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Impact of a cryopreservation program on the multiple pregnancy rate associated with assisted reproductive technologies

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Objective: To determine the impact of a cryopreservation program on pregnancy rates and multiple-pregnancy rates in ART cycles.

Design: Retrospective study.

Setting: University teaching hospital.

Patient(s): Women who underwent stimulation for in vitro fertilization at the Jones Institute for Reproductive Medicine between October 1987 and June 1999.

Intervention(s): Analysis of pregnancy and multiple-pregnancy rates based on the number of embryos transferred.

Main Outcome Measure(s): Implantation; pregnancy and multiple-pregnancy rates.

Result(s): Pregnancy rates per transfer increased from 9% when one embryo was transferred to 20% with two embryos, 35% with three embryos, 40% with four embryos, and 41% with five embryos. The rate of twin pregnancies increased to 21% with two embryos, 23% with three embryos, 21% with four embryos, and 22% with five embryos. The triplet pregnancy rates were 8% with three embryos, 9% with four embryos, and 2% with five embryos. A theoretical model limiting the number of embryos transferred to two with cryopreservation and subsequent transfer yields a cumulative pregnancy rate of 77%, a twin rate of less than 20%, and no triplet or higher-order pregnancies.

Conclusion(s): The use of a cryopreservation program can help maximize pregnancy rates while minimizing multiple-pregnancy rates. Cryopreservation should be considered in all assisted reproductive technology cycles. (Fertil Steril® 2001;75:147–51. ©2001 by American Society for Reproductive Medicine.)

Key Words: In vitro fertilization, embryo cryopreservation, multiple pregnancy

Since the introduction and clinical implementation of ovulation-induction agents in the late 1960s and assisted reproductive technologies in the late 1970s, the incidence of multiple gestation has risen dramatically. Between 1973 and 1990, the twin birth rate increased at twice that of singletons, whereas the incidence of triplet and higher-order births increased at 7 times that of single births.

The incidence of twins has increased from 1 in 55 to 1 in 43 births; for triplets and higher-order births, the numbers have risen from 1 in 3,323 to 1 in 1,341. An increase in the incidence of low-birth-weight infants has paralleled the increased incidence of multiple gestation. The occurrence of very-low and low-birth-weight infants is 24.2% higher among twins and 142.3% greater among triplet and higher-order births (1).

The introduction of assisted reproductive technologies has made a significant contribution to the multiple-pregnancy rate. This relationship is demonstrated in the 1997 Assisted Reproductive Technology Success Rate published by the Centers for Disease Control (CDC). According to the CDC; more than 335 assisted reproductive technology clinics in North America reported on more than 55,000 cycles that resulted in approximately 17,000 live births in 1997. During 1997, the percentage of pregnancies that resulted in multiple births among patients in these clinics was 38%, compared with a 2.7% multiple-birth rate in the general population. Of the pregnancies that re-

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sulted from assisted reproductive technologies, 25.9% were twin gestations and 5.3% were triplet or more (2).

Maternal complications from multiple gestations are directly related to the number of fetuses present. The most common complication of a twin gestation is preterm labor, which occurs 7 to 10 times more frequently than in singleton pregnancies. Twin gestations are also associated with a 2-to-5-fold increase in maternal hypertension, a 3-fold increase in placental abruption, a 2-to-3-fold increase in cesarean-section delivery, and a significantly higher incidence of postpartum uterine atony, placenta previa, and gestational diabetes (3).

With gestations of triplets or higher, the incidence of maternal complications increases even further. Triplet gestations have a very high incidence of preterm labor; the average delivery occurs after 32 to 34 weeks' gestation, and the average infant weight is 1,800 to 1,900 grams. Quadruplet and higher-order multiple pregnancies result in overdistention of the uterus and earlier-onset preterm labor. The average time for delivery is 30 to 31 weeks for quadruplet pregnancies, with an average fetal weight of 1,200 to 1,500 grams (3).

Infant complications also increase in direct correlation with the number of fetuses present. The fetal mortality rate for twins is increased 4-fold, the neonatal mortality rate is increased 6-fold, and the perinatal mortality is increased 10-fold over the rates for singleton pregnancies. Prematurity is the most common infant complication of a multiple pregnancy and can result in respiratory distress, sepsis, necrotizing enterocolitis, patent ductus arteriosus, intercranial hemorrhage, and more. Other significant complications can include an increased incidence of congenital abnormalities, vanishing-twin syndrome, monoamniotic twin pregnancy, dead-fetus syndrome, and locking twins (4).

