

Relationship between maternal age and aneuploidy in in vitro fertilization pregnancy loss

Steven D. Spandorfer, M.D., Owen K. Davis, M.D., Larry I. Barmat, M.D.,
Pak H. Chung, M.D., and Zev Rosenwaks, M.D.

The Center for Reproductive Medicine and Infertility, Weill Medical College of Cornell University, New York, New York

Objective: To determine the fetal loss rate after documented fetal cardiac activity (7-week sonogram) and to evaluate the chromosomal makeup of these losses in IVF pregnancies.

Design: Retrospective analysis.

Setting: University-based IVF center.

Patient(s): Two thousand fourteen consecutive IVF pregnancies with documented fetal cardiac activity.

Main Outcome Measure(s): Miscarriage rates and karyotypes of pregnancy losses were analyzed.

Result(s): The overall pregnancy loss rate after demonstrated fetal cardiac activity was 11.6% (233/2014). A highly significant increase in fetal loss with advancing maternal age was observed (<30 years = 5.3% vs. 31–34 years = 7.6% vs. 35–39 years = 12.8% vs. ≥40 years = 22.2%). Patients with a multiple gestation were more likely to deliver a live infant, compared with those with a singleton detected at a 7-week sonogram. Of the 233 losses in the study period, cytogenetic analyses were obtained for 74 (31.8%). Three specimens were nondiagnostic. Fifty-two patients had abnormal karyotypes (71.2% [52/71]). Eighty-two percent of the pregnancy losses in women aged ≥40 years were associated with chromosomally abnormal fetuses, compared with 65% of the losses in women aged <40 years (odds ratio, 3.35; 95% confidence interval, 0.96–11.97).

Conclusion(s): Pregnancy loss after documentation of fetal cardiac activity is >10%. This loss is significantly increased with advancing maternal age. The major underlying cause of these losses seems to be chromosomal aneuploidy. (Fertil Steril® 2004;81:1265–9. ©2004 by American Society for Reproductive Medicine.)

Key Words: IVF, miscarriage, maternal age, aneuploidy

Diminished fecundity occurs with increasing maternal age (1–5). In Tzietze's classic study of the Hutterites, sterility increased from 11% at age 34 years and 33% at age 39 years to >85% by age 44 years (1). Maternal age has a significant negative impact on IVF outcome (3–5). Liveborn delivery rates inversely correlate with maternal age. Furthermore, even after achieving a pregnancy through IVF-embryo transfer (ET), the older patient is at a higher risk for miscarriage (5–8). Many of these losses occur before the detection of fetal cardiac activity.

To date, the fetal loss rate of IVF pregnancies after detection of fetal cardiac activity at 7 weeks' gestation (defined as 49 days after the last menstrual period or 5 weeks postretrieval) is not well established. Data derived from studies of spontaneous conceptions report a miscar-

riage rate of 2%–5% after detection of fetal cardiac activity (9–12). These figures might be misleading, however, because they might reflect the loss rate of spontaneous conceptions in patients who are substantially younger than IVF patients. Moreover, most studies considered loss rates in women who had positive fetal cardiac activity late in the first trimester. For infertility patients, pregnancy loss is known to be highly associated with maternal age (13–15). Several studies have shown that increasing age is associated with significant pregnancy loss—up to 20% in women aged >40 years (13). Most of these studies have only included a few hundred women in their analyses. The present study includes more than 2,000 clinical pregnancies.

This study was undertaken to evaluate the miscarriage rate after detection of fetal cardiac

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Reprint requests: Steven D. Spandorfer, M.D., The New York Hospital/Cornell Medical Center, The Center for Reproductive Endocrinology and Infertility, 505 East 70th Street HT340, New York, New York 10021 (FAX: 212-746-8860; E-mail: sdspando@med.cornell.edu).

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activity in IVF pregnancies for which the date of conception was absolutely defined. In addition, in an attempt to understand the etiology of these losses, the chromosomal composition of the products of conception was analyzed when possible.

