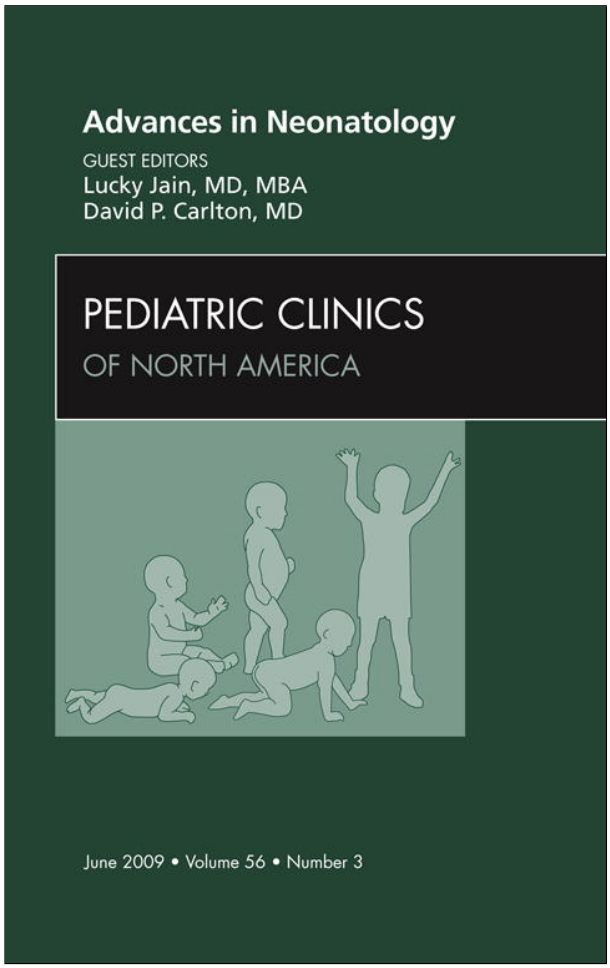

Current Status of the Approach to Assisted Reproduction

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KEYWORDS

• ART • IVF • Infertility • Pediatric • Oncofertility • Fertility preservation

Since the advent of in vitro fertilization (IVF) and the successful birth of Louise Brown on July 25, 1978, more than 3 million children have been born worldwide through assistive reproductive technologies (ART).[1] In 2005, the 422 fertility clinics in the United States reported performing 134,260 ART cycles resulting in 38,910 live births of 52,041 children.[2] Approximately 1% of all births and 18% of all multiple births in the United States are the result of assisted reproductive technologies.[2,3]

Assisted reproductive technology includes all treatments that involve manipulation of both eggs and sperm outside of the body. Most commonly, it refers to in vitro fertilization, although other forms of ART used include gamete intrafallopian transfer (GIFT), zygote intrafallopian transfer (ZIFT), tubal embryo transfer (TET), donor oocyte, and gestational carriers. Other commonly used strategies to treat infertility, including ovulation induction and intrauterine inseminations, are not considered forms of ART. Ovulation induction stimulates multifollicular development with the use of oral or injectable medication without retrieving oocytes. Intrauterine insemination involves the handling of only the male gametes outside of the body.

The purpose of this chapter is to describe the workup of infertility, provide an overview of the indications for ART, and explain the process of ovarian stimulation, sperm recovery, fertilization, and embryo transfer. Complications related to the use of ART

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will be discussed with an emphasis on topics related to the care of the pediatric population. In addition, the use of ART in the preservation of fertility in pediatric and adolescent population undergoing chemotherapy or radiation therapy will be explored.

INDICATIONS AND EVALUATION BEFORE IN VITRO FERTILIZATION

Infertility has been defined as the inability of a couple to conceive after 12 months of unprotected and frequent intercourse. Women who have never conceived a child are classified as having primary infertility, whereas women who have had a prior child and are unable to subsequently conceive are classified as having secondary infertility. According to the 2002 National Survey of Family Growth published by the Center for Disease Control and Prevention, 7.4% of married women not using contraception, ages 15 through 44, were unable to become pregnant in the previous 12 months and thus classified as infertile.[4] They also noted that 7.1% of childless women and 11.9% of all women between the ages of 15 and 44 had received infertility treatment. In practice, patients may present with the inability to achieve pregnancy before 12 months and desire treatment options. Earlier evaluation and treatment may be warranted in women older than 35, because fecundity decreases with increasing maternal age. In addition, earlier evaluation is indicated in patients with suspected reproductive disorders, such as amenorrhea or oligomenorrhea, tubal disease, endometriosis, prior history of chemotherapy, or impending treatment with chemotherapy or radiation to the pelvis.

Although IVF was first developed for the treatment of tubal disease, today IVF is commonly used to treat a variety of causes of infertility (**Box 1**). With the birth of the first child from a cryopreserved embryo in 1983, the use of IVF was expanded to include patients facing chemotherapy or radiation therapy to preserve fertility.[5] The development of intracytoplasmic sperm injection (ICSI) allowed for the treatment of severe male-factor infertility.[6] Advances in molecular genetics and embryo biopsy have permitted IVF to be used to screen for single gene defects and aneuploidy, with the first two reported clinical pregnancies after preimplantation genetic diagnosis reported in 1990.[7] Alternatively, patients who carry a genetic disorder may opt for donor oocytes. Current work in cryobiology in the areas of ovarian tissue freezing and oocyte cryopreservation may further expand the role of ART in fertility preservation in women wishing to delay childbearing and women undergoing chemotherapy or radiation therapy.

The basic evaluation of the female partner includes an assessment of ovarian reserve, uterine abnormalities, and tubal patency. A day three follicle-stimulating hormone, estradiol level, or antral follicle count by ultrasound scan have all been shown to correlate with ovarian responsiveness to gonadotropins in patients undergoing assisted reproductive technologies.[8–14] Recently, serum anti-Mullerian hormone has also been associated with ovarian response.[15] The uterus should also be evaluated for the presence of polyps, submucosal fibroids, and adhesions. This may be performed via a sonohysterogram (saline infusion sonogram), hysterosalpingogram, or hysteroscopy. A hysterosalpingogram has the benefit of evaluating for the presence of hydrosalpinges (abnormally distended fluid filled fallopian tubes), which are most often the result of a prior inflammatory process. If structural abnormalities are found they should be addressed before starting an ART cycle, because structural irregularities of the uterus may interfere with implantation or be responsible for miscarriages. A mock or trial embryo transfer has been advocated to reduce the number of difficult embryo transfers and has been shown to improve IVF outcomes.[16] Male factor infertility can be evaluated through performing a routine semen analysis.

Box 1
Indications for ART

- Tubal factor
- Male factor
- Endometriosis
- Diminished ovarian reserve
- Before radiation or chemotherapy treatment
- Genetically transmitted disease
- Fertility preservation
- Donor egg
- Uterine factor/gestational carrier
  - Uterine anomaly
  - Prior hysterectomy
  - Extensive fibroid disease
  - Asherman's syndrome–intrauterine adhesions
  - Maternal medical condition precluding pregnancy
  - Poor obstetric history
- Ovulatory dysfunction
  - Polycystic ovarian syndrome
  - Hypogonadotropic hypogonadism
- Unexplained infertility

OVARIAN STIMULATION

Assisted reproductive technologies refer to a large number of techniques by which a third party handles oocytes and sperm outside the body to create an embryo that is transferred to an intended recipient. IVF is a method of assisted reproduction whereby a woman's ovaries are stimulated with fertility medications; oocytes are aspirated from ovarian follicles, fertilized in the laboratory, and transferred to the uterus to implant and develop into a pregnancy.

The first IVF birth resulted from the retrieval of a single oocyte from a natural menstrual cycle.[1] To increase the number of embryos available for embryo transfer and cryopreservation, fertility medications often are given to enhance the number of oocytes. The goal of ovarian stimulation is to produce a cohort of uniform follicles that develop and mature in a controlled fashion to allow for retrieval of multiple mature oocytes.

There are various treatment regimens used for superovulation to stimulate multifollicular development in the ovaries. The use of clomiphene citrate and the combination of clomiphene citrate and exogenous gonadotropins have been used for ovarian stimulation; however, the most commonly used approach is exogenous gonadotropins alone. Clomiphene citrate is an oral medication that binds to nuclear estrogen receptors producing estrogen agonist and antagonist effects.[17] It interferes with estrogen negative feedback resulting in an augmentation of gonadotropin-releasing hormone (GnRH) secretion leading to increased pituitary release of gonadotropins, which stimulate ovarian follicular development. In addition to augmenting endogenous GnRH

production, exogenous gonadotropins can be given to promote follicular growth. Typically, exogenous gonadotropins are given in combination with GnRH analogs, either agonist or antagonists in various stimulation protocols. The ''long'' protocol involves the administration of a GnRH agonist during the luteal phase of the preceding menstrual cycle before IVF to down-regulate pituitary production of endogenous gonadotropins, preventing a premature luteinizing hormone (LH) surge during stimulation. Alternatively, the ''short'' or ''flare'' protocol involves starting the GnRH agonists at the beginning of the menstrual cycle to take advantage of the initial surge of endogenous gonadotropins released before down-regulation. Unlike GnRH agonists that down-regulate GnRH receptors, thereby inhibiting endogenous pituitary gonadotropin secretion, GnRH antagonist competitively bind to GnRH receptors suppressing endogenous gonadotropin production and preventing a premature LH surge.[18] Because GnRH antagonists are fast acting, they may be given later in the cycle, therefore, decreasing the number of injections, length of the cycle, and potentially cost.

