelled to transfer multiple embryos to maximize the chance of success with a single cycle as a means towards cost containment as some patients can only afford one try. Unfortunately, economic concerns drive many of our medical practices, but not always in the patient's best interest.[10-13]

As we improve our embryo selection techniques, clinicians can more persuasively recommend single embryo transfer, especially in younger, good-prognosis patients.

Studies have shown that if patients are well educated on the subject, they will be more likely to agree with this approach.[14] However, it can be time consuming as the clinician tries to explain why transferring embryos one at a time is the safer approach despite the trade-off in success rate for that cycle.

The nurse's role is critical here. Explaining the risks of multiples is very important. Many patients voice a preference for twins, unaware of the increased risks this implies.[15, 16] Our responsibility is to educate them and explain that transferring a single embryo and placing additional embryos in cryopreservation will result in the same number of implantations over time without the risks associated with multiples.

Patients are often surprised to learn that the average twin delivery occurs a full 5 weeks early, placing them at risk for the problems associated with prematurity with increased morbidity for both the mother and babies as well as a wide range of short- and potentially long-term problems.[17]

Assisted reproductive technology is now responsible for more than 1% of the babies born in the U.S. and more than 5 million babies worldwide.[2]

Experience has taught us that if a patient with an excellent prognosis has several high-grade embryos to transfer, transferring them one at a time will yield at least the same cumulative pregnancy rate as transferring them all at the same time.

In fact, a recent study suggests a higher cumulative pregnancy rate with this approach. The most likely explanation for this is that if a cycle fails, then the physician can make protocol adjustments at the next attempt, which may increase the odds of success in a frozen embryo transfer.[18]

If the endometrial preparation is not ideal, with SET only one embryo will be involved instead of losing several precious embryos in a single transfer. When seen from this perspective, spreading the risk over several cycles makes sense. Our job is to encourage the patient to make the most responsible decision with the least inherent risk.

What else can be done to facilitate implantation and improve success rates with SET? Until recently, emphasis was placed on embryo quality and chromosomal normalcy as the primary determinant of successful implantation and pregnancy. However, increasing attention has turned to two other important factors: endometrial receptivity and better methods for sperm selection.

Looking first at the importance of endometrial maturation and assessment, we understand that ensuring synchrony between the embryo and the uterus is a vital determinant of a healthy and ongoing implantation. There is a critical period called the "window of implantation," approximately 4-7 days after ovulation when a viable embryo is most likely to implant. Our protocols aim to ensure the proper timing of embryo transfer within this critical time frame. In fact, it has been estimated that 15% of failed cycles are due to a uterine receptivity problem, so this factor is receiving increased attention.

While most IVF protocols raise estradiol levels to supraphysiologic levels, there is a concern that beyond a certain point elevated estrogen levels may negatively affect the endometrium, possibly due to a change that affects the window of implantation's timing, causing it to close sooner than in a natural cycle. Similarly, if the progesterone level is raised in the follicular phase above a certain level the endometrium may also prematurely advance and impede implantation.[19, 20]

The importance of synchronization between the endometrium, the maturity of the embryo, and the timing of transfer has led to the development of diagnostic tools to better assess the preparedness of the uterine lining to accept an embryo. Some researchers suggest that the window of implantation may actually vary from patient to patient leading to the postulate that embryo transfers in the future may be personalized as a way to significantly improve implantation rates for a euploid embryo. This concept has been labeled personalized embryo transfer (PET). Further randomized clinical trials are necessary to study this concept.[21]

Another approach to improving the endometrial environment is a trend towards "freeze all," meaning all embryos resulting from a fresh cycle are intentionally cryopreserved for later transfer allowing the uterine lining to recover from any potential ill effects of gonadotropin stimulation. Single embryos can then be returned to the uterine cavity in either a natural cycle or at a later date to an endometrium that has been exogenous estrogen- and progesterone-prepared. In fact, several recent papers report a higher pregnancy rate in frozen versus fresh cycles.[22, 23]

Nurses and physicians may be challenged by patients who are accustomed to the recommendation of a fresh embryo transfer and freezing extra embryos for future use. In states where there is an insurance mandate, it is not permitted to intentionally freeze all embryos from a fresh cycle, so these patients would be counseled to proceed with the fresh cycle, and use their cryopreserved embryos for future cycles.[24]

Another popular trend around the globe is to encourage or only offer blastocyst transfer. While the pendulum has swung back and forth over the past decade regarding the superiority of blastocyst versus cleavage stage (day 3) embryos, currently we see a definite trend towards blastocyst transfer. In part this can be attributed to improvements in blastocyst culture media and methods of cryo-preservation. When performed by centers whose laboratories are proficient working with blastocysts, the pregnancy rates tend to be higher.[25]

Although it has been known for a long time that morphologic criteria for embryo selection has limitations for day 3 and day 5 embryos, there is still more confidence in an expanded blastocyst than a high grade day 3 embryo. By focusing on blastocysts, there is a higher chance of success with a single embryo and fewer additional embryos to cryopreserve. Restricting cryopreservation to the most promising embryos is also desirable given the vast numbers of embryos cryopreserved, many of which will never be used.

For the couple accustomed to freezing extra embryos on day 3, nurses need to prepare them for fewer to freeze. We should emphasis the reproductive efficiency of this approach where less competent embryos are eliminated, allowing the patient to focus efforts on the best embryos. The nurse's role is to set reasonable expectations with a focus on quality over quantity.[26]

There are exciting emerging tools for improved embryo assessment and selection. The concept has been available for years as noninvasive time-lapse imaging is gaining in popularity. Invasive diagnostic methods such as preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS) require the removal of polar bodies, blastomeres or trophectoderm to determine the genetic competency of the embryo.

Studies have shown that if patients are well educated on the subject, they will be more likely to agree with the [single embryo transfer] approach.

Alternative noninvasive screening methods seek to predict embryo competency without disturbing the embryo. In other words, instead of removing the embryo from its controlled and carefully calibrated environment or subjecting it to biopsy, time-lapse imaging devices are incubators that allow for embryo observation without interference. Traditionally, embryologists were limited to several viewings over several days, requiring the removal of the embryo from its incubator. In contrast, time-lapse imaging shoots video every 20 minutes providing 72 daily images detailing embryo development.[27]

We know that the embryo is sensitive to a multitude of variables including temperature and other environmental factors. Less manipulation and disruption is in theory preferable. Preliminary data suggest improved pregnancy rates with this approach, so we can expect to see more incubators that are capable of both noninvasive imaging as well as the ability to measure samples from the embryo's immediate environment to check for key metabolites correlated with a less stressed and higher prognosis embryo. As research reveals crucial markers correlated with euploidy—or normal chromosomes—we can expect to see improvements in culture media, tailored to address an embryo's particular and changing needs over time.[28-30]

In summary, we can anticipate time-lapse imaging offering further insight into noninvasive embryo selection, which will make the recommendation of single embryo transfer more realistic.

As previously mentioned, there is also interest in utilizing and developing better sperm assays beyond the tests we presently rely upon. Studies reveal that sperm play a role in a host of reproductive challenges, including miscarriage and recurrent pregnancy loss as well as (in the case of sperm from older men) development of autism, schizophrenia, and dwarfism.[31]

Until recently, the gold standard for determining the method of insemination, e.g., IVF versus intracytoplasmic sperm injection (ICSI), has been the semen analysis and karyotype. Most significant male factor problems can be detected this way. If either is significantly abnormal, a visit to a reproductive urologist is the proper next step.

However, we have all seen cases where embryo quality is decreased and not easily attributed to either male or female contribution. In some cases the problem

may reside in both parties, but there are now better sperm assessment tools for these difficult cases.

Similar to the limitations seen with the morphologic description of oocytes and embryos, there are also limits to morphologic assessment of sperm. In these cases, further testing may be warranted as the clinician seeks to give the patient or couple answers to why their gametes may be performing suboptimally.

One such tool is the hyaluronan-binding assay (HBA) test that measures hyaluronan-binding capacity. Hyaluronan-binding is an indicator of sperm health and maturity with a normal level >70%. If the level is decreased, further testing or a different approach at the time of ICSI may be helpful. Some centers will recommend physiological intracyto-plasmic sperm injection (PICSI), which enables picking the bound sperm that are more likely to be chromosomally normal.[32]

While PICSI allows for better sperm selection over routine morphologic inspection, this additional step can be costly, which may limit patient adoption for the time being until more cost-effective tests are available.

Let us turn our attention to recent trends in pharmacologic approaches. As our ability to override the normal mechanism of single follicle recruitment through advances in gonadotropin preparations became possible, an emphasis was placed on protocols that stimulated as large a cohort of follicles as possible without causing ovarian hyperstimulation syndrome (OHSS).

In the normal or high responder supra-physiologic estrogen levels are achieved from these multiple follicles. In addition to the risk of OHSS there are other possible untoward consequences of this approach. As previously mentioned, numerous papers describe the potential risk for an adverse affect on the endometrium from significantly elevated estradiol and progesterone levels, although there is no uniform agreement to a specific cut-off level and physician practices vary widely.

This has led to the question of whether milder stimulations might be more physiologic and might perhaps enhance pregnancy rates. Some centers offer mini IVF with an emphasis on gentle stimulation with oral agents such as clomiphene citrate or letrozole or very low-dose gonadotropins. It is interesting to note that this is the same approach used by the original IVF team that achieved success in a natural cycle with a single embryo transfer.[33]

NOTES *from the Program Director*

Monica Moore, MSN, RNC

Advances in cryopreservation techniques have improved the care of patients who are interested in fertility preservation. Now, cancer patients who are about to undergo chemotherapy or radiation treatments, which often damage the ovaries, or women who do not have a male partner, have viable options to preserve their fertility. Until recently, a large attrition rate was expected when thawing oocytes, reducing the number of embryos available to transfer once the eggs are fertilized unless multiple cycles can be undergone to "bank" oocytes. Patients who are diagnosed with cancer cannot "bank eggs" as they usually only have time to do one IVF cycle prior to their cancer treatment. Oocytes which are frozen via vitrification have an excellent thaw rate, now making oocyte cryopreservation a viable option for cancer patients or single women of advancing age who want to be proactive about their fertility.