MATERIALS AND METHODS

A retrospective study of all clinical pregnancies (positive fetal cardiac activity) after IVF-ET at The New York Hospital/Cornell Medical Center from October 1991 to June 1996 was conducted. Only pregnancies resulting from fresh ET were analyzed, excluding donor oocyte or frozen ET pregnancies. Patients who underwent selective reduction or voluntary termination for aneuploidy detected at amniocentesis or for fetal anomalies detected on ultrasound were excluded.

When possible, at the attending physician's discretion, cytogenetic analysis of the fetal products was performed. A pregnancy loss was defined as any fetal demise that occurred before delivery.

Patients were treated with standard ovulation induction protocols and underwent IVF-ET as previously described (5). In brief, most women were treated with luteal-phase leuprolide acetate (Lupron; Tap Pharmaceuticals, Deerfield, IL), 0.5–1 mg s.c. daily until ovarian suppression was achieved. Women not treated with luteal leuprolide acetate began stimulation on day 2 of their treatment cycle. Ovarian stimulation was then effected with a combination of gonadotropins (hMG and/or FSH [Pergonal or Metrodin; Serono, Waltham, MA]), according to a step-down protocol (5). Human chorionic gonadotropin was administered (3,300–10,000 IU) when at least two follicles reached or exceeded 16 to 17 mm mean diameter, as measured by transvaginal ultrasound. Oocytes were harvested by transvaginal ultrasound-guided follicular puncture 35 to 36 hours after hCG administration.

Conventional oocyte insemination or micromanipulation was performed as determined by the embryologist and the patient's attending physician. The best morphologically appearing embryos were transferred into the uterine cavity approximately 72 hours after retrieval. The number of embryos transferred depended on maternal age, according to our standard protocol (5). In general, three embryos were transferred to patients aged <35 years; patients aged 35–40 years received four embryos, and patients aged >40 years underwent transfer of up to five embryos, when available. Methylprednisolone (16 mg/day) and tetracycline (250 mg every 6 hours) were administered for 4 days to all patients, commencing on the day of oocyte retrieval. Progesterone supplementation was initiated on the third day after hCG administration (25–50 mg/day i.m.) and was continued until sonographic assessment of the pregnancy at 47–51 days' gestation, as determined by the day of oocyte insemination

(day 14) (5). Sonograms were performed with transvaginal techniques.

To evaluate the impact of age on fetal loss rates, results were evaluated in four distinct age groups. Group I included women aged ≤ 30 years; group II, aged 31–34 years; group III, aged 35–39 years; and group IV, aged ≥ 40 years. Total fetal loss was calculated as the final number of liveborn infants divided by the total number of live fetuses with cardiac activity at 7 weeks' gestation. Statistical analyses included χ^2 , Student's *t* test, Mann-Whitney test, and analysis of variance where appropriate. Significance was defined as $P < .05$.

RESULTS

A total of 2,014 consecutive pregnancies were included in the study. Of these, 233 patients (11.6%) experienced a pregnancy loss (spontaneous abortion, SAB) after detection of fetal cardiac activity. Patients whose pregnancies ended as an SAB were, on average, older than those patients who ultimately delivered a liveborn infant (37.3 ± 3.8 years vs. 35.1 ± 4.1 years [mean \pm SD], $P < .001$).

A clear association between age and pregnancy loss rate was demonstrated (5.3% [15/281; age ≤ 30 years] vs. 7.6% [47/622; age 31–34 years] vs. 12.8% [103/805; age 35–39 years] vs. 22.2% [68/306; age ≥ 40 years]; $P < .001$). More than 20% of all women aged >40 years with documented fetal cardiac activity eventually had a pregnancy loss.