One option for couples facing high-order multiple pregnancies is multifetal pregnancy reduction. This procedure may be performed to reduce the risk of serious prenatal morbidity and mortality, which is primarily associated with prematurity. The main risk associated with multifetal pregnancy reduction is that of pregnancy loss, which ranges from 10% to 26%. The benefit of multifetal reduction has been demonstrated in quadruplet and higher-order gestations by an increase in the time of gestation for surviving fetuses (5). The role of selective reduction is unclear in regard to the short- and long-term neonatal outcome of triplet gestations that are reduced to twins. In addition to these problems, selective reduction has a high psychological price, particularly in women who have had difficulty becoming pregnant.

In November 1994, the American Society for Reproductive Medicine (formerly known as the American Fertility Society) published the Ethical Considerations of Assisted Reproductive Technologies by the Ethics Committee of the American Fertility Society. The committee unanimously felt that "the goal of this procedure (IVF) is to maximize pregnancy rates while minimizing multiple gestation." The committee condemned the practice of transferring excessive numbers of pre-embryos with the intention of using selective reduction in the event of multiple pregnancies. The committee further recommended that the number of pre-embryos transferred should be limited with the goal of no quadruplet pregnancies and triplet pregnancies limited to 1% to 2% of all pregnancies. The committee further reinforced the role of cryopreservation, as it enables the transfer of a limited number of pre-embryos, thereby reducing the necessity of selective reduction.

Expanding on its statement in 1994, the American Society for Reproductive Medicine in 1998 published a committee opinion that further defined its recommendations regarding the number of embryos that should be transferred. Assuming an above-average prognosis, the number of good embryos transferred should usually be limited to no more than three in women under age 35; no more than four in patients 35–40 years of age; and no more than five in women 40 and over (6).

Two recent studies have addressed pregnancy and multiple-pregnancy rates in relation to the number of embryos transferred. Templeton and Morris (7) in 1998 analyzed 44,236 cycles within the United Kingdom to study the factors associated with an increased risk of multiple births. Their study also noted no statistically significant difference between the pregnancy rate with the transfer of two embryos and that resulting from transfer of three embryos in those patients with more than four fertilized embryos. However, the multiple pregnancy rate in women 30 to 35 years of age increased from 28.6% with the transfer of two embryos to 39.4% with the transfer of three embryos, a statistically significant change. This study was unable to examine elective transfer of high numbers of embryos because the United Kingdom limits to three the number of embryos that can be transferred (7).

Schieve et al. in 1999 examined the association between the number of embryos transferred after in vitro fertilization (IVF), and the live- and multiple-birth rate in 35,554 in vitro fertilization transfer procedures performed within the United States. As an increasing number of embryos are transferred, the pregnancy rate increases until three or more embryos are transferred. Transfer of three or more embryos generally causes a plateau in the pregnancy rate; however, the multiple-pregnancy rate continues to increase with each additional embryo transferred. The only exceptions are women aged 35 to 39 and 40 to 44, who demonstrate a continued increase in pregnancy rates before the plateau occurs (at four embryos transferred in the 35 to 39 year age group and five embryos in the 40 to 44 year age group). In a subcategory analysis that examined only those cycles in which excess embryos were cryopreserved, no statistically significant difference was found between the pregnancy rates resulting from transfer of

two versus three embryos in patients under 35 years of age (8).

On the basis of these studies and recent improvements in implantation and pregnancy rates with the use of IVF, the Practice Committee for the American Society for Reproductive Medicine in October 1999 amended the earlier guidelines. The amendment states that patients who have a favorable prognosis (age <35, good-quality embryos) should usually have no more than two good-quality embryos transferred. Patients under age 35 with an above-average prognosis should usually have no more than three good embryos transferred. Patients aged 35 to 39 with an above average prognosis should have no more than four embryos transferred, and those greater than 40 should have no more than five embryos transferred (9).