The advantages of milder stimulation include the potential for better embryo and uterine synchrony, fewer side effects, lower cost, and acceptable success rates in appropriately screened patients. It is difficult to foresee how this approach would be applicable to a majority of patients, but may be appealing to a subset of patients.[34]

Next, there are better screening tests to determine who is a candidate for ART and what the optimal gonadotropin starting dose and protocol would be.

For several decades, our ability to assess ovarian reserve, which refers to the relative quantity and quality of available oocytes, was dependent on day 3 or early follicular phase assessment of FSH and estradiol interpreted in the context of chronologic age. While many centers still rely on "day 3" assessment this is no longer the only or best test, as it is a late stage indicator of decreased ovarian reserve.

Some centers measure antral follicles or "the smallest measurable follicles visible on transvaginal ultrasound" in order to predict responsiveness to gonadotropins and for choosing the best dose for that patient. For example, if a patient has a high number of antral follicles and may be at risk for OHSS, a lower dose would be the judicious choice.

Even more popular is the AMH (anti Mullerian hormone) test, and while not seeking to replace the day 3 FSH blood test, it does provide several advantages. For example, AMH can be drawn on any day in the menstrual cycle, including while on oral contraceptives or when breastfeeding. This is especially convenient when there is a limited time to complete testing, as in the case of the fertility preservation candidates or with oocyte donors who often present for evaluation while on oral contraceptives.[35,36]

While an AMH level can vary both intra- and intercycle, and can be discordant when compared with an individual's FSH level, AMH's ability to predict ovarian reserve is still an improvement over other methods. It is an earlier indicator than FSH of waning ovarian reserve. There are recent data to suggest that in younger woman AMH can predict approximate age of onset of menopause, allowing women to consider pregnancy or fertility preservation at a younger age than they may have otherwise planned.[37] We can expect increased reliance on AMH to screen ovarian reserve given its advantages.

One of the most anxiety-inducing aspects of fertility treatments is the realization for patients that they will have to give themselves daily injections of medications for up to two weeks. Patients commonly ask if there is any way to achieve the same effect of the medications without all of the injections. While no oral medication is as potent as injections, at this point, there is a corifollitropin alpha, currently in phase 3 trials. In a prospective, randomized, double-blind study, the study group was administered a single injection of corifolliltropin alpha, as opposed to the control group who was given daily injections of rFSH for 7 days (on the 8th day, if more medication was needed, each group was given daily rFSH injections until the time of the trigger injection). Researchers found that the clinical pregnancy rate and mean number of mature oocytes retrieved was comparable in both groups. If approved by the FDA, this option may offer patients fewer injections and may prove useful in an REI setting.

Focusing now on the problem of aneuploidy and the inefficiency of human reproduction, it must be remembered that more than half of all oocytes and embryos used in IVF cycles from patients of advanced reproductive age are chromosomally abnormal, contributing to both failed cycles and the heart breaking miscarriage that our patients so frequently endure. The concept that embryos can be tested for aneuploidy prior to implantation is not a new one. In fact, as previously mentioned, the founding fathers of IVF envisioned this tool to maximize a healthy outcome.

Nurses and physicians may be challenged by patients who are accustomed to the recommendation of a fresh embryo transfer and freezing extra embryos for future use.

While advances in the field of PGD and PGS, now referred to as comprehensive chromosomal screening (CCS), are ongoing, there is still debate as to the applicability of these techniques for our patients. While some predict in the foreseeable future that all embryos will be tested prior to transfer, there are those who disagree. Certainly as with other advanced technologies, cost can be prohibitive for some, limiting its universal appeal. However, for the couple who carry a lethal single gene defect, or the couple who have experienced recurrent pregnancy loss, these patients often will not proceed without it.

Recent advances make the possibility of increased use more feasible. PGD worldwide experience includes close to 100,000 PGD cycles performed in more than 100 centers worldwide, the majority 80% for aneuploid testing.[38]

In the late 1980s early work in PGD focused on single gene disorder detection. Fluorescent in situ hybridization (FISH) was used to screen for a limited number of disorders as well as aneuploidy. In the next decade, the scope of PGD widened to help those with failed implantations, recurrent pregnancy loss and advanced reproductive age.

After years of experience with FISH, concerns were raised secondary to the problem of mosaicism, where some of the cells tested were normal and others abnormal, making reliable embryo diagnosis in some cases difficult. There was also concern for potential damage to the embryo by removing one of its few cells.

In the case of a day 3 embryo biopsy, one or two blastomeres are removed from an approximate 8-celled embryo. Concern that one or two blastomeres might not always be representative of the entire embryo led to the development of array-based comparative genomic hybridization (aCGH) with trophectoderm biopsy which offers many advantages over former techniques.

In contrast, sampling the trophectoderm provides many more cells for analysis than one or two blastomeres from cleavage stage embryos. Studies show that euploid blastocysts tested with aCGH have a higher success rate than those untested. Success with this approach suggests that the effect of advanced maternal age disappears when the embryo has under-gone full chromosomal analysis following the transfer of a euploid blastocyst.

There are other advanced techniques in the pipeline such as next generation gene sequencing that provides full chromosome testing, although more work is necessary before this is ready for widespread adoption.[39,40]

A new study in *Reproductive Biomedicine Online* shows that fluid from a blasto-cyst may provide DNA for genetic analysis, avoiding the need for biopsy.[41,42] Comprehensive, non-invasive testing, if affordable, would hold great appeal for our patients.

The freezing process of vitrification is also responsible for the improvement in blastocyst success rates around the world.

Although cryopreservation of gametes and embryos has been practiced for more than 50 years, the recent improvements in vitrification have had profound impact. Today, blastocysts can be cryopreserved and thawed with excellent survival and implantation rates with vitrification.[43]

The history of the process of vitrification is an interesting one and its application to embryos and oocytes has been widely credited with advances in both of these areas. In fact, the rise in private and commercial egg banks can be directly attributed to advances in vitrification.

While human sperm contain only minute amounts of water, the human oocyte is mostly water. The process of freezing water-filled eggs into a solid state resulted in the formation of damaging ice crystals. While the first birth that used a frozen human egg took place in Australia in 1986, the number of eggs required before achieving success was discouraging.

The challenge for researchers was to devise a way to freeze human eggs without the formation of ice crystals. We can thank our Italian colleagues for many of the early advances in this pursuit.[44]

In 1994, Italy passed a law prohibiting the insemination of more than 3 eggs. Scientists recognized that this would negatively impact success rates and cycle efficiency and worked diligently to find a way to freeze extra eggs without damaging them. They focused their efforts on 2 methods: slow freezing and vitrification.

While early research with slow freezing showed limited success, recent improvements and mastery of vitrification have led to the adoption of the technique by most IVF centers. Vitrification results in a glass-like solid without the forming of ice crystals by rapid, flash freezing. It uses more cryoprotectants (similar to antifreeze) than other methods to prevent ice crystal formation. The American Society for Reproductive Medicine (ASRM) issued a recent statement that endorses vitrification and asserts that it is no longer experimental.[45]

Results with vitrification are impressive, with delivery rates reported in experienced centers comparable to that of IVF using fresh eggs. More than 1,500 babies have been born from vitrified eggs worldwide, with no increase in congenital defects.[46]

Vitrification has the potential to revolutionize egg donation and fertility preservation, similar to the impact that ICSI had on treating male factor infertility.[47] It is the main reason for the growth of commercial and private egg banks in the U.S. For those who need third party assistance, the availability of vitrified eggs through egg banks provides an efficient way to proceed, eliminating the need for donor and recipient synchronization. In some cases, the cost is less, making this more affordable. When offered to appropriately screened younger women, it provides a way to preserve fertility until a later date when ready to procreate.[48]

Lastly, we can expect in the coming decade to see advances in the field of oocyte rejuvenation and regeneration as our research scientists take steps to improve the competency of the older oocyte. This will hopefully enable our patients to work with their own gametes while decreasing the risk of aneuploidy and other adverse advanced maternal age-related events.[49]

We can envision a day when this approach could also be applied to sperm so that preconceptually, the healthiest gametes would be selected for procreation. While this raises ethical issues and has the potential for abuse and misuse, we will trust that our field will apply prudence when considering such advancements in the service of helping our patients achieve a healthy pregnancy.

Finally, we should anticipate improvements in every aspect of the IVF cycle. This will hopefully increase the feasibility of a single embryo transfer that results in a healthy singleton pregnancy and increases the appeal and utilization of ART worldwide.

REFERENCES

1. Bavister BD. Early history of in vitro fertilization. *Reproduction*. 2002;124:181-196.

2. Nygren KG, Sullivan E, Zegers-Hochschild F, Mansour R, Ishihara O, Adamson GD, et al. For the International Committee for Monitoring Assisted Reproductive Technology (ICMART). World report: assisted reproductive technology. *Fertil Steril*. 2011;95:2209–2222.

3. Wright VC, Chang J, Jeng G, Macaluso M. Centers for Disease Control and Prevention (CDC). Assisted reproductive technology surveillance—United States, 2005. *MMWR Surveill Summ*. 2008;57:1–23.

4. BBC News, Test-tube baby pioneer Sir Robert Edwards dies April 10, 2013. Available at http://www.bbc.co.uk/news/uk-england-cambridgeshire-22091873. Accessed May 30, 2013.

5. BBC News. Birthday Honours: Test-tube baby pioneer knighted. June 10, 2011. http://www.bbc.co.uk/news/health-13727900. Accessed May 30, 2013.

6. Jones H, Gosden R. Professor Sir Robert Edwards. *Fertil Steril*. 2013;99(7):1799-1800.

7. Society for Assisted Reproductive Technology website. http://www.sart.org/Templates/SearchResults.aspx?q=400.

8. Edwards R, Steptoe P, Birth after the reimplantation of a human embryo. *Lancet*. 1978;2:366.

9. Maheshwari A, Griffiths S, Bhattacharya S. Global variations in the uptake of a single embryo transfer. *Hum Reprod Update*. 2011;17:107-130.