The stimulation protocol selected for a patient should take into account the patient's age, cause of infertility, ovarian reserve, and prior treatment history. It should also seek to minimize cost and risk while maximizing chance of pregnancy. A Cochrane meta-analysis of 22 trials comparing long and short GnRH agonist protocols found slight superiority in the number of clinical pregnancies using the long protocol; odds ratio (OR) 1.27 (95% confidence interval [CI], 1.04–1.56).[19] Both the short or flare protocol and GnRH antagonist protocols are used for patients with diminished ovarian reserve. A recent randomized study of 90 patients comparing microdose flare and GnRH antagonist protocols found a significantly higher number of oocytes retrieved and implantation rates, as well as a nonstatistically significant trend toward higher clinical pregnancy rates in the flare group.[20] However, GnRH antagonist protocols help suppress premature LH surges, preventing premature ovulation in poor responders, and can be used to decrease the incidence of ovarian hyperstimulation.[21,22]

Ovarian stimulation usually occurs for 8 to 12 days. Response to stimulation can be monitored by measuring serum estradiol levels and serial ultrasound measurements of follicular growth and endometrial thickness. The dose of gonadotropins given can be titrated up or down based on these findings to promote the desired follicular response. When the cohort of developing preovulatory follicles reaches an optimal size, human chorionic gonadotropin (hCG) is given to induce follicular maturation and the ovulatory cascade. Various criteria are used to judge when a cycle has reached the target threshold for hCG administration. Ideally, at least two follicles present measuring 17 to 18 mm in diameter with multiple other follicles 14 to 16 mm in diameter and serum estradiol concentrations consistent with the number of follicles in the cohort (approximately 200 pg/mL per follicle measuring greater than 14 mm) are optimal for hCG administration.[23]

OOCYTE RETRIEVAL

Oocyte retrieval occurs 34 to 36 hours after hCG administration. Oocyte retrievals were initially performed laparoscopically; however, this technique has been largely replaced by transvaginal ultrasound–guided oocyte aspiration, because of safety as well as increased number of oocytes retrieved. After analgesia, an ultrasound probe is inserted into the vagina and used to identify the follicles. A needle is then inserted though a needle guide, and the follicles are sequentially vacuum aspirated. Complications after oocyte retrieval are rare. Despite not being able to use antiseptics, which can be toxic to embryos, the risk of infection after retrieval is low, regardless of

whether prophylactic antibiotics are administered.[24] Hemorrhage from the needle puncture site is uncommon.

FERTILIZATION AND EMBRYO CULTURE

Once oocytes are identified, they are placed in culture medium in an incubator. Oocytes that have extruded the first polar body identify mature metaphase II oocytes from immature oocytes. Oocytes are inseminated 2 to 8 hours after retrieval depending on the method of insemination. Fertilization may be achieved by conventional means whereby each oocyte is incubated with 50,000 to 100,000 motile sperm for 12 to 18 hours. Alternatively, individually selected sperm may be injected into the ooplasm of the oocyte in a process called *intracytoplasmic sperm injection* (ICSI) (**Fig. 1**). Unlike conventional fertilization in which the sperm must penetrate the zona pellucida of the oocyte, undergo the acrosome reaction, and fuse with the oocyte membrane to activate the oocyte, ICSI directly activates the oocyte. ICSI is performed primarily for severe male factor infertility, couples who have previously failed conventional fertilization, to limit contamination with extraneous DNA when performing preimplantation genetic diagnosis, and when small quantities of sperm remain. Fertilization rates of approximately 70% are observed for both conventional fertilization and ICSI. Sperm is most commonly obtained from a partner by masturbation, but ICSI can achieve fertilization with sperm retrieved by other methods. Retrograde ejaculation can be treated with sympathomimetics, or sperm can be recovered from a postejaculatory void after alkalinization of the urine. Men with spinal cord injuries below T6 or psychogenic ejaculatory failure may produce ejaculate with the aid of vibratory stimulation or electroejaculation. In patients with congenital bilateral absence of the vas deferens or uncorrectable duct obstructions, epididymal sperm aspiration can be performed. Testicular sperm extraction and aspiration can be used to retrieve sperm in men with nonobstructive azoospermia.

Fertilization can be confirmed by visualization of two pronuclei and two polar bodies the day after fertilization. The first cleavage division occurs within 24 hours after fertilization. Two days after retrieval, the embryo consists of two to four cells and reaches

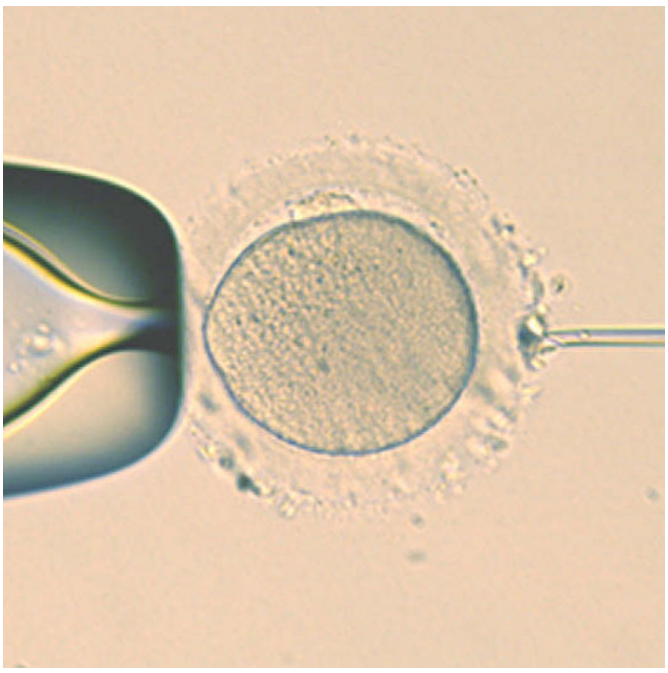

Fig. 1. Intracytoplasmic Sperm Injection (ICSI): a single sperm is injected into an oocyte.

eight cells by day 3.[25] DNA transcription begins between days 3 and 4 when compaction occurs at the 8 to 16 cells stage in which the previously visualized individual cells become an indistinguishable solid mass called a *morula*. By day 5, the embryo is called a blastocyst, which contains a fluid-filled cavity, an inner cell mass, and a trophoblast, which later develops into the placenta (**Fig. 2**).

Before implantation, the blastocyst hatches from the zona pellucida. A variety of embryo micromanipulation techniques called *assisted hatching* have been developed to artificially thin the zona to improve the interaction of the embryo and the endometrium. While multiple studies have failed to show that assisted hatching improves pregnancy rates in all patients, the procedure may be of value in selected populations.[26,27] Current guidelines support assisted hatching in patients with more than two failed IVF cycles, poor embryo quality, or women older than 37 years.[28]

EMBRYO TRANSFER

Embryo transfer typically is performed 3 days after oocyte retrieval at the cleavage stage or 5 days after retrieval at the blastocyst stage. The most common embryo transfer involves placing a catheter via the cervix into the uterus; however, embryos can also be placed in the fallopian tubes laparoscopically. The number of embryos transferred is based on the age of the patient, embryo quality and stage, and other patient characteristics that may influence success. A Cochrane meta-analysis of 16 trials comparing cleavage stage versus blastocyst embryo transfer showed no difference in live birth rates, clinical pregnancy rates, multiple gestations, higher-order multiple pregnancies, or miscarriages.[29] Blastocyst transfer was associated with a higher failure to transfer embryos and lower rates of embryo freezing. Blastocyst transfers have also been associated with a higher rate of monozygotic twins and may be associated with imprinting disorders caused by epigenetic alterations.[30–34] As technology has improved, the rates of success for patients undergoing ART has improved steadily throughout the years. In 2005, 34% of ART cycles resulted in a clinical pregnancy, and 28% of cycles resulted in