The cryopreservation of embryos after IVF provides numerous advantages to couples. The first is further possibilities for conception in addition to those achieved from the fresh cycle. Second, the cryopreservation of embryos provides a method for storing excess embryos. Because of this capability, infertile couples may be less inclined to pressure their physicians to transfer high numbers of embryos. Third, embryo cryopreservation plays an important role in the treatment of severe ovarian hyperstimulationby allowing embryo transfer to be delayed to a later cycle when hyperstimulation is less severe.

In this paper, we present a theoretical model of the fresh and multiple pregnancy rates in relation to the number of pre-embryos transferred. We then compare the model with the actual pregnancy and multiple-pregnancy rates in relation to the number of pre-embryos transferred from the Norfolk in vitro fertilization cycles between October 1987 and June 1999. Next, we demonstrate that cryopreservation can mitigate or eliminate the multiple-pregnancy problems while maintaining an acceptable pregnancy rate. These pregnancy rates are based on the number of embryos transferred for our patients who were considered to be "good responders" on the basis of previous stimulations or day 3 FSH, LH, and estradiol levels. The cryopreservation concept is then applied to these data to demonstrate the impact of a cryopreservation program on the pregnancy and multiple-pregnancy rates.

MATERIALS AND METHODS

Patients at the Jones Institute between October 1987 and June 1999 who were considered to be "good responders" were stimulated for in vitro fertilization. The patients then began a luteal lupron protocol that included suppression with 0.5 mg of lupron starting on day 21 of the preceding cycle. This dosage was decreased to 0.25 mg with the onset of menses and continued until the administration of human chorionic gonadotropin (hCG).

Patients were stimulated with a combination of human menopausal gonadotropin (hMG), highly purified follicle-stimulating hormone (hp-FSH), and recombinant follicle-stimulating hormone (rFSH, Gonal-F). The stimulation has been described in detail previously (10). In brief, the stimulation was by a step-down protocol and generally consisted of a starting dose of 2 to 4 ampules, which was reduced as indicated by the patient's response. This treatment continued until three or more follicles of 16 or 17 mm in diameter were determined to be present. Next, hCG was administered in dosages ranging from 5,000 to 10,000 units i.m. Approximately 33 hours later, after hCG administration, 2,040 oocyte harvests were performed.

TABLE 1

Theoretical fresh and multiple pregnancy rates assuming an implantation rate of 14%.

# Transferred	Pregnancy rate	Singleton	Twin	Triplet	Quadruplet
1	14.0%	100%			
2	26.04%	92.5%	7.5%		
3	36.39%	85.4%	13.9%	0.8%	
4	45.30%	78.6%	19.2%	2%	0.1%

Schnorr. Cryopreservation and multiple pregnancies. Fertil Steril 2001.

Physicians are commonly confronted with pressure from patients and competing programs to transfer more embryos in an effort to increase pregnancy rates. The result of these pressures is illustrated through the use of simple calculations to estimate the rates of pregnancy and multiple gestations on the basis of the number of embryos transferred. In the theoretical example that follows, an implantation rate of 14% for all fresh transfers is used. This rate is based on our average fresh-cycle implantation rate at the Jones Institute for Reproductive Medicine between October 1987 and June 1999.

RESULTS

In the theoretical model described above, the transfer of one embryo yields a pregnancy rate of 14%; 100% of those pregnancies are singleton gestations. When two embryos are transferred, the theoretical pregnancy rate increases to 26.04%; of those, 92.47% are singleton and 7.53% are twin gestations. The transfer of three embryos results in a pregnancy rate of 36.39%, of which 85% are singleton, 13.89% are twins, and 0.75% are triplets. The transfer of four embryos results in a pregnancy rate of 45.3%, of which 78.63% are singletons, 19.2% are twins, 2.8% are triplets, and 0.08% are quadruplets (Table 1).

The transfer of one embryo in 46 cycles resulted in four pregnancies, or a pregnancy rate per transfer of 9%. The transfer of two embryos in 96 cycles resulted in 19 pregnancies, for a pregnancy rate of 20%. Of those pregnancies, four, or 21%, were twins. The transfer of three embryos resulted

TABLE 2

Actual fresh multiple pregnancy rates at the Jones Institute between October 1987 and June 1999.