10. Practice Committee of the Society for Assisted Reproductive Technology and Practice Committee of the American Society for Reproductive Medicine. Elective single embryo transfer. *Fertil Steril*. 2012;97:835-842.

11. Kalu E, Thum MY, Abdalla H. Reducing multiple pregnancy in assisted reproductive technology: toward a policy of single blastocyst transfer in younger women. *BJOG*. 2008;115:1143-1150.

12. Henman M, Catt JW, Wood T, Bowman MC, de Boer KA, Jansen RP. Elective transfer of single fresh blastocysts and later transfer of cryostored blastocysts reduces the twin pregnancy rate and can improve in vitro fertilization live birth rate in younger women. *Fertil Steril*. 2005;94:1620-1627.

13. Hojgaard A, Ottosen LD, Kesmodel U, Ingerslev HJ. Patient attitudes towards twin pregnancies and single embryo transfer, a questionnaire study. *Human Reprod*. 2007;22:2673-2678.

14. Hope N, Rombauts L. Can an educational DVD improve the acceptability of elective single embryo transfer? A randomized controlled study. *Fertil Steril*. 2010;94:489–95.

15. Newton C, et al. Factors affecting patients attitudes toward single and multiple-embryo transfer. *Fertil Steril*. 2007;87(2):269-278.

16. Ryan GL, Zhang SH, Dokras A, Syrop CH, van Voorhis BJ. The desire of infertile patients for multiple births. *Fertil Steril*. 2004;81:500-504.

17. Melzer K, McCaffrey C, Berkeley A, Grifo J. Double the transfer equals double the risk: the benefits of elective single embryo transfer. *Fertil Steril*. 2012;97:835-842.

18. Maheshwari A, et al. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: A systematic review and meta-analysis. *Fertil Steril*. 2012 Aug;98(2):368-377.

19. Xu B, Li Z, Zhang H, et al. Serum progesterone level effects on the outcome of in vitro fertilization in patients with different ovarian response: an ananlysis of more than 10,000 cycles. *Fertil Steril*. 2012;97:1321-1327.

20. Elnashar AM. Progesterone rise on the day of hCG administration (premature leutinization) in IVF: an overdue update. *J Assist Reprod Genet*. 2010:27:149-155.

21. Bosch E, Laharia E. Crespo J, et al. Circulating progesterone levels and ongoing pregnancy rates in controlled ovarian stimulation cycles in in vitro fertilization: an analysis of over 4,000 cycles. *Human Reprod*. 2010;25:2092-2100.

22. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfer in normal responders. *Fertil Steril*. 2011;96:344–348.

23. Roque M, Lattes K, Serra S, et al. Fresh transfer versus frozen embryo transfer in in vitro fertilization cycles: a systematic review and meta-analysis. *Fertil Steril*. 2013;99:156-162.

24. Checa M. Freezing all embryos in IVF with transfer in a later non-stimulated cycle may improve outcome. Presented at European Society of Human Reproduction and Embryology, July 2012.

25. The Practice Committee of the American Society of Reproductive Medicine. Blastocyst culture and transfer in clinical assisted reproduction: a committee opinion. *Fertil Steril*. 2013;99:667-672.

26. le Lannou D, Griveau JF, Laurent MC, Gueho A, Veron E, Morcel K. Contribution of embryo cryopreservation to elective single embryo transfer in IVF-ICSI. *Reprod Biomed Online*. 2006;13:368–375.

27. Meseguer M, Rubio I, Cruz M, Basile N, Marcos J, Requena A. Embryo incubation and selection in a time-lapse monitoring system improves pregnancy outcome compared with a standard incubator: a retrospective cohort study. *Fertil Steril*. 2012;98:1481-1489.e10.

28. Katz-Jaffe MG, Gardner DK. Symposium: innovative techniques in human embryo assessment. Can proteomics help to shape the future of human assisted reproduction? *Reprod Biomed Online*. 2008;17:497-501.

29. Campbell A, Fishel S, Bowman N, Duffy S, et al. Retrorospective analysis of outcomes after IVF using an aneuploidy risk model derived from time-lapse imaging without PGS. *Reprod Biomed Online*. 13 May, 2013 (in press)

30. Herrero J, Mesequer M. Selection of high potential embryos using time-lapse imaging: The era of morphokinetics. *Fertil Steril*. 2013;99(4):1030-1034.

31. Schubert C. Male biological clock possibly linked to autism, other disorders. *Nat Med*. 2008;14:1170.29.

32. Sakkas D. Novel technologies for selecting best sperm for IVF and ICSI. *Fertil Steril*. 2013;94(5):1023-1029.

33. Gordon J. Utilization and success rates of unstimulated in vitro fertilization in the United States: an analysis of the Society for Assisted Reproductive Technology database. *Fertil Steril*. 2013. article in press.

34. Verberg MF, Macklon NS, Nargund G, Milder Stimu-lation with IVF. *Human Reprod Update*. 2009.15:13-29.

35. Nelson S. Antimüllerian hormone: Is the writing on the wall for AF count? *Fertil Steril*. 2013;99:1563-1564.

36. Toner JP, Seifer DB. Why we may abandon basal follicle-stimulating hormone testing: a sea change in determining ovarian reserve using antimüllerian hormone. *Fertil Steril*. 2013;99(7):1825-1830.

37. Tehrani FR, Solaymani-Dodaran M, Tohidi M, Gohari MR, Azizi F. Modeling age at menopause using serum concentration of anti mullerian hormone. *J Clin Endocrin Metab*. 2013;98:729-735.

38. Preimplantation Genetic Diagnosis International Society (PGDIS) http://www.pgdis.org/, Accessed June 4, 2013.

39. Treff NR, Fedick A, Tao X, Taylor D, Scott RT. Evaluation of targeted next-generation sequencing-based preimplantation genetic diagnosis of monogenic disease. *Fertil Steril*. 2013;99:1376-1384.

40. Simpson JL, Rechitsky S, Kuliev A. Next generation sequencing for PGD. *Fertil Steril*. 2013;99:1203-1204.

41. Pallini S, Galluzzi L, De Stefani S et al. Genomic DNA in human blastocele fluid. *Reproductive Biomedicine Online*. March 13, 2013.

42. Simpson JL. Cell-free DNA and maternal serum analytes for monitoring embryonic and fetal status. *Fertil Steril*. 2013;99(4):1124-1134.

43. Chen C. Pregnancy after human oocyte cryopreservation. *Lancet*. 1986;1(8486):884-886.

44. Fabbri R, Porcu E, Marsella T, et al. Oocyte cryopreservation. *Human Reprod*. 1998;13 (suppl 4):98-108.

45. The Practice Committee of the American Society of Reproductive Medicine and the Society for the Assisted Reproductive technology. Mature oocyte cryopreservation: a guideline. *Fertil Steril*. 2013;99:37

46. Edgar DH, Gook DA. A critical appraisal of cryopreservation (slow cooling versus vitrification) of human oocytes and embryos. *Human Reprod Update*. 2012;18:536-554.

47. Grifo J, Noyes N. Delivery rate using cryopreserved oocytes is comparable to conventional in vitro fertilization using fresh oocytes: potential fertility preservation for female cancer patients. *Fertil Steril*. 2010;93:391-396.

48. Nagy ZP, Chang CC, Shapiro DB et al. Clinical evaluation of the efficiency of an oocyte donation program using donor egg-banking. *Fertil Steril*. 2009;92:520-526.

49. Gura T. Reproductive biology: fertile mind. *Nature*. 2012;491:318-320.

Ovarian Stimulation Considerations for Special Needs Patients: Polycystic Ovary Syndrome (PCOS), Hypothalamic Amenorrhea, and Cancer Patients

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The individual response to gonadotropins can be highly variable in patients who are considered to be "normal responders." This variability can be even more profound in patients with underlying medical conditions. This article will review ovarian stimulation considerations for patients with polycystic ovary syndrome (PCOS), hypothalamic menorrhea (HA), and cancer.

Polycystic Ovary Syndrome

The European Society of Human Reproduction and Embryology (ESHRE) and the American Society of Reproductive Medicine (ASRM) sponsored PCOS Consensus Workshop Groups in 2003, 2007, 2010, and 2012 which brought together teams of world experts.

In 2003 the group established the diagnostic criteria for PCOS. The consensus for diagnosis was to have 2 of the 3 following criteria: oligoovulation or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries.[1]

In 2007, the focus of the PCOS workshop was the management of infertility. The group of specialists agreed that the importance of lifestyle modifications—especially weight reduction and exercise in overweight women—was imperative as a weight loss as little as 5% of initial body weight improved outcomes.[1]

The first-line treatment for ovulation induction continues to be clomiphene citrate. The second-line of intervention is either the use of gonadotropins or laparoscopic ovarian surgery.[1] If using gonadotropins, the starting dose of 150 IU of follicle stimulating hormone (FSH) is no longer recommended in women with PCOS. A safer protocol is the step-up regimen in which a chronic low dose is used to determine the FSH threshold for follicle development. The concept is to start with a low dose of 37.5 – 50 IU of FSH for 7 – 14 days. If no follicular growth is noted, then increase the dose of FSH, but only by 50% of the initial or previous dose.[1] A step-down approach may also be considered, which achieves the FSH threshold through a loading dose of FSH. However, this approach may require more experienced monitoring to prevent the recruitment of multiple follicles and increased risk of multiple pregnancy. The third-line treatment for patients with PCOS is IVF. Many different stimulation regimens were proposed during the workshop and the most standard protocol from this meeting was the long gonadotropin-releasing-hormone (GnRH) agonist protocol with FSH.[1]

The last PCOS Consensus Workshop Group met in Amsterdam in 2012 and the focus was on current knowledge and gaps in knowledge regarding other health aspects of PCOS. Participants agreed that women with PCOS are at higher risk for adverse outcomes, such as gestational diabetes and hypertension, and infants born to these women may have increased morbidity and mortality.[2] Gaps in our current knowledge brought up interesting points requiring further research. For example, should PCOS patients be screened for diabetes earlier in pregnancy and should they have increased perinatal monitoring?[2] Another important conclusion from this meeting was that there is no evidence for improved live birth rates or decreased pregnancy complications with the use of metformin and that this medication should be restricted to only patients with impaired glucose tolerance.[2]

Prevention of Ovarian Hyperstimulation Syndrome(OHSS)

When undergoing ovarian stimulation for IVF, PCOS patients have a higher rate of cancellation and incidence of OHSS. Thus, the prevention of OHSS is critical for these patients as there are many considerations for ovarian stimulation protocols when faced with the PCOS patient. For example, limiting the FSH starting dose to 150 IU is one approach to fewer small follicles which contribute significantly to OHSS.