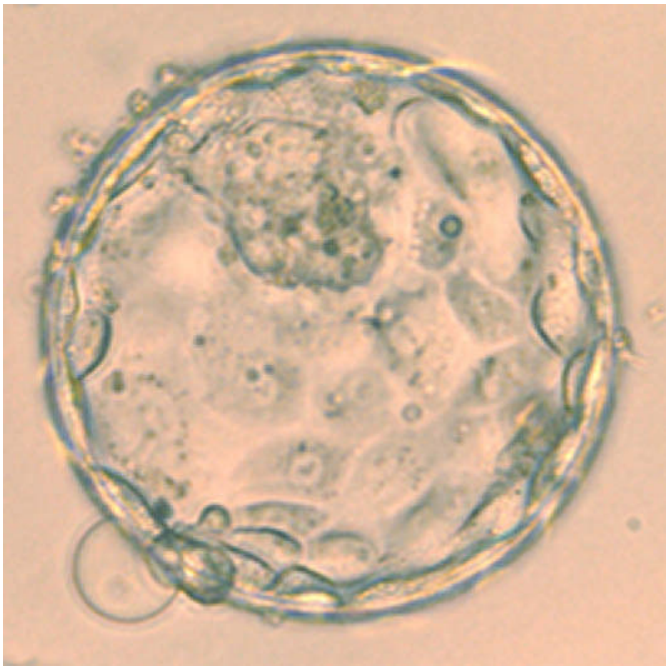

Fig. 2. Day 5 blastocyst: a blastocyst is composed of a fluid filled cavity, an inner cell mass, and an outer ring of trophoblastic cells.

a live birth.[2] Success rates correlate with the age of the patient, with the highest pregnancy rates observed in patients under the age of 35 (**Table 1**).

The ability to cryopreserve embryos has increased the success rate of ART. In patients with a high yield of good-quality embryos, freezing increases the cumulative pregnancy rate per retrieval and decreases the risk of higher-order multiples. Cryopreservation has also been used to decrease the risk of ovarian hyperstimulation syndrome, as the syndrome is worsened and prolonged in conception cycles.[35,36] Success rates of a frozen embryo transfer cycle are approximately one half to two thirds that observed for fresh cycles.[23] The lower pregnancy rate observed in frozen embryo transfers is likely secondary to cell damage from the freezing and thawing process as well as the best quality embryos most likely to result in pregnancy were transferred during the fresh cycle.

PREIMPLANTATION GENETIC DIAGNOSIS

The ability to biopsy embryos and advances in the field of molecular genetics have allowed for pretransfer analysis of the genetic make-up of embryos created through in vitro fertilization in a process called preimplantation genetic diagnosis (PGD). This technique can reduce the risk of conceiving a child with a genetic abnormality as long as the genetic abnormality has been identified and can be tested for in a single cell. Although PGD involves evaluating embryos for a specific known mutation or chromosomal rearrangement carried by one or both of the parents, preimplantation genetic screening (PGS) is a similar technology that screens embryos of presumed chromosomally normal parents for aneuploidy. One of the key advantages of preimplantation genetic testing over conventional prenatal diagnosis is that it allows for early detection of affected embryos before embryo transfer. This reduces the risk of having to decide to terminate an affected pregnancy before it has been established.

Although various methods of biopsying the embryo for PGD or PGS exist, the most commonly used process involves removing one or two cells from an eight-cell cleavage-stage embryo for genetic analysis. Gene analysis typically is performed using either fluorescence in situ hybridization (FISH) or polymerase chain reaction (PCR) for PGD. FISH and comparative genomic hybridization (CGH) are used for PGS. Although PGD has been a major advance for couples at risk of conceiving a child with an inheritable disease, the technology is limited by several factors, including the short amount of time available for analysis before the embryos must be transferred, limited genetic material available for amplification, and false-positive and false-negative results secondary to genetic mosaicism.[37] To limit the risk of misdiagnosis,

Table 1
2005 Centers for Disease Control Assistive Reproductive Technology national success rates

Age	% Pregnancy	% Live Birth
<35	43	37
35–37	36	29
38–40	27	20
41–42	18	11
>42	8	4
All cycles[a]	34	28
Donor egg[a]	55	47

[a] Regardless of age.

chorionic villus sampling (CVS) or amniocentesis is recommended during the pregnancy to confirm PGD results.

PGD was first used for sex selection to prevent the transmission of X-linked diseases to male offspring.[7] PGD has evolved and is now able to detect a variety of chromosomal abnormalities and gene mutations encompassing more than 100 inheritable genetic conditions (**Box 2**). PGS was proposed initially to increase the effectiveness of IVF in women of advanced maternal age by screening for aneuploidies. However, it is not routinely recommended because a recent randomized, controlled study found PGS reduced pregnancy rates and live births.[38] PGD has been used in IVF cycles of couples with known autosomal recessive single gene disorders, such as cystic fibrosis, beta-thalassemia, and sickle cell anemia, as well as autosomal dominant conditions such as hemophilia and myotonic dystrophy.[39–43] This technology has also recently been used to screen for mutations with high penetrance that predispose to cancer. PGD has been used to identify mutations in the APC gene that cause familial adenomatous polyposis coli, the BRCA1 gene that predisposes to breast and ovarian cancer, the NF2 mutation that causes neurofibromatosis 2, and mutations in the tumor suppressor gene p53.[44–48] PGD has detected early adult-onset syndromes associated with gene mutations such Huntington's disease and Alzheimer disease caused by a mutation in valine to leucine at codon 717.[49,50] Rhesus (Rh) D–negative embryos identified by PGD were transferred to an RhD alloimmunized mother with a heterozygous RhD-positive father to prevent hemolytic disease of a newborn.[51] PGD combined with human leukocyte antigen (HLA) typing has been used to establish unaffected donor progeny for cord blood cell transplantation for a sibling affected with Fanconi anemia.[52]

Couples at risk of having a child with a genetic disorder or parents with a child with an affected disorder should be made aware of the possibility of preimplantation genetic diagnosis before attempting to become pregnant. The advantage of PGD over prenatal diagnosis (amniocentesis or chorionic villus sampling) is that the

Box 2
Potential indications for preimplantation genetic diagnosis

- X-linked disorders
- Single gene disorders
  - Autosomal recessive
  - Autosomal dominant
- Structural chromosome abnormalities
  - Translocations
  - Inversions
  - Deletions
- Detection of genetic susceptibility and late-onset disease
  - Huntington's
  - BRCA1/BRCA2
- Human leukocyte antigen (HLA) typing to establish potential donor progeny
  - Fanconi anemia
  - Leukemia

detection of the condition is possible before the pregnancy is established. Unaffected embryos can be transferred, eliminating the need to make the difficult decision whether to terminate the pregnancy in the event of an affected fetus. For parents faced with a child needing a bone marrow transplant who desire further children, PGD offers a unique opportunity to preselect unaffected embryos that can be HLA-compatible with the sibling. Although currently not performed, preimplantation gene therapy may be possible as technology advances.

RISK OF ART

Risks of IVF can be divided into risks associated with the procedure and risks to the pregnancy. Relatively minor bruising at the site of injections and abdominal discomfort as the ovaries enlarge are common. Multiple studies have been performed to evaluate the effect of fertility medication on the risk of ovarian cancer. Although some of the early studies suggested a possible link of fertility medications to ovarian cancer, the most recent studies have failed to establish an association.[53,54] A poor response to medication can result in cycle cancellation of some women, whereas an exaggerated ovarian response is seen in some patients resulting in ovarian hyperstimulation syndrome (OHSS). Bleeding, anesthesia complications, or injury to bowel, bladder, or blood vessels at the time or retrieval are uncommon. Infection related to oocyte retrieval or embryo transfers is uncommon, and prophylactic antibiotics can be given to further minimize risks.

OHSS is one of the more serious complications of IVF with the potential for critical morbidity and death. OHSS is more common in younger patients and correlates with the number of developing follicles, high or rapidly rising serum estradiol levels, and number of retrieved oocytes.[55–57] The degree of ovarian hyperstimulation can be classified into three levels and five grades based on signs, symptoms, ultrasound, and laboratory findings.[58] The use of hCG for inducing oocyte maturation or for luteal support, as well as pregnancy, increases the risk of OHSS.[56,59] Mild hyperstimulation is common and occurs in approximately 30% of IVF cycles. Moderate OHSS is seen in 3% to 6% of cycles, and severe OHSS is observed in 0.25% to 1.8% of IVF cycles. Symptoms may include abdominal distention and discomfort with accompanying nausea, vomiting, and diarrhea. Moderate to severe OHSS can be associated with significant weight gain, ascites, pleural or pericardial effusions, hemoconcentration, electrolyte imbalance, hypovolemia, and thrombosis. If severe enough, this can place a patient at risk for respiratory distress, renal failure, stroke, and death. Hospitalization often is required for intravenous fluid replacement, correction of electrolyte disturbances, initiation of thrombosis prophylaxis, and drainage of third-spaced fluid if symptomatic. Ovarian enlargement seen in OHSS may place the patient at increased risk of ovarian torsion, necessitating urgent surgical correction. OHSS is self-limiting and typically resolves within 14 days.[60] Cryopreservation of all embryos, coined a ‘‘freeze all’’ cycle, with delayed interval transfer decreases the risk of ovarian hyperstimulation yet maintains pregnancy and live birth rates.[36,61]