# Transferred	Pregnancy rate	Singleton	Twin	Triplet	Quadruplet
1	9.0%	100%			
2	20.0%	79%	21%		
3	35.0%	68.4%	23%	8%	0.6%
4	40.0%	68%	21%	9%	2%
5	41%	75%	22%	2%	1.2%
6	30%	86%	7%	7%	

Schnorr. Cryopreservation and multiple pregnancies. Fertil Steril 2001.

in 152 pregnancies from 436 transfers, or a pregnancy rate of 35%, of which 23% were twins, 8% triplets, and 0.6% quadruplets. The transfer of four embryos resulted in 393 pregnancies from 989 transfers, for a pregnancy rate of 40%, 21% of which were twins, 9% triplets, and 2% quadruplets. The transfer of five embryos in 384 cycles resulted in 158 pregnancies, or a pregnancy rate of 41%; of these, 22% were twin gestations, and one quadruplet and one quintuplet gestation resulted in an incidence of 0.6% for each. The transfer of six embryos occurred in 89 cycles, resulting in 27 pregnancies, or a pregnancy rate of 30%. Of those pregnancies, 7% were twins and 7% were triplets (Table 2).

Because of the retrospective implementation of the recommendations by the Ethics Committee of the American Fertility Society in 1994 (i.e., limiting the number of preembryos transferred, with the goal of no quadruplet pregnancies and triplet pregnancies limited to 1% to 2%), and also as a result of the studies by Templeton and Morris (7) and by Schieve et al. (8), the Jones Institute was able to transfer only two embryos. Transfers of three embryos at the Jones Institute resulted in a triplet pregnancy rate of 8%, which, on the basis of the ethical guidelines, is unacceptable. Thus, in our theoretical model, any embryos in excess of two will be cryopreserved and used for a cryopreserved transfer in the event that pregnancy does not occur during the fresh cycle.

The Jones Institute database was unable to provide (as part of the tracking of cryopreserved material) cumulative pregnancy rate data for those individuals who had two fresh embryos transferred. Accordingly, we have resorted to a theoretical analysis using the Jones Institute actual fresh implantation rate of 14% between October 1987 and June 1999. The impact of using additional cryopreserved embryos in subsequent cryopreserved cycles was assessed on the basis of actual cryopreservation survival and implantation rates of 70% and 7.7%, respectively.

Accordingly, patients with one embryo had a fresh pregnancy rate of 9%, all of which were singleton gestations. Patients with two embryos had a pregnancy rate of 20%, of which 79% were singleton gestations and 21% twin gestations. Patients with three embryos had a fresh pregnancy rate identical to that for individuals with two embryos (20% singleton and 21% twin, respectively). With the addition of the single remaining cryopreserved embryo in a subsequent cycle, the pregnancy rate for all patients with three embryos increased to 27.7%, with a 15.16% incidence of twins.

Once the fresh transfer pregnancy rates and cryo transfer of the two remaining embryos were considered, individuals with four embryos had a total pregnancy rate of 34.21%, of which 13.94% were twins. Individuals with five embryos had fresh transfer, one cryopreserved transfer with two embryos, and one cryopreserved transfer with one embryo. These individuals had a resultant pregnancy rate of 41.91%, of which 11.38% were twins. Individuals with six embryos had two embryos in a fresh transfer, two cryopreserved embryos in the first transfer, and two cryopreserved embryos in a second cryopreservation transfer, which resulted in a pregnancy rate of 48.42% and a twin gestation rate of 11.02% (see Fig. 1).

DISCUSSION

Ovulation induction and assisted reproductive technologies have had a dramatic impact on multiple-pregnancy rates since their introduction in clinical use in the early 1960s. The higher rates of multiple pregnancy have resulted in considerable maternal morbidity and neonatal morbidity and mortality.

Physicians continue to experience significant pressures from patients and competing programs to raise pregnancy rates while lowering multiple pregnancy rates. To this end, physicians are faced with multiple options, which include selective fetal reduction, blastocyst transfer, and cryopreservation programs to limit the number of embryos transferred.