Coasting

In past years a method used to prevent OHSS involved coasting. Coasting consist of discontinuing gonadotropins while continuing GnRH agonist therapy when the lead follicle has a mean diameter of 16 mm. The thought is that the mature follicles will progress since they are no longer FSH dependent and the smaller follicles will enter atresia as they are FSH dependent. The trigger with human chorionic gonadotropin (hCG) for final oocyte maturation is then given when the estradiol level is considered safer (<3,000 pg/mL). In 2011, a Cochrane Review of coasting concluded that there was no difference in moderate or severe OHSS with coasting and significantly fewer eggs were retrieved in the coasting group compared to the non-coasting group (P=0.01).[3] Furthermore, there is a significant decrease in implantation rates if coasting is done for 4 or more days; therefore it is not advised to coast more than 3 days.[3-5] Studies by Aboulghar and colleagues concluded that there is evidence coasting may be beneficial but providers should seek other strategies to reduce OHSS rather than coasting.[3-5]

GnRH Trigger for Final Oocyte Maturation

Another strategy to prevent OHSS in patients with PCOS is the use of GnRH agonist therapy to trigger ovulation. GnRH agonist trigger can only be utilized in an antagonist cycle. GnRH induces both a FSH and luteinizing hormone (LH) surge before it down regulates the pituitary. It has a much shorter half-life compared to hCG and causes rapid corpus luteum degeneration, a drop in both LH and FSH from the pituitary, and thereby prevents OHSS.[6-7] This dramatic drop in gonadotropins however, causes a corpus luteal deficiency and a defective luteal phase. Additionally, gonadotpropins used in the follicular phase for ovarian stimulation reduce endogenous LH. In most cycles using the hCG trigger for final oocyte maturation, the hCG can outbalance the low LH because of its long half-life.

In contrast, a GnRH agonist trigger requires aggressive luteal phase support with both estrogen and progesterone. Of note, it is important to point out that the GnRH agonist trigger does not appear to have an impact on oocytes and embryo quality and therefore may be an excellent option for egg donors and the prevention of OHSS in that patient population.[8]

A Cochrane Review of GnRH agonist trigger in 2011 concluded that OHSS was significantly lower; however, there was a decrease in live birth rate, decrease in ongoing pregnancy rate, and increase in miscarriage rate.[3] GnRH agonist trigger was not recommended in fresh autologous cycles, except for patients at high risk of OHSS.[6] Kummer and colleagues looked at factors that predict the probability of a successful clinical outcome after GnRH agonist trigger. They found that patients with a peak estradiol level ≥4,000 pg/mL had statistically significant higher serum LH and higher clinical pregnancy rate than those with a peak estradiol level <4,000 pg/mL.[9]

Women with PCOS are at higher risk for adverse outcomes, such as gestational diabetes and hypertension, and infants born to these women may have increased morbidity and mortality.

GnRH agonist trigger with a "freeze all" is another option to prevent OHSS in high-risk patients with a subsequent frozen embryo transfer. This however may be difficult and inconvenient for patients and may incur additional costs. Therefore, it is important to have the discussion about a potential oocyte or embryo "freeze all" prior to cycle start.

Metformin

Metformin does not improve pregnancy outcomes, but a meta-analysis by Costello and colleagues found that the incidence of OHSS was decreased in patients with PCOS who took metformin.[10] Another randomized, controlled trial by a team led by Palomba looked just at PCOS patients at risk for OHSS.[11] Metformin, 500 mg three times a day, was started with GnRH agonist and stopped with the pregnancy test or start of menses. The authors concluded total OHSS and cancellation rates were significantly reduced in patients treated with metformin.[11] The mechanism of action is unclear but several hypotheses have been suggested. Vascular Endothelial Growth Factor (VEGF) is thought to be one of the main contributors in OHSS. Insulin increases VEGF production, therefore metformin may help by decreasing serum insulin levels which decrease VEGF production.[11]

Low-dose Aspirin

Low-dose aspirin has also been considered for the prevention of OHSS in high-risk patients. Varnagy and colleagues did a randomized clinical trial to evaluate the effect of low-dose aspirin therapy on OHSS.[12] In their study, 2 patients in the OHSS high-risk group had OHSS on low-dose aspirin versus 43 patients who did not receive aspirin.[12] The authors discussed that increased platelet activation strongly correlates with VEGF levels and aspirin may help by inhibiting platelet activation.

Dual Trigger

Lastly, the option of a dual trigger for final oocyte maturation with GnRH agonist and low-dose hCG has been under review. The low-dose hCG may be enough to rescue the luteal function and improve live birth rates as compared with hCG trigger alone. In patients with a peak estradiol level of <4,000 pg/mL, GnRH

agonist was supplemented with 1,500 IU hCG 35 hours after trigger[6, 9, 13] or 1,000 IU hCG at the same time as the GnRH agonist.[9, 13, 14] The dual trigger still requires intensive luteal phase support to optimize pregnancy.[14] Additionally, one must use this process with caution as the addition of hCG still carries a risk of OHSS development. Further studies are needed to evaluate the effectiveness of dual trigger in patients at risk for OHSS.

PCOS patients are at an increased risk for OHSS and stimulation protocols need to be tailored accordingly. Frequent monitoring, low-dose stimulation, and antagonist protocols are effective initial strategies.[15] Having a back-up plan and prior discussion with the patient are also important. For example, a patient may be more receptive to a "freeze all" if the OHSS risks were clearly communicated prior to stimulation start. Alternatively, if a patient is responding lower than expected a dual trigger with close monitoring could be utilized.

Hypothalamic Amenorrhea (HA)

Patients with hypothalamic amenorrhea present a unique challenge to ovarian stimulation because of their dysfunctional hypothalamic-pituitary-ovarian (HPO) axis. In a normal menstrual cycle, FSH and LH stimulate follicular growth in the ovary. The rising estrogen level from the growing follicle provides a negative feedback to the hypothalamus to further regulate FSH and LH from the pituitary. The high estrogen level stimulates a positive feedback and LH release from the pituitary, i.e., the LH surge. The drop in progesterone and estrogen with menses allows FSH secretion from the pituitary to rise again (SEE FIGURE 1).

Patients under chronic stress including excessive exercise, weight loss, or insufficient caloric intake have an overproduction of corticotropin-releasing hormone in the hypothalamus which reduces pulse frequency of FSH and LH.[16] These patients have low circulating estradiol levels and do not get the normal negative feedback in the HPO axis or the positive feedback with the LH surge. HA patients have decreased or absent GnRH, FSH and LH pulses, may have a thin endometrium, and often will not bleed in response to progesterone because they lack the estrogen primed endometrium from estrogen deficiency.[17, 18]

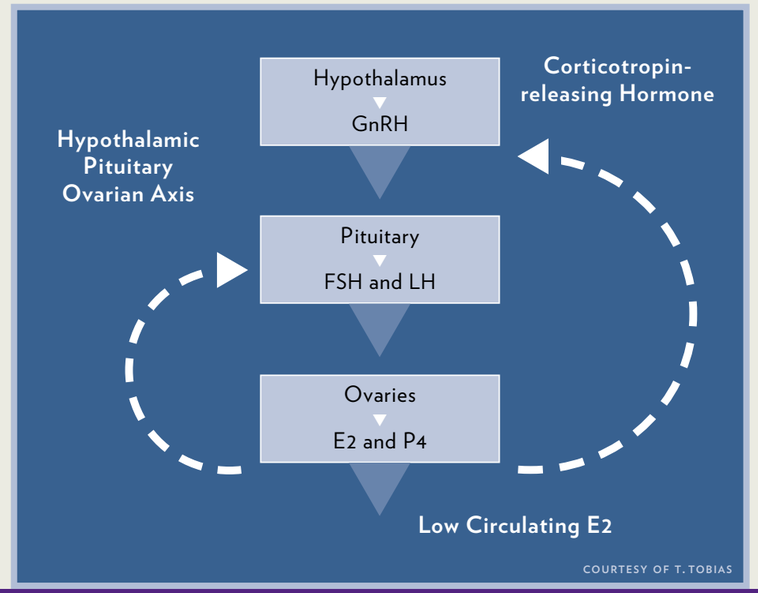
HA patients are also less likely to respond to oral ovulation medications such as clomiphene citrate because it blocks the negative feedback in the HPO axis, which then causes an increase in FSH secretion from the pituitary. Recall that the HPO axis is dysfunctional in these patients and their estrogen level is already very low; thus these medications are seldom effective.