First trimester bleeding before 13 weeks’ gestation may occur in patients that conceive via ART. Bleeding can be clinically insignificant or may signal an impending miscarriage or ectopic pregnancy. First trimester bleeding requires medical evaluation to determine the cause. Very early spotting within a week after transfer may be associated with implantation bleeding. First trimester bleeding is associated with a twofold relative risk of a spontaneous miscarriage.[62] Bleeding may be associated with a subchorionic hemorrhage in which bleeding occurs between the uterine wall and the chorionic membranes that may leak through the cervical canal resulting in vaginal

bleeding. Subchorionic hemorrhage is associated not only with an increased risk of miscarriage, but also stillbirth, placental abruption, and preterm labor.[63] Vaginal spotting, with or without unilateral pain, may be a harbinger of an ectopic pregnancy. In 2005, 0.6% of all ART cycles corresponding to 1.7% of all resulting pregnancies ended in an ectopic pregnancy.[2] Rates of ectopic pregnancy are increased in women electing for ZIFT in lieu of IVF and those with tubal factor infertility or endometriosis and are related to location of embryo transfer.[64,65] Studies have also found that rates are lower in women who have had a prior live birth, when embryos with a high implantation potential are transferred, in donor egg cycles, and when a gestational carrier or surrogate is used.[64] Although the rate of a spontaneous heterotopic pregnancy, where there is both an intrauterine and extrauterine pregnancy, is a rare event, occurring in 1 in 10,000 pregnancies, this condition can be observed in approximately 1 in 100 ART pregnancies.[66,67]

It has been estimated that 12% to 15% of clinically recognized pregnancies result in miscarriage.[23] This number is an underestimate of the miscarriage rate given the number of early pregnancies that end before they are detected clinically.[68] The risk of miscarriage for pregnancies conceived by ART is comparable with that observed in spontaneous pregnancies, with data from the Centers for Disease Control (CDC) 2005 Assisted Reproductive Technology Success Rate report indicating 15.8% of all pregnancies resulting from ART ended in miscarriage.[2] The risk of miscarriage increases as the age of the mother increases. Although miscarriage rates in 2005 were less than 13% for women under the age of 33 undergoing ART, the miscarriage rate reached 27% by age 40 and was 64% for women over the age of 43.[2] Pregnancies resulting from frozen embryo transfers have a higher rate of miscarriage when compared with pregnancies conceived with freshly fertilized embryos.[69]

ART increases the risk of multiple gestations. Although ART is responsible for approximately 1% of all births in the United States, it accounts for almost 18% of multiple births. In 2005, fresh nondonor ART cycles produced 33,101 pregnancies resulting in 60.4% singleton pregnancies, 28.5% twin pregnancies, and 4.4% triplet and higher-order multiple pregnancies; 6.7% of pregnancies were unknown secondary to early miscarriage.[2] Of the resulting 27,047 births, 68.0% were singletons, 29.6% twins, and 2.4% triplets and higher order multiples. Although most twins are dizygotic, ART increases the risk of monozygotic twinning. This may be related to ovulation induction, assisted hatching, and blastocyst transfer.[31–33,70,71] Multiple-order births pose significant risks to both the mothers and resulting infants of these pregnancies.[72] Mothers are more prone to pregnancy complications including hyperemesis, gestational diabetes, preeclampsia, preterm labor, cesarean delivery, and postpartum hemorrhage. Pregnancies are at a higher risk for intrauterine growth restriction and preterm delivery with increased rates of perinatal and infant morbidity and mortality. Monozygotic twins also have higher rates of congenital anomalies.[73]

The goal of IVF is to maximize chances of pregnancy while minimizing the risk of higher-order multiple gestations. The live birth rate increases as the number of embryos transferred increases to a threshold, after which, only the multiple pregnancy rate increases.[74] The most important factor in predicting success is the age of a women or oocyte donor undergoing IVF retrieval and embryo quality.[75] Other patient characteristics including prior IVF cycle response are also important in guiding the decision of how many embryos to transfer. Therefore, the American Society of Reproductive Medicine (ASRM) has established guidelines that delineate the ideal number of embryos to transfer based on age and prognosis (**Table 2**).[76] The current recommendation for women less than 35 years with a favorable prognosis is to transfer no more than two embryos. Women with a favorable prognosis and a high risk of multiple

Table 2
2006 American Society of Reproductive Medicine embryo transfer guidelines

	Cleavage-Stage Embryos		Blastocysts	
Age[a]	Favorable Prognosis[b]	Others	Favorable Prognosis	Others
<35	1–2	2	1	2
35–37	2	3	2	2
38–40	3	4	2	3
>40	5	5	3	3

In patients with two or more failed cycles or other unfavorable circumstances, additional embryos may be transferred with informed consent.
[a] In donor egg cycles use age of donor.
[b] First IVF cycle, good quality embryos, excess embryos for cryopreservation.

pregnancy may elect for single embryo transfer.[77,78] In the event of a multifetal pregnancy, selective reduction can be performed to reduce fetal number. The decision to perform the procedure is not a suitable option for some couples. Although reduction is associated with higher birth weights and lower rates of preterm delivery, this must be balanced against an approximately 5% risk of loss of the entire pregnancy.[79]

ART is associated with an increased risk of preterm delivery and low birth weight. It has been estimated that the cost to society per preterm birth is $51,600, with the medical cost in the first year being ten times greater for children born preterm compared with full-term babies.[80] Multiple studies and systematic reviews have found an increase in preterm delivery and low birth weight independent of the increase seen in multiple gestations.[81–86] A comparison of the 42,463 infants conceived with assisted reproductive technologies between 1996 and 1997 with the almost 3.4 million infants born in 1997 in the United States showed a 2.6 times (95% CI 2.3–2.6) increase in the risk of a low birth weight term (>37 weeks) infants for ART babies when compared with the general population.[81] This trend was also observed in preterm (<37 weeks) infants with a risk ratio of 1.4 (95% CI 1.3–1.5) but was not observed in twin ART pregnancies with a risk ratio of 1.0 (95% CI 1.0–1.1).[81] This observation has been supported by a recent meta-analysis that compared perinatal outcomes of in vitro fertilization twins with spontaneously conceived twins.[87] It showed that although IVF was associated with a very mild, if any, increase in preterm birth, it was not associated with an increase in low birth weight IVF twins.[87] Although the association between preterm birth and low birth weight infants has been established, the etiology remains unclear. The elevated risk ratio for preterm labor and low birth weight infants for singleton ART pregnancies in 2002 persisted when compared with the general population when analyzed by the cause for infertility, number of embryos cryopreserved, days of embryo culture, or the use of IVF, ICSI, or assisted hatching.[88] Proposed explanation for the difference in outcomes of ART pregnancies when compared with spontaneous pregnancies include the subset of infertility, maternal–fetal exposures to medications and ART procedures, treatment biases and obstetric practices of ART pregnancies, differences in the socioeconomic forces of the two populations, and altered circulating ovarian or uterine protein levels unique to ART pregnancies.

Although early studies failed to show an increased risk of congenital malformations among patients undergoing IVF and ICSI, more recent studies show a modest increase.[89,90] An Australian study found 26 of 301 infants conceived with ICSI (8.6%), 75 of 837 infants conceived with IVF (9.0%), and 168 of 4000 naturally conceived infants (4.2%) had a major birth defect identified before 1 year of age.[91]

This study highlighted that infants conceived with ICSI or IVF were twice as likely to have a major birth defect when compared with spontaneously conceived infants. This increase in congenital birth defects was confirmed by a recent meta-analysis of 25 studies that suggested a 30% to 40% increased risk of birth defects associated with ART.[90] A Swedish study that compared congenital malformations in 9175 infants born via IVF from 1982 to 1997 to the population base control group of 1,690,577 infants born in Sweden over the same period showed IVF was related with an almost threefold increase in neural tube defects, esophageal atresia, small gut atresia, anal atresia, omphalocele, and hypospadias.[92] The excess risk of hypospadias was seen only in infants that resulted from ISCI and was thought to be secondary to paternal subfertility. However, limitations of these studies, as well as other studies that have looked at the risk of congenital malformations in children born as a result of ART, include the relatively small number of defects detected and the possible increased diagnostic vigilance in ART-conceived pregnancies compared with spontaneously pregnancies. Furthermore, the increased risk of birth defects observed may be because of the underlying cause of infertility. Although most studies have used spontaneously conceived pregnancies from women without infertility as controls, a more appropriate control group would be spontaneously conceived pregnancies from women who sought infertility treatment or pregnancies resulting from couples undergoing ART after failed reversal of a tubal ligation or vasectomy.