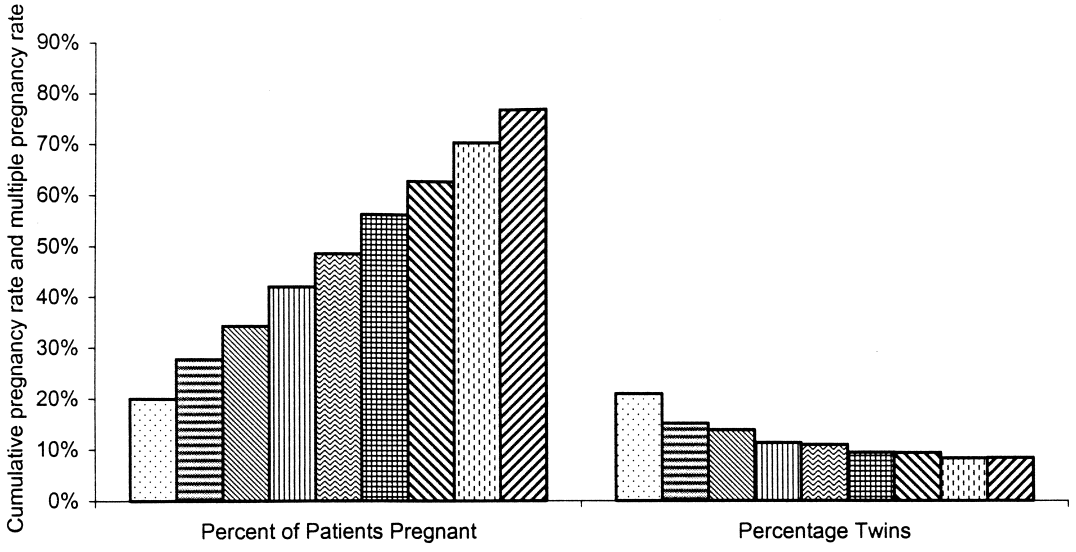
Blastocyst transfer is a newer technique which, in theory, allows nature to select those embryos that will have a higher implantation rate. Accordingly, physicians can transfer a smaller number of embryos while maintaining a higher pregnancy rate and a lower multiple-pregnancy rate. Programs have experienced mixed results with blastocyst transfer, and a significant percentage of patients are not eligible for this procedure because of their age, number of mature eggs at retrieval, or other factors.

The data from the Jones Institute between 1987 and 1999 aptly demonstrate that pregnancy rates rise on the basis of the number of embryos transferred. However, when three or more embryos are transferred, pregnancy rates plateau and multiple-pregnancy rates continue to increase, often leading to high-order multiple pregnancies. The 1996 Assisted Reproductive Technology Success Rates published by the Centers for Disease Control reinforce the validity of these data. The CDC statistics demonstrate that pregnancy rates slowly increase with the transfer of one to three embryos. With the

FIGURE 1

1 embryo 2 embryos 3 embryos 4 embryos 5 embryos 6 embryos 7 embryos 8 embryos 9 embryos

Cumulative and multiple pregnancy rates based on the number of mature embryos per retrieval, assuming the fresh transfer of only two embryos and subsequent transfer of the cryopreserved embryos remaining.



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transfer of four or more embryos, the pregnancy rate plateaus and the multiple-pregnancy rate continues a slow increase. It is exceedingly important that both physician and patient understand that transferring a large number of embryos does not substantially increase the overall pregnancy rate, but has a significant impact on the multiple-pregnancy rate, specifically the rate of high-order multiple gestations.

A considerable difference is seen between the theoretical and actual rates of pregnancy and multiple pregnancy. The increased frequency of multiple pregnancies relative to the frequency predicted on the basis of a fixed implantation rate suggests that an "all-or-nothing" effect tends to result when multiple embryos are transferred. This effect could be due to "extra embryo" factors that significantly affect the embryos transferred within a given cycle. These "extra embryo" factors could include the endometrium's receptiveness to embryo implantation via the presence or absence of specific adhesion molecules, the hormonal milieu, and embryo transfer techniques.

The use of a cryopreservation program to limit the number of embryos transferred and cryopreserve any excess embryos results in pregnancy rates that continue to increase on the basis of the number of mature embryos available. However, the multiple-pregnancy rate includes only twins, and it actually decreased with the number of embryos available because of the dilution from the high percentage of singleton gestations. Cryopreservation results in the maximum benefit to the patient in terms of both high pregnancy rates and low multiple-pregnancy rates. Accordingly, a cryopreservation program should be considered in all assisted reproductive technology cycles because of the many advantages it offers to both the patient and the physician.

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