Genazzani and colleagues from Italy evaluated the impact of estriol administration on the HPO axis function and the secretion of gonadotropin in patients with HA.[19] Patients in the study underwent endocrine testing before and after treatment consisting of 8 weeks of oral estriol at 2 mg/day. This study found an increase in LH plasma levels and improved GnRH-induced LH secretion with estriol administration and the authors suggest that weak estrogen therapy may improve HPO dysfunction in patients with HA. Noteworthy, endometrial thickness did not change and none of the patients resumed their menstrual cycle during the 8-week treatment.[19]

Ovulation induction with gonadotropins is often used for HA patients. Ideally, it is preferable to use gonadotropins with both FSH and LH. FSH stimulates granulosa cells and LH stimulates the production of androgens in thecal cells. The androgens are then converted to estrogen by the granulosa cells via aromatization.[17, 18] The goal is a single dominant follicle; therefore, a step-down protocol which more closely mimics a natural cycle is favored. When a lead follicle on ultrasound is >10 mm in mean diameter, the dose is decreased. Ovulation is triggered with hCG as the LH surge may not reliably occur. Additionally, these patients have a true luteal phase deficiency because of their dysfunctional HPO axis and luteal progesterone support is indicated.[20]

FIGURE 1

The Challenge of Treating Patients with Hypothalamic Amenorrhea



COURTESY OF T. TOBIAS

Although patients with hypothalamic amenorrhea pose a unique challenge, one study found that women with this disorder undergoing IVF have a favorable prognosis despite higher stimulation requirements.[21] In this study, 27 hypothalamic amenorrhea patients were compared with tubal factor patients. The authors report total gonadotropin dose and a longer stimulation for hypothalamic amenorrhea patients but there was no difference in the number of oocytes retrieved, fertilization rates, pregnancy rates, multiples, or spontaneous loss.[21] Amenorrheic patients often get discouraged from slow or lack of response to treatment and this study, although small, offers encouragement.

In summary, the treatment of patients with hypothalamic amenorrhea is complicated by a dysfunctional HPO axis and they have a true luteal phase deficiency. More often than not, these patients fail to respond to oral medications and frequently require more intense and expensive treatment. This adds to the overall stress for these patients as well as the increased risk of multiples which must not be overlooked. Furthermore, the treating clinician must be aware of a patient's bone health and risk for osteoporosis because of prolonged low circulating estrogen.

Female Cancer Patients

The diagnosis of cancer is life changing and cancer patients should be informed of available options for fertility preservation and future reproduction. Potential treatment options and considerations for treating cancer patients include age, type of cancer, time to onset of treatment, partner status, and current state of health. Currently, cryopreservation of embryo or oocytes are the only treatments not considered experimental.[22]

The diagnosis of cancer is life changing and cancer patients should be informed of available options for fertility preservation and future reproduction.

Treatment Options

Embryo freezing is a mature technology and clinicians can use available data from the Society for Assisted Reproductive Technologies (SART) and clinical data to counsel patients on success rates. Potential drawbacks to embryo cryopreservation include that it can only be used on post-pubertal females and may require selection of a male partner or the use of donor sperm at a stage in the patient's life when she may not be prepared and/or capable of making long-range plans.

Oocyte cryopreservation is no longer considered experimental and avoids the quandary of embryo storage which may be a concern for some patients. Success rates are steadily improving. In fact, four randomized controlled trials of fresh versus vitrified and warmed oocytes demonstrate similar implantation and clinical pregnancy rates.[22] This option would be for post-pubertal women; however, no decision concerning a partner or donor sperm is required.

Ovarian tissue freezing is considered experimental and involves the removal of an ovary, dividing it into small strips, and then cryopreserving the tissue. Successful pregnancies have been reported worldwide after orthotopic transplantation. Ovarian function resumes between 60–130 days post-transplant and lasts up to 3 years or more.[22] One concern with this method is the possibility of reseeding cancer cells; however, this is the only option for pre-pubertal girls.

Another option, in vitro maturation, is investigational and involves the collection of immature eggs without ovarian stimulation.[23] The immature oocytes are matured for 24–48 hours in vitro and then fertilized by intracytoplasmic sperm injection (ICSI). Live births have been reported with this technology.[22] One consideration with this method would be to do the egg retrieval during the late follicular phase in hopes of retrieving one mature egg as well as the immature ooctytes. This method would require a partner or donor sperm.

If pelvic radiation is a concern, ovarian transposition may be an alternative treatment. The ovaries are surgically moved to sites away from radiation exposure. This procedure may require future transabdominal egg retrieval if IVF is required.

Lastly, GnRH analog treatment has been utilized during chemotherapy with the intent to limit damage to immature follicles and reduce the chance of infertility. This off-label use would be for post-pubertal females and administered as a monthly injection. This too is considered experimental and more studies are needed in regards to benefit on fertility outcomes. However, it is noted that this treatment may help prevent heavy bleeding in patients with thrombocytopenia related to their cancer treatment.[22]

Ovarian Stimulation

The existence of cancer may have a negative impact on the reproductive system.[24] The increased stress hormone and catabolic state associated with malignancy may affect the HPO axis and decrease fertility. A recent meta-analysis compared untreated cancer patients with healthy

age-matched patients and reported lower numbers of total oocytes and mature oocytes.[24] Although more studies are needed, the authors conclude that cancer patients should be counseled that the expected number of oocytes retrieved may be lower compared with healthy patients of similar age.

The stimulation protocol to use for cancer patients is also challenging because there are many additional considerations the clinician must weigh as these patients may not get another chance for a stimulation cycle prior to their cancer treatments. The need to procure a sufficient number of oocytes to maximize their chance of a successful pregnancy in the future is critical. However, the prevention of OHSS is of utmost importance. OHSS in cancer patients could be very serious and potentially delay or complicate their cancer treatment. Additionally, these patients are at higher risk of thromboembolic events because of a hypercoagulable state from their malignancy.[25]

There are numerous ovarian stimulation protocols used in IVF centers. Most cancer patients are treated with a GnRH antagonist-based protocol which provides the shortest interval to egg retrieval as they do not require the 10–14 days of down regulation prior to the initiation of gonadotropin therapy.[25] Nevertheless, a gonadotropin-based protocol still requires waiting for day one of the woman's menstrual cycle before administration of gonadotropins. Noteworthy, a study compared low-dose antagonist (150 IU FSH) and high-dose (FSH >150 IU) regimens in cancer patients. The authors found that higher dose FSH stimulation does not improve outcomes and their findings may support minimal stimulation in young non-fertile women with breast cancer.[26]

Another interesting approach to ovarian stimulation is the concept of starting at any point in the menstrual cycle. Studies are emerging on the use of this method and its potential advantage when time is of the essence. In a random start, stimulation is initiated in either the follicular or luteal phase and allows for egg retrieval within 2 weeks regardless when in the patient's menstrual cycle the initial consult at the infertility practice occured.[27, 28]

The concept of starting therapy during the luteal phase is compelling. In this protocol, the GnRH antagonist down regulates LH and causes a rapid degeneration of the corpus luteum. FSH is started at the same time to avoid exogenous LH that may further support the corpus luteum.

NOTES *from the Program Director*

Monica Moore, MSN, RNC

Ovarian hyperstimulation syndrome (OHSS) is a complication of fertility treatments that continues to challenge those of us who work in an REI setting. Although uncommon, severe OHSS is dangerous, sometimes causing patients to be hospitalized. For those patients who are only mildly or moderately affected, it can still be frustrating because it may necessitate frequent office visits and can delay future pregnancy attempts while waiting for it to resolve. Most clinicians agree that the best way to manage it is to actually prevent it by choosing stimulation protocols that reduce its risk, and possibly using agonist trigger (with or without a reduced dose of hCG), as Tamara Tobias, RN, describes in this article. Cryopreservation techniques have improved to the point that the fresh and frozen/thawed pregnancy rates are similar in many REI centers. Since pregnancy can exacerbate OHSS, clinicians should be proactive in offering embryo cryopreservation as a viable option to reduce OHSS risk while maintaining good pregnancy rates.

The GnRH antagonist causes a rapid fall in progesterone and a menstrual cycle starts within 2–4 days. More studies are needed to evaluate if oocytes/embryos obtained during the luteal phase have pregnancy rates similar to conventional IVF.[27, 28]

Which medication to use for final oocyte maturation is one more important decision for cancer patients. As discussed earlier, it is imperative to obtain a sufficient number of oocytes or embryos for future pregnancy attempts, but on the other hand, OHSS must be avoided. HCG has a longer half-life and may potentiate endogenous production of estrogen in the luteal phase which is not desirable, especially in estrogen-sensitive cancers, and may increase the risk of OHSS.[25] Since all oocytes or embryos are cryopreserved, the concern for luteal-phase deficiency induced with GnRH agonist trigger is not a problem. GnRH agonist trigger may also shorten the luteal phase, which may be advantageous if there is enough time for a possible second cycle and it may reduce the likelihood of residual cysts.[29]

Estrogen-sensitive Cancers

A final hazard regarding patients with estrogen-sensitive cancers should also be addressed: during ovulation hyperstimulation there is a risk that the elevated estradiol level from ovarian stimulation may promote the growth of estrogen-sensitive tumors.[25, 29] Aromatase inhibitors such as letrozole, have been used to reduce the risk of cancer recurrence in postmenopausal women with breast cancer because they minimize circulating estrogen levels in the body. Aromatase inhibitors function by preventing aromatization (thus the name) of androgens to estrogens in granulosa cells, thereby decreasing circulating estrogen.[30] There is a decrease in the negative feedback on the HPO axis and gonadotropin secretion from the pituitary increases. Studies have shown that during ovarian stimulation, aromatase inhibitors may be used with gonadotropins to decrease serum estradiol levels to be closer to that seen in natural cycles.[25, 29] The dose typically used is 2.5–5 mg/day starting during the first 3 days and continuing until the trigger for final oocyte maturation is administrated. Letrozole may be restarted after the egg retrieval and continued until estradiol levels are <50 pg/mL. This medication is well-tolerated and minimizes the risk of high estrogen exposure during ovarian stimulation.

To conclude, cancer patients have several considerations and treatment options which depend on patient age, overall current health status, and type of cancer. Prompt intervention with ovarian stimulation, and eliminating OHSS—thereby maximizing patients' prospects for a future successful pregnancy—are critical components of patient care.

REFERENCES

1. Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Consensus on infertility treatment related to polycystic ovary syndrome. *Fertil Steril*. 2008;89:505-522.

2. Fauser BC, Tarlatzis BC, Rebar RW et al. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. *Fertil Steril*. 2012;97:28-38.

3. D'Angelo A, Brown J, Amso NN. Coasting (withholding gonadotropins) for preventing ovarian hyperstimulation syndrome. *Cochrane Database Syst Rev.* 2011;15(6):CD002811.