Men with extreme oligozoospermia or azoospermia have an approximately 25% increased risk of genetic abnormalities.[93] ICSI in this population can result in an increased risk of unbalanced translocations, subsequent infertility of male offspring from inheritance of Y-chromosomal microdeletions, and cystic fibrosis if both parents are carriers of the CFTR gene mutation. A daughter conceived via ICSI from a father with an androgen-receptor gene defect caused by expansion of CAG trinucleotide repeats on the gene located on the X chromosome could have a son in the following generation affected by infertility and Kennedy's disease (spinal and bulbar atrophy).[94,95]

Many studies have looked at both short- and long-term neurologic sequelae in children born after IVF. IVF babies are at an increased risk of developing neurologic problems, especially cerebral palsy. However, this increase is likely secondary to the high frequency of twin pregnancies with associated increased risk of low birth weights and prematurity and not related to IVF directly.[96] A recent meta-analysis of nine studies reported that IVF had an increased risk of cerebral palsy associated with preterm delivery with an odds ratio of 2.18 (95% CI, 1.71–2.77), whereas eight studies looking at autism spectrum disorders, and 30 studies examining developmental delay failed to show any difference.[97] A Danish nationwide cohort study found similar rates of neurologic sequelae and cerebral palsy when twins conceived by assisted reproductive technologies were compared with spontaneously conceived twins or singleton pregnancies conceived by ART.[98] This same study also found similar rates of neurologic sequelae and cerebral palsy in children conceived with ICSI compared with children conceived by IVF with an odds ratios of 1.1 (95% CI, 0.7–1.7) and 0.9 (95% CI, 0.5–1.7), respectively. Multiple studies comparing children born with ICSI to spontaneously conceived children or children conceived by IVF have shown no difference in cognitive, psychomotor, and neurodevelopmental outcomes when examined 2, 5, and 10 years after birth.[99–103]

Assisted reproductive technologies may also influence imprinting disorders through epigenetic modifications. Rather than altering the DNA sequence, epigenetic changes involve modifications in DNA methylation of either maternal or paternal alleles, resulting in altered gene expression. Although nine human imprinting disorders exist, only 3

have been potentially linked to assisted reproductive technologies.[104] Angelman syndrome, a neurogenetic disorder characterized by mental retardation, developmental delays, seizures, jerky movements, hand-flapping, absence of speech, and a happy disposition, is caused by a loss of function of a gene on maternal chromosome 15.[105] A study of two children with Angelman syndrome conceived with ICSI found hypomethylation of the maternal chromosome 15, suggesting ICSI may increase the risk of imprinting disorders in children conceived by ART.[106] Epigenetic alterations LIT1 and H19 on chromosome 11 have also been seen in children with Beckwith-Wiedemann syndrome who were conceived by ART.[107] This syndrome is characterized by macroglossia, macrosomia, midline abdominal wall defects, ear creases/ear pits, and neonatal hypoglycemia.[108] It has been suggested that maternal hypomethylation syndrome may be associated with abnormal imprinting in patients conceived by ART.[104] Animal data in mice has suggested that the length of time in culture and certain culture conditions may predispose to imprinting disorders; however, it is still uncertain whether there is an association in humans.[109–112] Additionally, while the possible association between ART and imprinting disorders has been raised by the above studies, the infrequency of imprinting disorders makes them difficult to study and prone to selection bias. Further research on the effect of embryo culture conditions in humans, as well as large-scale epidemiologic studies with appropriate control groups are needed to clarify the relationship of imprinting disorders with impaired fertility and ART.

FERTILITY PRESERVATION AND ASSISTIVE REPRODUCTIVE TECHNOLOGIES

ARTs offer a unique opportunity to preserve fertility in pediatric and adolescent populations with cancer. Fortunately, survival and cure rates for childhood cancers have increased dramatically. However, both systemic chemotherapy and radiation therapy directed to areas that contain the gonads may result in premature gonadal failure, subfertility, or infertility. Various treatment strategies exist to minimize risk to the gonads and preserve fertility either before or during treatment. Discussions regarding fertility preservation and the available modalities should involve the patient and family and take into account the child's age and cancer type.

Various treatment strategies exist to preserve male fertility both during and before treatment. Although findings from studies in rats suggested gonadotropin-releasing agonist could protect the male gonads from cytotoxic chemotherapy, no benefit has been observed in humans.[113–117] For male patients undergoing radiation therapy, gonads can be shielded or relocated outside the radiation field to the thigh or anterior abdominal wall. The most common technique to preserve male fertility is cryopreservation of sperm obtained from masturbation. Cryopreserved sperm can be used later for intrauterine insemination or intracytoplasmic sperm injection in combination with in vitro fertilization. In boys not psychologically ready to produce a specimen for cryopreservation, penile vibratory stimulation and electroejaculation can be performed under general anesthesia.[118] Sperm production occurs around age 13, with one study showing the rate of spermaturia, a marker for spermarche, occurs in almost 69% of adolescent boys by age 13 compared with less than 1% of boys at age 11.[119] Discussions of masturbation and future fertility should be approached with sensitivity, as this can be a source of embarrassment for adolescent boys. The use of testicular cryopreservation, spermatogonial stem cell transplantation, and in vitro maturation of sperm are experimental. These are areas of ongoing research and may be available in the future when these techniques are developed further.

Similarly, there are a variety of approaches to preserving female fertility during chemotherapy, radiation treatment, and surgery for early-stage tumors. The use of gonadotropin-releasing hormone agonists to reduce chemotherapy-induced ovarian damage may be of benefit. A recent prospective, randomized study in young adult women undergoing combination chemotherapy for breast cancer found women that received gonadotropin-releasing agonists were more likely to have resumption of menses (90% versus 33%), return to spontaneous ovulation (69% versus 26%), and decreased risk of premature ovarian failure (11% versus 67%) compared with controls.[120] Ovarian shielding or ovarian transposition can be performed to minimize radiation exposure for those receiving pelvic radiation.[121–123] Children and women who have not completed childbearing undergoing surgery for ovarian stromal tumors, germ cell tumors, and borderline and stage Ia epithelial ovarian cancers should be offered conservative fertility-sparing surgery.[124–127] This includes exploratory laparotomy with unilateral salingo-oophorectomy and surgical staging, with preservation of the contralateral ovary and uterus.

Before treatment, cryopreservation of embryos, oocytes, and ovarian tissue may be performed.[128] Cryopreservation of embryos and mature oocytes requires postponement of treatment as well as hormonal stimulation. These methods are not recommended for individuals with advanced stage or aggressive cancers or in cases of estrogen receptor sensitive tumors. Although cryopreservation of embryos is a well-established technique commonly used to store surplus embryos from in vitro fertilization cycles, it requires an immediately available partner or the use of donor sperm. Oocyte cryopreservation circumvents the need for a current partner. This procedure is investigational with limited long-term follow-up studies. Although pregnancies have been reported from cryopreserved oocytes, pregnancy rates are lower than those observed using cryopreserved embryos.[129,130] Ovarian tissue cryopreservation is also an investigational technique for fertility preservation with limited safety and efficacy data. The advantage of this method over embryo and oocyte cryopreservation is that ovarian tissue can be obtained quickly without delaying treatment and does not require ovarian stimulation. Similar to oocyte cryopreservation, ovarian cryopreservation does not require a current partner. Immature oocytes from ovarian tissue can be harvested and matured in vitro either before freezing or after thawing. Alternatively, ovarian tissue can be reimplanted after treatment is complete. In addition to possible fertility preservation, autotransplantation also preserves the endocrine function of the ovary.[131–133] Although the use of both oocyte and ovarian tissue cryopreservation holds promise in preserving fertility, their use is investigational. These procedures should only be performed in a research setting with informed consent and institutional review board approval until optimal protocols have been developed and the safety of these methods has been validated.[134,135]

The approach to fertility preservation in children with cancer is a complex issue and requires a comprehensive approach from an interdisciplinary team. It is imperative that the child and family be explained the potential impact of treatment on future fertility potential and made aware of available fertility preservation options. Both informed assent by the child and informed consent of the parents are paramount in establishing a plan for cancer treatment and fertility preservation.[136,137] This requires close communication between the family and the medical team, which may involve pediatricians, medical oncologists, radiation oncologists, oncologic surgeons, reproductive endocrinologists, reproductive biologists, geneticists, embryologists, psychiatrists, and medical ethicists.

SUMMARY

Assisted reproductive technologies are important tools in the clinical armamentarium used to treat both female and male infertility disorders. Preimplantation genetic diagnosis offers couples at risk of having children with inheritable disorders the ability to analyze the genetic make-up of embryos before transfer. For patients undergoing treatment of cancer with chemotherapy or radiation therapy, these technologies offer the potential for the preservation of future fertility. Assisted reproductive technologies are not without risks, and it is imperative to continue to perfect treatment strategies to increase success rates, while at the same time reducing the risk of treatment complications, higher-order multiple pregnancies, and preterm birth. Concerns regarding the possible association of assisted reproductive technologies with congenital malformations and epigenetic modifications have been proposed in the literature. Further research with large-scale, carefully controlled studies is necessary to analyze the outcomes of these pregnancies. As technology evolves, it is likely the clinical applications of assisted reproduction will continue to develop and expand in the future to enhance fertility.