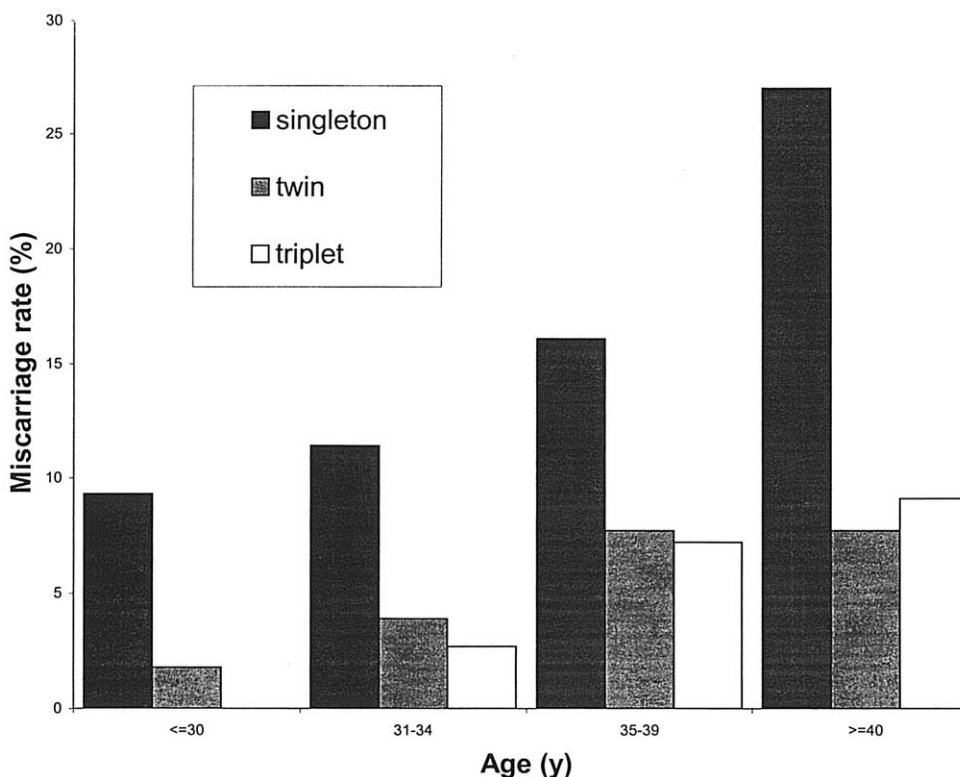
Patients with a singleton pregnancy were more likely to have a pregnancy loss (loss of the entire pregnancy) as compared with patients with a multiple gestation (16.1% [190/1,177; one fetal heart] vs. 5.4% [35/653; two fetal hearts] vs. 4.3% [8/184; three fetal hearts]; $P < .0001$). However, patients with a singleton pregnancy were significantly older than those with multiple pregnancies (36.0 vs. 34.4 years, $P < .001$). Patients with a twin gestation were no more likely to have a pregnancy loss than were patients with triplet gestations ($P = .7$).

Given that both maternal age and the number of fetal hearts detected seem to be significant factors in determining pregnancy loss risk, an analysis of the pregnancy loss rate as a function of both factors was undertaken (Fig. 1). In patients with a singleton pregnancy, an increase in the pregnancy loss rate with advancing age was demonstrated. Of note, more than 25% of all patients aged ≥ 40 years found to have a viable singleton at 7 weeks' gestation did not deliver a liveborn infant. Patients with a multiple pregnancy also had a significant increase in their pregnancy loss rate with advancing age; however, the SAB rate for multiple gestations was lower for every age group than that for singleton pregnancies.

Figure 2 depicts the total fetal heart loss as a function of age. No difference in losses was noted in groups I and II, but a significant increase in the total fetal heart loss rate in

FIGURE 1

Miscarriages increase with maternal age. Patients with multiple pregnancies had a lower overall miscarriage rate for each age group.



Spandorfer. Miscarriage rates and maternal age. *Fertil Steril* 2004.

groups III and IV was noted. More than 25% of all fetuses with proven cardiac activity in patients aged ≥ 40 years subsequently miscarried.