4. Aboulghar, M. Agonist and antagonist coast. *Fertil Steril*. 2012;97:523-526.

5. Aboulghar M, Mansour R, Amin Y, Al-Inany H, Aboulghar M, Serour G. A prospective randomized study comparing coasting with GnRH antagonist administration in patients at risk for severe OHSS. *Reprod Biomed Online*. 2007;15:271-279.

6. Humaidan P, Papanikolaou EG, Tarlatzis BC. GnRHa to trigger final oocytes maturation: a time to reconsider. *Hum Reprod*. 2009;24:2389-2394.

7. Kolibianakis EM, Kyrou D, Venetis, CA, Sfontouris I, Lainas TG, Tarlatzis BC. Triggering final oocytes maturation with GnRH agonist in patients with polycystic ovaries undergoing IVF. *Fertil Steril*. 2012;98:S259.

8. Youssef MAFM, Van der Veen F, Al-Inany HG, et al. Gonadotropin-releasing hormone agonist versus HCG for oocytes triggering in antagonist assisted reproductive technology cycles (Review). *Cochrane Database Syst Rev.* 2011;(1):CD008046.

9. Kummer N, Benadiva C, Feinn R, Mann J, Nulsen J, Engmann L. Factors that predict the probability of a successful clinical outcome after induction of oocytes maturation with gonadotropin-releasing hormone agonist. *Fertil Steril*. 2011;96:63-68.

10. Costello MF, Chapman M, Conway U. A systematic review and meta-analysis of randomized controlled trials on metformin co-administration during gonadotropin ovulation induction or IVF in women with polycystic ovary syndrome. *Hum Reprod*. 2006;21:1387-1399.

11. Palomba, S, Falbo A, Carrillo L, et al. Metformin reduces risk of ovarian hyperstimulation syndrome in patients with polycystic ovary syndrome during gonadrotropin-stimulated in vitro fertilization cycles: a randomized, controlled trial. *Fertil Steril*. 2011;96:1384-1390.

12. Varnagy A, Bodis J, Manfai Z, Wilhelm F, Busznyak C, Koppan M. Low-dose aspirin therapy to prevent ovarian hyperstimulation syndrome. *Fertil Steril*. 2010;93:2281-2284.

13. Lee HS, Jeong HJ, Kim MH, Chung MK. GnRH agonist trigger with low dose human chorionic gonadotropin successfully rescues Luteal phase, prevents ovarian hyperstimulation syndrome and improves IVF outcomes. *Fertil Steril*. 2012;98:S52.

14. Engmann, Lawrence. Agonist trigger: what is the best approach? Agonist trigger with aggressive Luteal support. *Fertil Steril*. 2012;97:531-533.

15. Meldrum, David. Preventing severe OHSS has many different facets. *Fertil Steril*. 2012;97:536-538.

16. Kondoh Y, Uemura T, Murase M, Yokoi N, Ishikawa M, Hirahara F. A longitudinal study of disturbances of the hypothalamic-pituitary-adrenal axis in women with progestin-negative functional hypothalamic amenorrhea. *Fertil Steril*. 2001;76:748-752.

17. Practice Committee of the American Society for Reproductive Medicine. Use of exogenous gonadotropins in anovulatory women: a technical bulletin. *Fertil Steril*. 2008;90:S7-S12.

18. Practice Committee of the American Society for Reproductive Medicine. Current evaluation of amenorrhea. *Fertil Steril*. 2008;90:S219-S225.

19. Genazzani A, Meczekalski B, Podfigurna-Stopa A, et al. Estriol Administration modulates luteininzing hormone secretion in women with functional hypothalamic amenorrhea. *Fertil Steril*. 2012;97:483-488.

20. Practice Committee of the American Society for Reproductive Medicine. The clinical relevance of luteal phase deficiency: a committee opinion. *Fertil Steril*. 2012;98:1112-1117.

21. Ruman J, Luna M, Messenger C, Copperman A. Women with hypothalamic amenorrhea undergoing IVF have favorable prognosis despite higher stimulation requirements. *Fertil Steril*. 2008.90:386.

22. Practice Committee of the American Society for Reproductive Medicine. Fertility Preservation. Draft Guidance. In press.

23. Practice Committee of the American Society for Reproductive Medicine. In vitro maturation: a committee opinion. *Fertil Steril*. 2012. In press.

24. Friedler S, Koc O, Gidoni Y, Raziel A, Ron-El R. Ovarian response to stimulation for fertility preservation in women with malignant disease: a systematic review and meta-analysis. *Fertil Steril*. 2012;97:125-133.

25. Cakmak, Hakan and Rosen, Mitchell. Ovarian stimulation in cancer patients. *Fertil Steril*. 2013;99:1476-1484.

26. Lee S, Oktay K. Does higher starting dose of FSH stimulation with letrozole improve fertility preservation outcomes in women with breast cancer? *Fertil Steril*. 2012;98:961-964.

27. Nayak S, Wakim A. Random-start gonadotropin-releasing hormone (GnRH) antagonist-treated cycles with GnRH agonist trigger for fertility preservation. *Fertil Steril*. 2011;96:e51-e54.

28. Von Wolff M, Thaler C, Frambach T, et al. Ovarian stimulation to cryopreserve fertilized oocytes in cancer patients can be started in the luteal phase. *Fertil Steril*. 2009;92:1360-1365.

29. Reddy J, Oktay K. Ovarian stimulation and fertility preservation with the use of aromatase inhibitors in women with breast cancer. *Fertil Steril*. 2012; 98:1363-1369.

30. Garcia-Velasco, Juan. The use of aromatase inhibitors in in vitro fertilization. *Fertil Steril*. 2012;98:1356-1358.

Preimplantation Genetic Diagnosis: Clinical Update

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Since the first application of embryonic testing for X-linked recessive disorders in 1990,[1] two different testing pathways have been developed to evaluate embryos prior to embryo transfer. The first of these pathways, preimplantation genetic diagnosis (PGD), is dedicated to the discovery of disease-causing genetic abnormalities in the human embryo prior to implantation. The second application of preimplantation embryonic diagnosis involves screening embryos for an abnormal number of chromosomes—termed aneuploidy—the most prevalent abnormality found in human embryos. Therefore, preimplantation genetic testing includes two arms; PGD and preimplantation genetic screening (PGS). PGS is an application which involves the biopsy of eggs or embryos prior to transfer into the uterus.

Although there have been tremendous advances in ART worldwide, only approximately 30% of IVF cycles produce a pregnancy. The dominant method for selecting embryos for transfer is the use of visual screening and morphologic assessment by the embryologist and the laboratory. Overall, approximately 80% of embryos transferred do not implant. This issue is a demonstration of the limits of modern embryology methods to assess the implantation potential of an embryo. Historically, the solution to low implantation rates has been to transfer multiple embryos to overcome this issue. A complication from this treatment pathway has been a higher than acceptable rate of multiple pregnancies. Approximately 20% to 30% of all IVF conceptions are multiple pregnancies, which are at increased risk for maternal, fetal, and neonatal complications.

PGD was first used to diagnose embryos carrying an X-linked disorder. Since that time it has been used successfully to evaluate embryos for thousands of genetic disorders. The basis for any preimplantation genetic testing involves completing a biopsy on either an egg or an embryo. There are three different methods of biopsy currently used: polar body biopsy, blastomere biopsy, or trophectoderm biopsy.

Polar Body Biopsy

Polar body biopsy involves removing the first and second polar body which represent the genetic material of the maternal compartment. During the process of egg development, two meiotic divisions occur which result in two sets of haploid maternal DNA that are extruded from the maternal oocyte. These polar bodies are easily accessible for biopsy and the process is not associated with harm to the egg or embryo. Unfortunately, secondary to the low sensitivity and accuracy of this testing, its usefulness in assessing the quality of an embryo is limited. It can, however, have a significant role if the disease process being evaluated is an X-linked disease. Polar body biopsy can be accurate in determining the presence or absence of disease-causing genes derived from the maternal genome. However, secondary to issues of polar body degradation, premature separation of sister chromatids, and lack of representation of the paternal component, is not widely used. In countries where embryo testing has been banned because of ethical concerns, polar body biopsy can be performed prior to fertilization and is the only form of PGD available. There are ongoing clinical trials investigating modern techniques to assess aneuploidy to measure the effectiveness and sensitivity of polar body biopsy. If proven effective, this would represent a method of biopsy which does not affect the embryo and would resolve the ethical issues which are presented by embryo biopsy.[2]

Blastomere Biopsy

After fertilization, the single-cell embryo (zygote) completes multiple mitotic divisions. By the third day of embryonic development, the embryo achieves the 6–8 cell cleavage stage. Cleavage stage biopsy (blastomere biopsy) involves the removal of one blastomere (sometimes two) to obtain a representative sample of the developing embryo. This is currently the most widely used method of biopsy for all forms of preimplantation genetic testing. The process involves removing 10% to 15% of the developing embryo at a time of embryonic development when all cells are considered pluripotent. One limitation of this technique is the small amount of genetic material evaluated. In addition, secondary to mitotic errors a condition known as mosaicism can occur. In this situation the biopsy specimen is not representative of the entire embryo. Concerns about embryo damage and mosaicism have led to the recent use of blastocyst-stage biopsy. In a recent publication a 20% rate of embryo harm was reported with the use of blastomere biopsy.[3] Therefore, secondary to concerns about harm to the embryos and accuracy of the results, blastomere biopsy may be abandoned in favor of trophectoderm biopsy.