REFERENCES

1. Steptoe PC, Edwards RG. Birth after the reimplantation of a human embryo. Lancet 1978;2(8085):366.
2. Available at: www.cdc.gov/art/art2005/. Accessed Jan 19, 2009.
3. Hamilton BE, Martin JA, Ventura SJ. Births: preliminary data for 2005. Natl Vital Stat Rep 2006;55(11):1–18.
4. Chandra A, Martinez GM, Mosher WD, et al. Fertility, family planning, and reproductive health of U.S. women: data from the 2002 National Survey of Family Growth. Vital Health Stat 2005;23(25):1–160.
5. Trounson A, Mohr L. Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo. Nature 1983;305(5936):707–9.
6. Palermo G, Joris H, Devroey P, et al. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 1992;340(8810):17–8.
7. Handyside AH, Kontogianni EH, Hardy K, et al. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. Nature 1990;344(6268):768–70.
8. Muasher SJ, Oehninger S, Simonetti S, et al. The value of basal and/or stimulated serum gonadotropin levels in prediction of stimulation response and in vitro fertilization outcome. Fertil Steril 1988;50(2):298–307.
9. Scott RT, Toner JP, Muasher SJ, et al. Follicle-stimulating hormone levels on cycle day 3 are predictive of in vitro fertilization outcome. Fertil Steril 1989;51(4):651–4.
10. Toner JP, Philput CB, Jones GS, et al. Basal follicle-stimulating hormone level is a better predictor of in vitro fertilization performance than age. Fertil Steril 1991; 55(4):784–91.
11. Licciardi FL, Liu HC, Rosenwaks Z. Day 3 estradiol serum concentrations as prognosticators of ovarian stimulation response and pregnancy outcome in patients undergoing in vitro fertilization. Fertil Steril 1995;64(5):991–4.
12. Smotrich DB, Widra EA, Gindoff PR, et al. Prognostic value of day 3 estradiol on in vitro fertilization outcome. Fertil Steril 1995;64(6):1136–40.
13. Tomas C, Nuojua-Huttunen S, Martikainen H. Pretreatment transvaginal ultrasound examination predicts ovarian responsiveness to gonadotrophins in in-vitro fertilization. Hum Reprod 1997;12(2):220–3.

14. Chang MY, Chiang CH, Hsieh TT, et al. Use of the antral follicle count to predict the outcome of assisted reproductive technologies. Fertil Steril 1998;69(3):505–10.
15. Seifer DB, MacLaughlin DT, Christian BP, et al. Early follicular serum mullerian-inhibiting substance levels are associated with ovarian response during assisted reproductive technology cycles. Fertil Steril 2002;77(3):468–71.
16. Mansour R, Aboulghar M, Serour G. Dummy embryo transfer: a technique that minimizes the problems of embryo transfer and improves the pregnancy rate in human in vitro fertilization. Fertil Steril 1990;54(4):678–81.
17. Clark JH, Markaverich BM. The agonistic-antagonistic properties of clomiphene: a review. Pharmacol Ther 1981;15(3):467–519.
18. Matikainen T, Ding YQ, Vergara M, et al. Differing responses of plasma bioactive and immunoreactive follicle-stimulating hormone and luteinizing hormone to gonadotropin-releasing hormone antagonist and agonist treatments in postmenopausal women. J Clin Endocrinol Metab 1992;75(3):820–5.
19. Daya S. Gonadotropin releasing hormone agonist protocols for pituitary desensitization in in vitro fertilization and gamete intrafallopian transfer cycles. Cochrane Database Syst Rev 2000;(2):CD001299.
20. Demirol A, Gurgan T. Comparison of microdose flare-up and antagonist multiple-dose protocols for poor-responder patients: a randomized study. Fertil Steril 2008; [epub ahead of print].
21. Leroy I, d'Acremont M, Brailly-Tabard S, et al. A single injection of a gonadotropin-releasing hormone (GnRH) antagonist (Cetrorelix) postpones the luteinizing hormone (LH) surge: further evidence for the role of GnRH during the LH surge. Fertil Steril 1994;62(3):461–7.
22. Ludwig M, Felberbaum RE, Devroey P, et al. Significant reduction of the incidence of ovarian hyperstimulation syndrome (OHSS) by using the LHRH antagonist Cetrorelix (Cetrotide) in controlled ovarian stimulation for assisted reproduction. Arch Gynecol Obstet 2000;264(1):29–32.
23. Speroff L, Fritz MA. Clinical gynecologic endocrinology and infertility. 7th edition. Philadelphia: Lippincott Williams & Wilkins; 2005.
24. Bennett SJ, Waterstone JJ, Cheng WC, et al. Complications of transvaginal ultrasound-directed follicle aspiration: a review of 2670 consecutive procedures. J Assist Reprod Genet 1993;10(1):72–7.
25. Veeck LL. An atlas of human gametes and conceptuses: an illustrated reference for assisted reproductive technology. New York: Parthenon Pub. Group; 1999.
26. Edi-Osagie E, Hooper L, Seif MW. The impact of assisted hatching on live birth rates and outcomes of assisted conception: a systematic review. Hum Reprod 2003;18(9):1828–35.
27. Seif MM, Edi-Osagie EC, Farquhar C, et al. Assisted hatching on assisted conception (IVF & ICSI). Cochrane Database Syst Rev 2006;(1):CD001894.
28. The role of assisted hatching in in vitro fertilization: a review of the literature. A Committee opinion. Fertil Steril 2008;90(Suppl 5):S196–8.
29. Blake D, Proctor M, Johnson N, et al. Cleavage stage versus blastocyst stage embryo transfer in assisted conception. Cochrane Database Syst Rev 2005;(4):CD002118.
30. Sheiner E, Har-Vardi I, Potashnik G. The potential association between blastocyst transfer and monozygotic twinning. Fertil Steril 2001;75(1):217–8.
31. Milki AA, Jun SH, Hinckley MD, et al. Incidence of monozygotic twinning with blastocyst transfer compared to cleavage-stage transfer. Fertil Steril 2003; 79(3):503–6.

32. Jain JK, Boostanfar R, Slater CC, et al. Monozygotic twins and triplets in association with blastocyst transfer. J Assist Reprod Genet 2004;21(4):103–7.
33. Wright V, Schieve LA, Vahratian A, et al. Monozygotic twinning associated with day 5 embryo transfer in pregnancies conceived after IVF. Hum Reprod 2004; 19(8):1831–6.
34. Blastocyst culture and transfer in clinical-assisted reproduction. Fertil Steril 2008;90(Suppl 5):S174–7.
35. Amso NN, Ahuja KK, Morris N, et al. The management of predicted ovarian hyperstimulation involving gonadotropin-releasing hormone analog with elective cryopreservation of all pre-embryos. Fertil Steril 1990;53(6):1087–90.
36. Ferraretti AP, Gianaroli L, Magli C, et al. Elective cryopreservation of all pronucleate embryos in women at risk of ovarian hyperstimulation syndrome: efficiency and safety. Hum Reprod 1999;14(6):1457–60.
37. Preimplantation genetic testing: a Practice Committee opinion. Fertil Steril 2008; 90(Suppl 5):S136–43.
38. Mastenbroek S, Twisk M, van Echten-Arends J, et al. In vitro fertilization with preimplantation genetic screening. N Engl J Med 2007;357(1):9–17.
39. Handyside AH, Lesko JG, Tarin JJ, et al. Birth of a normal girl after in vitro fertilization and preimplantation diagnostic testing for cystic fibrosis. N Engl J Med 1992;327(13):905–9.
40. Ray PF, Kaeda JS, Bingham J, et al. Preimplantation genetic diagnosis of beta-thalassaemia major. Lancet 1996;347(9016):1696.
41. Xu K, Shi ZM, Veeck LL, et al. First unaffected pregnancy using preimplantation genetic diagnosis for sickle cell anemia. JAMA 1999;281(18):1701–6.
42. Grifo JA, Tang YX, Cohen J, et al. Pregnancy after embryo biopsy and coamplification of DNA from X and Y chromosomes. JAMA 1992;268(6):727–9.
43. Sermon K, Lissens W, Joris H, et al. Clinical application of preimplantation diagnosis for myotonic dystrophy. Prenat Diagn 1997;17(10):925–32.
44. Ao A, Wells D, Handyside AH, et al. Preimplantation genetic diagnosis of inherited cancer: familial adenomatous polyposis coli. J Assist Reprod Genet 1998;15(3):140–4.
45. Spits C, De Rycke M, Van Ranst N, et al. Preimplantation genetic diagnosis for cancer predisposition syndromes. Prenat Diagn 2007;27(5):447–56.
46. Abou-Sleiman PM, Apessos A, Harper JC, et al. First application of preimplantation genetic diagnosis to neurofibromatosis type 2 (NF2). Prenat Diagn 2002;22(6):519–24.
47. Verlinsky Y, Rechitsky S, Verlinsky O, et al. Preimplantation diagnosis for neurofibromatosis. Reprod Biomed Online 2002;4(3):218–22.
48. Verlinsky Y, Rechitsky S, Verlinsky O, et al. Preimplantation diagnosis for p53 tumour suppressor gene mutations. Reprod Biomed Online 2001;2(2):102–5.
49. Schulman JD, Black SH, Handyside A, et al. Preimplantation genetic testing for Huntington disease and certain other dominantly inherited disorders. Clin Genet 1996;49(2):57–8.
50. Verlinsky Y, Rechitsky S, Verlinsky O, et al. Preimplantation diagnosis for early-onset Alzheimer disease caused by V717L mutation. JAMA 2002;287(8): 1018–21.
51. Seeho SK, Burton G, Leigh D, et al. The role of preimplantation genetic diagnosis in the management of severe rhesus alloimmunization: first unaffected pregnancy: case report. Hum Reprod 2005;20(3):697–701.
52. Verlinsky Y, Rechitsky S, Schoolcraft W, et al. Preimplantation diagnosis for Fanconi anemia combined with HLA matching. JAMA 2001;285(24):3130–3.