The rate of spontaneous reductions is depicted in Table 1. In five pregnancies in which a twin gestation was noted at the 7-week ultrasound, a triplet gestation was found at a later sonographic examination. Of the 653 initial twin pregnancies,

529 (79.6%) ultimately delivered at least two liveborn infants. There was a higher rate of spontaneous reductions in triplet gestations. Of the 184 patients initially found to have a triplet gestation, 101 (54.8%) delivered three infants. On the other hand, 40.8% of patients (75/184) initially diagnosed with a triplet gestation underwent a spontaneous reduction and delivered either a liveborn infant or liveborn twins. The remaining eight triplet gestations ended in pregnancy loss.

Cytogenetic testing was attempted in 31.7% (74/233) of all the miscarriages. In three instances (4.1%; 3/74), analysis was precluded owing to lack of fetal cell growth in vitro. Chromosomal analysis in six twin gestations revealed congruent results. One set had 46,XX karyotypes in both fetuses. The other five patients with twin miscarriages had abnormal results in both fetuses, whereas all the others exhibited different aneuploidies.

In total, chromosomal aneuploidy was found in 71.2% of pregnancy losses (52/71) examined. The incidence of aneuploidy was lower in karyotyped products of conception in women aged < 40 years (65%) when compared with women

TABLE 1

Spontaneous reduction in multiple pregnancies detected at 7 weeks' gestation.

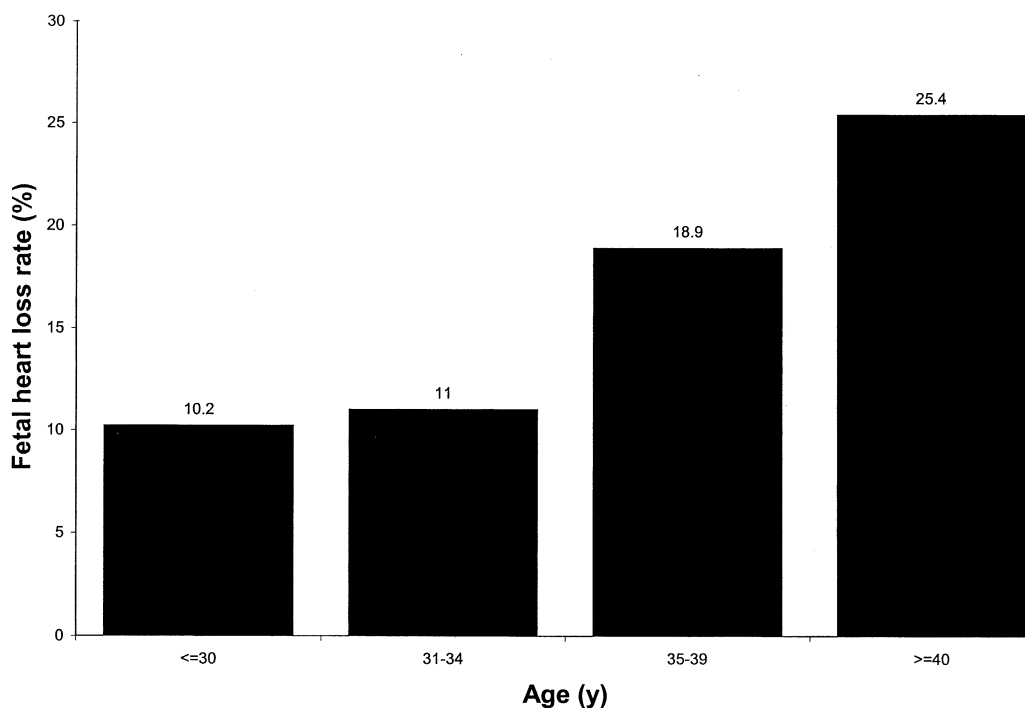
Fetal hearts at 7-wk ultrasound	No. delivered (%)			
	SAB	Singleton	Twin	Triplet
Twins	5.4	15.0	78.9	0.8 ^a
Triplets	4.3	6.5	34.2	54.9

^a Five patients initially thought to have a twin gestation at 7 wk were later found to have triplets.