Trophectoderm Biopsy

Between the fifth and sixth day of embryonic development, the embryo develops into a blastocyst. The blastocyst is the first step of embryonic differentiation and represents a concept known as genomic activation. To reach this stage an embryo must have specific male and female genes activate. The blastocyst contains two distinct types of cells: the inner cell mass which develops into the fetus, and the trophectoderm which develops into the placenta. Trophectoderm biopsy involves creating a breach in the shell of a cleavage-stage embryo. As pressure develops within the shell-encased embryo, the cells protrude through the breach in the zona pellucida and are easily available for biopsy. This technique allows the collection of 3–10 cells, which is a significantly larger sample than obtained during blastomere biopsy. In addition, this technique does not seem to be associated with embryonic damage as trophectoderm cells are rapidly dividing and the cells that have been removed are quickly replaced.[3] Moving into the future, this method of biopsy is likely to become the most commonly used method. Mosaicism remains a significant concern as to the validity of individual biopsy results, but owing to a larger sample size, it is considered to be less of an issue at this stage than at the cleavage stage (blastomere biopsy).

Technology for Embryonic Testing

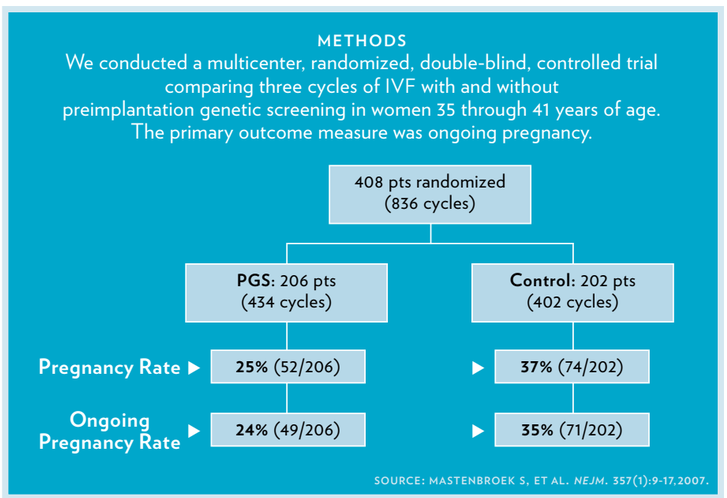
The technology used to complete PGD testing has been developing over the past 25 years. The initial technology relied on a fluorescent probe with which unique aspects of chromosomes were labeled. These colored probes were then viewed under a microscope after bonding with individual chromosomes. This technique is now known as fluorescent in situ hybridization (FISH), and this technology can determine whether there are copies of disease-causing genes present in an embryo. PGD has also been used extensively for patients with chromosomal translocations and inversions. Once again, FISH is used to label the chromosomal segment of interest to ensure the embryo transferred is "balanced." PGD, used to define embryos with disease-causing genes, has proven to be highly successful for families carrying recessive genes, X-linked disorders, translocations, and/or inversions. In situations such as these, a limited amount of signal is needed to identify whether there are 1 or 2 copies of disease genes in place of the proper complement of chromosomal material.

Subsequent to the application of FISH technology, a similar application was used to complete PGS. PGS focuses on identifying aneuploidy in embryos prior to embryo transfer. Initially PGS was used to identify 4 or 5 chromosomal errors, eventually expanded to include as many as 14 chromosomal anomalies. The chromosomes identified were those found commonly in miscarriage specimens and also associated with live births of children with chromosomal abnormalities. The chromosomes involved in these abnormalities were 13, 16, 18, 21, X, and Y.

For approximately 10 years this technology was applied in situations such as advanced maternal age, recurrent pregnancy loss, and recurrent implantation failure. This was despite a lack of prospective randomized trials showing benefit of this technology in helping achieve a live birth. In 2007 a seminal publication by Mastenbroek and colleagues demonstrated in a prospective, randomized fashion that couples using PGS did not have higher live birth rates than patients that did not complete any testing.[4] This paper, along with other reports that failed to support use of FISH technology for PGS, led to the American College of Obstetrics and Gynecology (ACOG), the American Society for Reproductive Medicine (ASRM), and the European Society of Human Reproduction and Endocrinology (ESHRE) to issue statements not recommending the use of PGS. (SEE FIGURE 1)

Researchers were already investigating other methods of PGS at the time of this publication. These involved 24-chromosome screening using amplification of

FIGURE 1
IVF With and Without Preimplantation Genetic Screening



genetic signals to obtain enough information to test for aneuploidy from a single cell or a group of cells. In addition, another technique, comparative genomic hybridization, was developed. This technique involved using fluorescent technology and amplification. Concurrently, methods in the laboratory developed to grow embryos after the cleavage stage to the blastocyst stage, which represented approximately a 250-cell embryo, allowed for the removal of a smaller percentage of cells (5/250=2%) for testing as compared to blastomere biopsy at the cleavage stage (where 10% to 15% of the cells creating the embryo are removed). In addition, there was a dramatic advance in cryopreservation technology centering around vitrification, which allowed embryos to be cryopreserved and thawed with very high rates of survival. These three advances combined with the development of trophectoderm biopsy and amplification technology allowed researchers to obtain good results from a developing preimplantation embryo.

Comprehensive chromosome screening (CCS) is another method for preimplantation aneuploidy assessment. CCS was validated demonstrating significantly increased pregnancy rates following 24-chromosome screening.[5-7] Patients were randomized to transfer either a single screened embryo (single embryo transfer: SET) using CCS as compared to transferring two (double embryo transfer: DET) unscreened embryos. Comparable pregnancy rates (61% for SET and 65% for DET) with significantly higher twin pregnancy rates for DET as opposed to SET (53% vs. 0%, respectively) were observed. Additionally, newborns after SET had a lower rate of admissions to the neonatal intensive care unit (NICU) than those following DET (8% vs. 35%, respectively) and less total time in the NICU as compared to the DET group (13 days vs. 280 days, respectively). For this work Forman and colleagues recently won third-place honors for their paper at the annual 61st ACOG clinical meeting.[8]

It is clear that PGD for the prevention of illness in the context of recessive diseases and chromosomal rearrangements is an essential tool for any in vitro fertilization laboratory.

The technology used in this study involves identifying chromosomes with unique SNPs and quantitative-reverse transcriptase-polymerase chain reaction (qRT-PCR). Using this technology these investigators were able to complete testing on individual blastocysts in less than 4 hours, allowing for a fresh embryo transfer in study patients. At this point, CCS is the most clinically validated form of PGD. It is important to note that CCS utilizes trophectoderm biopsy, which currently is not the dominant form of biopsy for PGD worldwide. The advantages of trophectoderm biopsy include an increased number of cells within the biopsy (which should increase reliability and precision), less invasive nature of the biopsy, the removal of a smaller percentage of the cellular content of the embryo, and the procedure is performed following embryonic genomic activation (i.e., the blastocyst stage, not the cleavage cell stage).

Other forms of 24-chromosome screening include comparative genomic hybridization (CGH) using MicroArray technology, which requires the use of color-coded control DNA, as compared to sample DNA, at multiple sites. This technology is also dependent on whole genomic amplification to obtain enough sample to accurately measure and compare the test sample to the control sample. A developing option for aneuploidy involves a new technology named Next Generation (Next-Gen) sequencing. When one considers that the human genome involves approximately three billion base pairs and is approximately three meters long, it is easy to appreciate that many unique sequences are possible. This approach involves breaking apart the genome into many small pieces, using complementary codes to amplify individual segments, then sequencing those segments, and using a computerized model to re-assemble the codes to identify the presence of 24-chromosomes. This technology, if validated, will provide a less expensive, rapid method of analysis for aneuploidy. Whether the future of 24-chromosome screening involves the MicroArray, qRT-PCR, CGH, or Next-Gen sequencing remains to be defined, but it seems clear that 24-chromosome screening is an essential tool in embryonic assessment. New 24-chromosome screening options support abandoning FISH-based technologies that do not involve 24-chromosome screening. Furthermore, with the ability to safely complete trophectoderm biopsy it is likely to become the route of embryonic biopsy in the future.[7]

Discussion

The development of new technologies with improved accuracy is opening the door for the greater use of preimplantation genetic diagnosis. PGD to test embryos for disease-causing genes and chromosomal inversions and translocations is already considered by many as standard of practice. Subsequent to the publication in 2007 by Mastenbroeks and colleagues,[4] there were concerns about the utilization of PGS in positively affecting clinical outcomes. The patient groups that could benefit from PGS include those of advanced maternal age (>35 years), recurrent pregnancy loss, recurrent IVF failure, and those wanting a single embryo transfer. Since a significant problem associated with ART involves the complications associated with multiple pregnancies, perhaps the single greatest application for PGS is to move the field towards single embryo transfer while maintaining very high pregnancy rates and minimizing the risk of miscarriage.

More widespread preimplantation genetic testing will need continued validation. Biopsy at the blastocyst stage, which only removes trophectoderm cells, the early placenta and avoids the inner cell mass (the cells which become the fetus), in theory should not increase the incidence of infants born with certain medical conditions. However, as embryo biopsy and PGD are relatively new procedures, the possibility of deleterious effects in the long-term can only be ruled out by evaluating the outcome of infants and children born using this technique well into their childhood and beyond. Results from prospective studies observing cohorts of children born following PGD have been reported and are reassuring.[9]

Looking to the future, there are possible societal implications that may hinder widespread acceptance of preimplantation testing, including moral, ethical, and legal concerns. In many countries legislation has been put in place limiting the use of certain types of PGD. As data accumulate with regards to embryo testing and its benefits, these laws will likely be reevaluated. A significant complicating factor associated with 24-chromosome preimplantation screening is the ability to determine gender, in addition to whether the embryo has a normal number of chromosomes. Therefore, preimplantation genetic testing for the purpose of sex selection is available, and it is particularly controversial. There are significant concerns that in cultures where a male child is perceived to be preferable, this technology could lead to gender imbalances on an extremely wide scale. This concern led many countries including Australia, China, India, and Thailand to prohibit the use of gender as a criterion when selecting embryos for transfer. Additionally, there are concerns that the use of ART with PGD will further exacerbate the gap between "the haves and have nots." The issues of income disparity and use of medical technologies represents an ongoing moral and ethical issue worldwide.