53. Klip H, Burger CW, Kenemans P, et al. Cancer risk associated with subfertility and ovulation induction: a review. Cancer Causes Control 2000;11(4): 319–44.
54. Venn A, Watson L, Lumley J, et al. Breast and ovarian cancer incidence after infertility and in vitro fertilisation. Lancet 1995;346(8981):995–1000.
55. Navot D, Relou A, Birkenfeld A, et al. Risk factors and prognostic variables in the ovarian hyperstimulation syndrome. Am J Obstet Gynecol 1988;159(1):210–5.
56. Enskog A, Henriksson M, Unander M, et al. Prospective study of the clinical and laboratory parameters of patients in whom ovarian hyperstimulation syndrome developed during controlled ovarian hyperstimulation for in vitro fertilization. Fertil Steril 1999;71(5):808–14.
57. Haning RV Jr, Austin CW, Carlson IH, et al. Plasma estradiol is superior to ultrasound and urinary estriol glucuronide as a predictor of ovarian hyperstimulation during induction of ovulation with menotropins. Fertil Steril 1983;40(1):31–6.
58. Golan A, Ron-el R, Herman A, et al. Ovarian hyperstimulation syndrome: an update review. Obstet Gynecol Surv 1989;44(6):430–40.
59. Herman A, Ron-El R, Golan A, et al. Pregnancy rate and ovarian hyperstimulation after luteal human chorionic gonadotropin in in vitro fertilization stimulated with gonadotropin-releasing hormone analog and menotropins. Fertil Steril 1990;53(1):92–6.
60. Whelan JG III, Vlahos NF. The ovarian hyperstimulation syndrome. Fertil Steril 2000;73(5):883–96.
61. Pattinson HA, Hignett M, Dunphy BC, et al. Outcome of thaw embryo transfer after cryopreservation of all embryos in patients at risk of ovarian hyperstimulation syndrome. Fertil Steril 1994;62(6):1192–6.
62. Pezeshki K, Feldman J, Stein DE, et al. Bleeding and spontaneous abortion after therapy for infertility. Fertil Steril 2000;74(3):504–8.
63. Ball RH, Ade CM, Schoenborn JA, et al. The clinical significance of ultransonographically detected subchorionic hemorrhages. Am J Obstet Gynecol 1996; 174(3):996–1002.
64. Clayton HB, Schieve LA, Peterson HB, et al. Ectopic pregnancy risk with assisted reproductive technology procedures. Obstet Gynecol 2006;107(3):595–604.
65. Nazari A, Askari HA, Check JH, et al. Embryo transfer technique as a cause of ectopic pregnancy in in vitro fertilization. Fertil Steril 1993;60(5):919–21.
66. Reece EA, Petrie RH, Sirmans MF, et al. Combined intrauterine and extrauterine gestations: a review. Am J Obstet Gynecol 1983;146(3):323–30.
67. Tal J, Haddad S, Gordon N, et al. Heterotopic pregnancy after ovulation induction and assisted reproductive technologies: a literature review from 1971 to 1993. Fertil Steril 1996;66(1):1–12.
68. Wilcox AJ, Weinberg CR, O'Connor JF, et al. Incidence of early loss of pregnancy. N Engl J Med 1988;319(4):189–94.
69. Schieve LA, Tatham L, Peterson HB, et al. Spontaneous abortion among pregnancies conceived using assisted reproductive technology in the United States. Obstet Gynecol 2003;101(5 Pt 1):959–67.
70. Derom C, Vlietinck R, Derom R, et al. Increased monozygotic twinning rate after ovulation induction. Lancet 1987;1(8544):1236–8.
71. Schieve LA, Meikle SF, Peterson HB, et al. Does assisted hatching pose a risk for monozygotic twinning in pregnancies conceived through in vitro fertilization? Fertil Steril 2000;74(2):288–94.
72. Schieve LA, Peterson HB, Meikle SF, et al. Live-birth rates and multiple-birth risk using in vitro fertilization. JAMA 1999;282(19):1832–8.

73. Schinzel AA, Smith DW, Miller JR. Monozygotic twinning and structural defects. J Pediatr 1979;95(6):921–30.
74. Templeton A, Morris JK. Reducing the risk of multiple births by transfer of two embryos after in vitro fertilization. N Engl J Med 1998;339(9):573–7.
75. Shulman A, Ben-Nun I, Ghetler Y, et al. Relationship between embryo morphology and implantation rate after in vitro fertilization treatment in conception cycles. Fertil Steril 1993;60(1):123–6.
76. Guidelines on number of embryos transferred. Fertil Steril 2006;86(Suppl 5): S51–2.
77. Strandell A, Bergh C, Lundin K. Selection of patients suitable for one-embryo transfer may reduce the rate of multiple births by half without impairment of overall birth rates. Hum Reprod 2000;15(12):2520–5.
78. Martikainen H, Tiitinen A, Tomas C, et al. One versus two embryo transfer after IVF and ICSI: a randomized study. Hum Reprod 2001;16(9):1900–3.
79. Stone J, Ferrara L, Kamrath J, et al. Contemporary outcomes with the latest 1000 cases of multifetal pregnancy reduction (MPR). Am J Obstet Gynecol 2008; 199(4):e401–4.
80. Behrman RE, Butler AS. Institute of medicine (US). Committee on understanding premature birth and assuring healthy outcomes. Preterm birth: causes, consequences, and prevention. Washington, DC: National Academies Press; 2007.
81. Schieve LA, Meikle SF, Ferre C, et al. Low and very low birth weight in infants conceived with use of assisted reproductive technology. N Engl J Med 2002; 346(10):731–7.
82. McGovern PG, Llorens AJ, Skurnick JH, et al. Increased risk of preterm birth in singleton pregnancies resulting from in vitro fertilization-embryo transfer or gamete intrafallopian transfer: a meta-analysis. Fertil Steril 2004;82(6):1514–20.
83. Jackson RA, Gibson KA, Wu YW, et al. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. Obstet Gynecol 2004;103(3):551–63.
84. Helmerhorst FM, Perquin DA, Donker D, et al. Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. BMJ 2004;328(7434):261.
85. Halliday J. Outcomes of IVF conceptions: are they different? Best Pract Res Clin Obstet Gynaecol 2007;21(1):67–81.
86. Wang YA, Sullivan EA, Black D, et al. Preterm birth and low birth weight after assisted reproductive technology-related pregnancy in Australia between 1996 and 2000. Fertil Steril 2005;83(6):1650–8.
87. McDonald S, Murphy K, Beyene J, et al. Perinatal outcomes of in vitro fertilization twins: a systematic review and meta-analyses. Am J Obstet Gynecol 2005; 193(1):141–52.
88. Schieve LA, Ferre C, Peterson HB, et al. Perinatal outcome among singleton infants conceived through assisted reproductive technology in the United States. Obstet Gynecol 2004;103(6):1144–53.
89. Van Steirteghem A. Outcome of assisted reproductive technology. N Engl J Med 1998;338(3):194–5.
90. Hansen M, Bower C, Milne E, et al. Assisted reproductive technologies and the risk of birth defects–a systematic review. Hum Reprod 2005;20(2):328–38.
91. Hansen M, Kurinczuk JJ, Bower C, et al. The risk of major birth defects after intracytoplasmic sperm injection and in vitro fertilization. N Engl J Med 2002; 346(10):725–30.
92. Ericson A, Kallen B. Congenital malformations in infants born after IVF: a population-based study. Hum Reprod 2001;16(3):504–9.