Spandorfer. Miscarriage rates and maternal age. *Fertil Steril* 2004.

FIGURE 2

Overall fetal heart loss rate increased significantly in women aged >35 years.



Spandorfer. Miscarriage rates and maternal age. *Fertil Steril* 2004.

aged ≥ 40 years (82%) (odds ratio, 3.35; 95% confidence interval, 0.96–11.97).

The specific karyotypic findings of all examined abortuses are presented in Table 2. Thirteen of the normal results (65%) were 46,XX, whereas only seven (35%) were 46,XY. Trisomies represented 80.7% of all abnormal karyotypes

(46/57). The most frequently observed abnormalities were trisomies of chromosome 21 (11/46; 23.9%), chromosome 16 (6/46; 13%), chromosome 22 (5/46; 10.9%), chromosome 15 (5/46; 10.9%), and chromosome 18 (4/46; 8.7%).

DISCUSSION

In this large series of consecutive IVF pregnancies (for which fetal cardiac activity was accurately documented), we have clearly demonstrated a linear increase in miscarriage rates with advancing maternal age. Indeed, whereas in women younger than 30 years the spontaneous loss rates was less than 6%, the miscarriage rate in women aged ≥ 40 years was almost quadrupled (22.2%). For women aged >40 years, the singleton pregnancy loss rate exceeded 27%.

Although other studies have reported similar or even higher pregnancy loss rates with advancing age (16, 17), these investigations did not exclude missed abortions and other early losses, because they relied on histologic tissue confirmation and/or documentation of a gestational sac on ultrasound. In this exhaustive series from a single unit, we demonstrate that pregnancy loss rates are higher than rates that had been reported after fetal heart documentation in spontaneous pregnancies in the late first trimester (9). This might in part be explained by the fact that our population was

TABLE 2

Breakdown of cytogenetic results obtained from abortus after demonstration of fetal cardiac activity.

Cytogenetic result	No.	%
Normal	20	26.0
46,XX	13	16.9
46,XY	7	9.1
Abnormal	57	74.0
Trisomy	46	59.7
48 chromosomes	4	5.2
Turner's	2	2.6
Mosaic	2	2.6
Translocation	1	1.3
47,XXY	1	1.3
Triploidy	1	1.3

Spandorfer. Miscarriage rates and maternal age. *Fertil Steril* 2004.

older, as well as the fact that fetal cardiac activity was documented at 7 weeks' gestation in a population for which the gestational age was clearly delineated.

Equally important, it has been clearly demonstrated that pregnancies in which multiple implantations exist are more likely to deliver at least one liveborn infant, when compared with age-matched controls with a singleton pregnancy documented by fetal cardiac activity at a 7-week vaginal ultrasound examination. This deserves particular emphasis because we have demonstrated that there is a significant rate of natural fetal loss, which is related to advancing maternal age. Indeed, only 55% of women with three heartbeats at 7 weeks' gestation delivered triplets. This information is critical for formulating clinical decisions and policies regarding the number of embryos to be transferred after IVF, as well as for counseling women before decisions are made for selective reduction.

We have thoroughly documented the pregnancy attrition rate after the detection of fetal cardiac activity at 7 weeks' gestation. These data are particularly compelling because they are derived from a single institution after precise documentation of fetal age by the day of conception. Clearly, fetal loss is greatly impacted by maternal age. Chromosomal aneuploidy, as expected, was most often associated with this observed phenomenon. Unfortunately, because of the retrospective nature of this study, we do not have cytogenetic analyses for all of the miscarriages. The significant pregnancy loss rate should be carefully considered when patients are counseled regarding the number of embryos to be transferred after IVF, as well as when fetal reductions are considered for older women.

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