NOTES *from the Program Director*

Monica Moore, MSN, RNC

Until recently, it was thought that one way to improve pregnancy rates was to increase the number of embryos transferred. The unfortunate complication of this practice was the resulting increase in the rate of multiple pregnancies in infertility patients. Although many infertile couples think that they desire twins in order to "complete their family" in a single, successful cycle, multiple pregnancies (twins or greater) are the cause of many obstetric and neonatal complications. This is especially true in older (>35 y/o) women, ironically the same subset of infertility patients in whom we would transfer the most embryos. The advances in Preimplantation Screening technologies now allow physicians to offer patients an excellent chance at a healthy pregnancy by transferring just one embryo.

Conclusions

Since the first utilization of preimplantation genetic testing in 1990, more than 20 years have passed, resulting in rapid advances in ART. The advances in technologies have, in turn, led to the development of many techniques and testing options for use on embryos. It is clear that PGD for the prevention of illness in the context of recessive diseases and chromosomal rearrangements is an essential tool for any in vitro fertilization laboratory. PGS remains a modality in development. At what developmental stage should biopsy be performed remains a point of controversy in the literature. Trophectoderm biopsy may be the preferred technique moving forward secondary to the advantages of obtaining more cells for testing, removing only cells that are destined to become placental cells, minimizing the effect of mosaicism, testing only embryos that develop to this stage, and supported by additional documentation of no embryonic harm. With 24-chromosome screening there are clear advantages over previous techniques that tested only a limited amount of chromosomes.

Since the birth of the first IVF child in 1978, the use of technology to overcome infertility has been a controversial topic. The use of preimplantation genetic testing is poised to expand in the future, arising from a desire to decrease multiple pregnancies, reduce the incidence of miscarriage, and to maximize IVF success rates.

REFERENCES

1. 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.

2. Geraedts J, Collins J, Gianaroli L, et al. What next for preimplantation genetic screening? A polar body approach! *Hum Reprod*. 2010;25:575-577.

3. Treff NR, Ferry KM, Zhao T et al. Cleavage stage embryo biopsy significantly impairs embryonic reproductive potential while blastocyst biopsy does not: a novel paired analysis of contransferred biopsied and non-biopsied sibling embryos. *Fertil Steril*. 2011: 96:S2.

4. Mastenbroek S, Twisk M, van Echten-Arends J, et al. In vitro fertilization with preimplantation genetic screening. *N Engl J Med*. 2007;357:9-17.

5. Treff NR, Su J, Tao X, Levy B, Scott RT Jr. Accurate single cell 24 chromosome aneuploidy screening using whole genome amplification and single nucleotide polymorphism microarrays. *Fertil Steril*. 2010;94:2017-2021.

6. Scott RT Jr, Ferry K, Su J, Tao X, Scott K, Treff NR. Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study. *Fertil Steril*. 2012;97:870-875.

7. Scott RT Jr, Tao X, Talyler D, Ferry K, Treff N. A prospective randomized controlled trial demonstrating significantly increased clinical pregnancy rates following 24 chromosome aneuploidy screening: Biopsy and analysis on day 5 with fresh transfer. *Fertil Steril*. 2010;94:S2.

8. Forman EJ. ACOG 61st annual clinical meeting: May 4-8, 2013.

9. Harper JC, Wilton L, Traeger-Synodinos J, et al. The ESHRE PGD Consortium: 10 years of data collection. *Hum Reprod Update*. 2012;18:234-247.

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1. ASRM has recently issued a statement that the following is no longer considered experimental and should be offered to women when appropriate:

O Assisted hatching

O Aromastase inhibitors for ovulation induction

O Growth hormone for improved oocyte recruitment

O Oocyte cryopreservation with vitrification

2. Please rate your level of agreement with the following statements:
(5 = Strongly agree, 1 = Strongly disagree)

A. I understand the role of agonist vs. antagonist in stimulation protocols
5 4 3 2 1

B. I am able to identify IVF protocols for different patient types
5 4 3 2 1

C. I am able to determine the appropriate administration of progesterone in IVF patients in order to increase the success rate of implantation and early embryogenesis
5 4 3 2 1

3. How capable are you in implementing management approaches for the following situations:
(5 = Extremely capable, 1 = Not at all capable, N/A)

A. Low to high responders
5 4 3 2 1

B. Avoidance of OHSS
5 4 3 2 1

C. ER positive breast cancer patients
5 4 3 2 1

4. Please rate your ability to understand the following methods of biopsy currently used for preimplantation genetic testing.
(5 = Extremely capable, 1 = Not at all capable, N/A)

A. Polar body biopsy
5 4 3 2 1

B. Blastomere biopsy
5 4 3 2 1

C. Trophectoderm biopsy
5 4 3 2 1

5. Which of the following is an application which involves the biopsy of eggs or embryos prior to transfer into the uterus:

O Preimplantation genetic diagnosis (PGD)

O Preimplantation genetic screening (PGS)

6. At this point time comprehensive chromosome screening CCS is the most clinically validated form of preimplantation genetic diagnosis.

O True

O False

7. Please rate your confidence in understanding the ethical issues involved in preimplantation testing.
(5 = Extremely confident, 1 = Not at all confident, N/A)
5 4 3 2 1

8. Please rate your ability to counsel patients on the reasoning behind single embryo transfer.
(5 = Extremely capable, 1 = Not at all capable, N/A)
5 4 3 2 1

9. While many tests are available to measure and predict ovarian reserve, the best overall predictor in addition to chronologic age is:

O Clomiphene citrate challenge test

O Inhibin B

O AMH

O FSH and Estradiol on cycle days 1, 2 or 3

10. Based on these articles, what two new patient care strategies do you plan to use that you have not used before?

11. What challenges or barriers might you face as you work to implement these strategies?

Continued on Page 22

ACTIVITY EVALUATION

Answer each question using a scale of **5-1**
(**5** = Strongly agree, **3** = Agree, **1** = Strongly disagree)

1. The articles met the stated objectives. 5 4 3 2 1

2. The articles are relevant to my current clinical practice needs.
5 4 3 2 1

3. Disclosure of faculty relationships with commercial organizations was made available to me before the articles.
O True O False

4. The commercial supporter was acknowledged in print.
O True O False

5. The articles were balanced and free of commercial bias.
O True O False

6. If trade names were used, all product trade names were discussed.
O True O False

7. Any off-label drug use, and/or investigational drug use not yet approved by the FDA was disclosed before or during the activity.
O True O False

8. If you answered "false" to any of the above questions, please provide details below.

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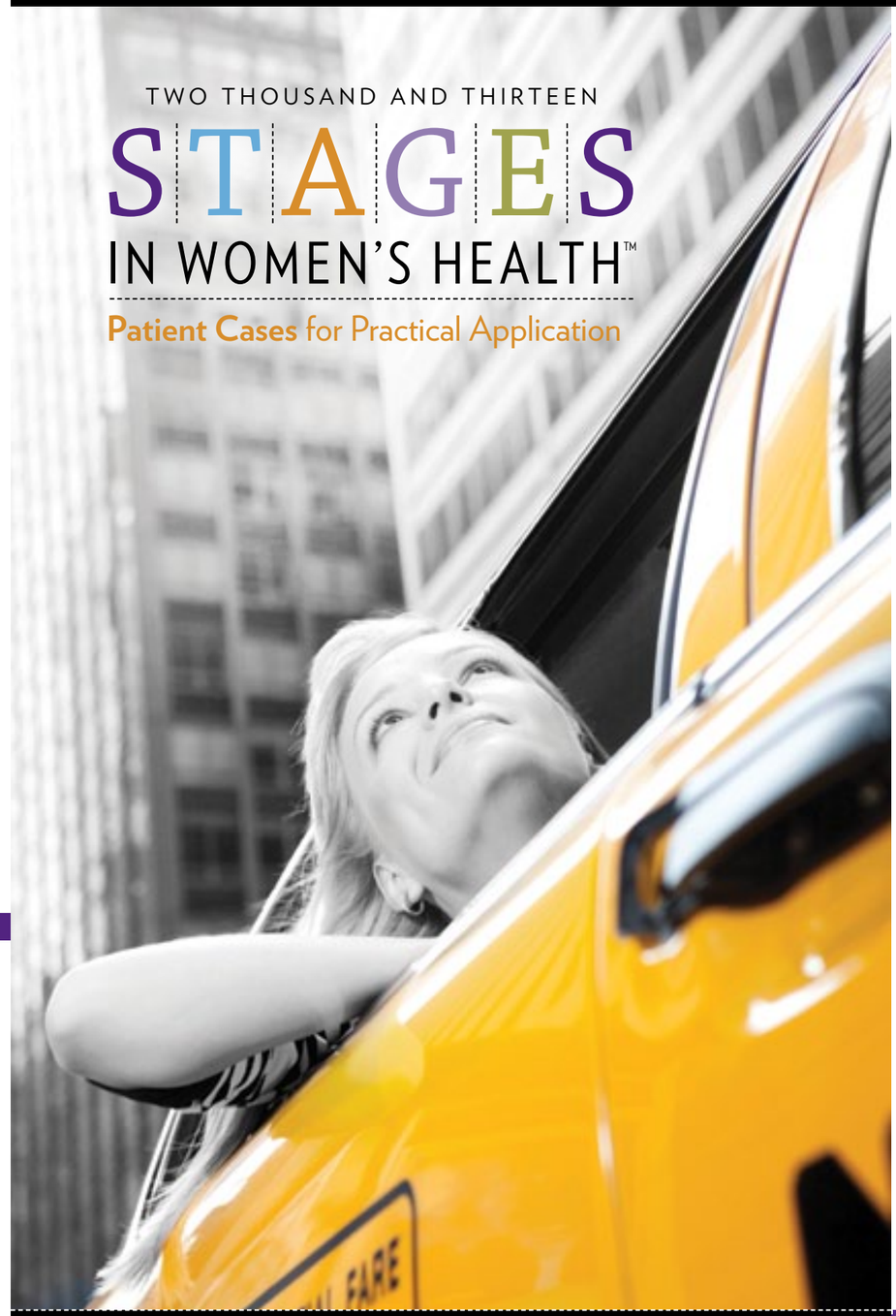

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