93. Dohle GR, Halley DJ, Van Hemel JO, et al. Genetic risk factors in infertile men with severe oligozoospermia and azoospermia. Hum Reprod 2002; 17(1):13–6.
94. Dowsing AT, Yong EL, Clark M, et al. Linkage between male infertility and trinucleotide repeat expansion in the androgen-receptor gene. Lancet 1999; 354(9179):640–3.
95. Patrizio P, Leonard DG, Chen KL, et al. Larger trinucleotide repeat size in the androgen receptor gene of infertile men with extremely severe oligozoospermia. J Androl 2001;22(3):444–8.
96. Stromberg B, Dahlquist G, Ericson A, et al. Neurological sequelae in children born after in-vitro fertilisation: a population-based study. Lancet 2002; 359(9305):461–5.
97. Hvidtjorn D, Schieve L, Schendel D, et al. Cerebral palsy, autism spectrum disorders, and developmental delay in children born after assisted conception: a systematic review and meta-analysis. Arch Pediatr Adolesc Med 2009; 163(1):72–83.
98. Pinborg A, Loft A, Schmidt L, et al. Neurological sequelae in twins born after assisted conception: controlled national cohort study. BMJ 2004;329(7461):311.
99. Agarwal P, Loh SK, Lim SB, et al. Two-year neurodevelopmental outcome in children conceived by intracytoplasmic sperm injection: prospective cohort study. BJOG 2005;112(10):1376–83.
100. Bonduelle M, Ponjaert I, Steirteghem AV, et al. Developmental outcome at 2 years of age for children born after ICSI compared with children born after IVF. Hum Reprod 2003;18(2):342–50.
101. Place I, Englert Y. A prospective longitudinal study of the physical, psychomotor, and intellectual development of singleton children up to 5 years who were conceived by intracytoplasmic sperm injection compared with children conceived spontaneously and by in vitro fertilization. Fertil Steril 2003;80(6):1388–97.
102. Ponjaert-Kristoffersen I, Bonduelle M, Barnes J, et al. International collaborative study of intracytoplasmic sperm injection-conceived, in vitro fertilization-conceived, and naturally conceived 5-year-old child outcomes: cognitive and motor assessments. Pediatrics 2005;115(3):e283–9.
103. Leunens L, Celestin-Westreich S, Bonduelle M, et al. Follow-up of cognitive and motor development of 10-year-old singleton children born after ICSI compared with spontaneously conceived children. Hum Reprod 2008;23(1):105–11.
104. Amor DJ, Halliday J. A review of known imprinting syndromes and their association with assisted reproduction technologies. Hum Reprod 2008;23(12):2826–34.
105. Knoll JH, Nicholls RD, Magenis RE, et al. Angelman and Prader-Willi syndromes share a common chromosome 15 deletion but differ in parental origin of the deletion. Am J Med Genet 1989;32(2):285–90.
106. Cox GF, Burger J, Lip V, et al. Intracytoplasmic sperm injection may increase the risk of imprinting defects. Am J Hum Genet 2002;71(1):162–4.
107. DeBaun MR, Niemitz EL, Feinberg AP. Association of in vitro fertilization with Beckwith-Wiedemann syndrome and epigenetic alterations of LIT1 and H19. Am J Hum Genet 2003;72(1):156–60.
108. DeBaun MR, Niemitz EL, McNeil DE, et al. Epigenetic alterations of H19 and LIT1 distinguish patients with Beckwith-Wiedemann syndrome with cancer and birth defects. Am J Hum Genet 2002;70(3):604–11.
109. Doherty AS, Mann MR, Tremblay KD, et al. Differential effects of culture on imprinted H19 expression in the preimplantation mouse embryo. Biol Reprod 2000;62(6):1526–35.

110. Khosla S, Dean W, Brown D, et al. Culture of preimplantation mouse embryos affects fetal development and the expression of imprinted genes. Biol Reprod 2001;64(3):918–26.
111. Ecker DJ, Stein P, Xu Z, et al. Long-term effects of culture of preimplantation mouse embryos on behavior. Proc Natl Acad Sci U S A 2004;101(6):1595–600.
112. Fernandez-Gonzalez R, Moreira P, Bilbao A, et al. Long-term effect of in vitro culture of mouse embryos with serum on mRNA expression of imprinting genes, development, and behavior. Proc Natl Acad Sci U S A 2004;101(16):5880–5.
113. Ward JA, Robinson J, Furr BJ, et al. Protection of spermatogenesis in rats from the cytotoxic procarbazine by the depot formulation of Zoladex, a gonadotropin-releasing hormone agonist. Cancer Res 1990;50(3):568–74.
114. Johnson DH, Linde R, Hainsworth JD, et al. Effect of a luteinizing hormone releasing hormone agonist given during combination chemotherapy on post-therapy fertility in male patients with lymphoma: preliminary observations. Blood 1985;65(4):832–6.
115. Waxman JH, Ahmed R, Smith D, et al. Failure to preserve fertility in patients with Hodgkin's disease. Cancer Chemother Pharmacol 1987;19(2):159–62.
116. Krause W, Pfluger KH. Treatment with the gonadotropin-releasing hormone agonist buserelin to protect spermatogenesis against cytotoxic treatment in young men. Andrologia 1989;21(3):265–70.
117. Kreuser ED, Hetzel WD, Hautmann R, et al. Reproductive toxicity with and without LHRHA administration during adjuvant chemotherapy in patients with germ cell tumors. Horm Metab Res 1990;22(9):494–8.
118. Schmiegelow ML, Sommer P, Carlsen E, et al. Penile vibratory stimulation and electroejaculation before anticancer therapy in two pubertal boys. J Pediatr Hematol Oncol 1998;20(5):429–30.
119. Hirsch M, Lunenfeld B, Modan M, et al. Spermarche–the age of onset of sperm emission. J Adolesc Health Care 1985;6(1):35–9.
120. Badawy A, Elnashar A, El-Ashry M, et al. Gonadotropin-releasing hormone agonists for prevention of chemotherapy-induced ovarian damage: prospective randomized study. Fertil Steril 2009;91(3):694–7.
121. Husseinzadeh N, Nahhas WA, Velkley DE, et al. The preservation of ovarian function in young women undergoing pelvic radiation therapy. Gynecol Oncol 1984;18(3):373–9.
122. Covens AL, van der Putten HW, Fyles AW, et al. Laparoscopic ovarian transposition. Eur J Gynaecol Oncol 1996;17(3):177–82.
123. Morice P, Castaigne D, Haie-Meder C, et al. Laparoscopic ovarian transposition for pelvic malignancies: indications and functional outcomes. Fertil Steril 1998; 70(5):956–60.
124. Perrin LC, Low J, Nicklin JL, et al. Fertility and ovarian function after conservative surgery for germ cell tumours of the ovary. Aust N Z J Obstet Gynaecol 1999; 39(2):243–5.
125. Zanetta G, Bonazzi C, Cantu M, et al. Survival and reproductive function after treatment of malignant germ cell ovarian tumors. J Clin Oncol 2001;19(4): 1015–20.
126. Morice P, Camatte S, El Hassan J, et al. Clinical outcomes and fertility after conservative treatment of ovarian borderline tumors. Fertil Steril 2001;75(1):92–6.
127. Morice P, Wicart-Poque F, Rey A, et al. Results of conservative treatment in epithelial ovarian carcinoma. Cancer 2001;92(9):2412–8.
128. Jeruss JS, Woodruff TK. Preservation of fertility in patients with cancer. N Engl J Med 2009;360(9):902–11.

129. Porcu E, Venturoli S. Progress with oocyte cryopreservation. Curr Opin Obstet Gynecol 2006;18(3):273–9.
130. Oktay K, Cil AP, Bang H. Efficiency of oocyte cryopreservation: a meta-analysis. Fertil Steril 2006;86(1):70–80.
131. Oktay K, Karlikaya G. Ovarian function after transplantation of frozen, banked autologous ovarian tissue. N Engl J Med 2000;342(25):1919.
132. Donnez J, Dolmans MM, Demylle D, et al. Live birth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet 2004;364(9443):1405–10.
133. Meirow D, Levron J, Eldar-Geva T, et al. Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. N Engl J Med 2005;353(3):318–21.
134. Ovarian tissue and oocyte cryopreservation. Fertil Steril 2008;90(Suppl 5): S241–6.
135. ACOG Committee Opinion No. 405: Ovarian tissue and oocyte cryopreservation. Obstet Gynecol 2008;111(5):1255–6.
136. Dudzinski DM. Ethical issues in fertility preservation for adolescent cancer survivors: oocyte and ovarian tissue cryopreservation. J Pediatr Adolesc Gynecol 2004;17(2):97–102.
137. Patrizio P, Butts S, Caplan A. Ovarian tissue preservation and future fertility: emerging technologies and ethical considerations. J Natl Cancer Inst Monogr 2005;34:107–10.