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# Live-birth rates and multiple-birth risk of assisted reproductive technology pregnancies conceived using thawed embryos, USA 1999–2000

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**BACKGROUND: Increasing use of assisted reproductive technology treatments has been associated with the current rise in multiple births in the USA. Embryo cryopreservation and subsequent thawed embryo transfer may favourably impact the multiple-birth risk by relieving some pressure that patients and providers may feel to transfer several embryos in a single cycle. The study objective was to examine both live-birth rates and multiple-birth risk in thawed cycles. METHODS: The authors used a population-based sample of 21 555 assisted reproductive technology procedures performed in US clinics in 1999 and 2000 that used thawed embryos derived from the patient's oocytes. RESULTS: Both patient age and the number of embryos transferred were independent predictors of live birth. Even among women aged 20–29 years, the transfer of three embryos resulted in an increase in the live-birth rate compared with cycles in which one or two embryos were transferred. This increase in success was accompanied by an increased multiple-birth risk. In all age groups up to 40 years, the transfer of just two embryos resulted in a multiple-birth risk of 16–17%. The multiple-birth risk increased with the number of embryos transferred. CONCLUSIONS: Patient age and the number of embryos transferred significantly affect live-birth and multiple-birth rates among women who use thawed embryos.**

*Key words:* assisted reproductive technology/embryo cryopreservation/multiple birth/thawed embryo transfer

## Introduction

The rise in multiple births in the USA has been considerable over the past decade. The number of twin births rose 29% and the number of triplet and higher-order births increased 147% from 1990 to 2001 (Ventura *et al.*, 2001; Martin *et al.*, 2002). Delayed childbearing and an increased use of assisted reproductive technologies have been associated with this increase (Wilcox *et al.*, 1996; Martin *et al.*, 1997; Centers for Disease Control and Prevention, 2002). Over 50% of infants born as a result of assisted reproductive technology in the USA are multiple births (Centers for Disease Control and Prevention, 2002). In comparison, 3% of infants in the general population are multiple births (MacDorman *et al.*, 2002). Multiple gestation pregnancies are at risk for a number of adverse fetal and infant health outcomes (Spellacy *et al.*, 1990; Gardner *et al.*, 1995; Martin *et al.*, 1997; Kiely, 1998; Keith *et al.*, 1999). Approximately half of all twin births and 90% of triplet and higher-order multiple births are born preterm and with low birthweight (Martin *et al.*, 1997; MacDorman *et al.*, 2002). These infants are at increased risk for infant mortality and long-term disability among survivors (Martin *et al.*, 1997; Keith *et al.*, 1999). Thus, it is important to identify primary prevention strategies to reduce the incidence of multiple gestation pregnancies (Keith *et al.*, 1999; Jones, 2003).

Embryo cryopreservation is one option for patients to reduce their risk for a multiple birth during a stimulated cycle, as they can transfer fewer embryos to the uterus and freeze excess embryos for future cycles (Mandelbaum *et al.*, 1998; Mandelbaum, 2000). The subsequent thawed embryo cycles are less expensive and less invasive than other assisted reproductive technology procedures, as neither ovarian stimulation nor oocyte retrieval is necessary. However, these cycles have lower success rates than fresh embryo transfer. In 2000, the live-birth rate was 20.3% for thawed embryo transfers and 31.6% for fresh embryo transfers (Centers for Disease Control and Prevention *et al.*, 2002). As a result, multiple embryos are commonly transferred during thawed embryo cycles. Thus, thawed embryo cycles in themselves present a risk for multiple births. In 2000, the multiple-birth rate was 26% for thawed embryo transfer and 35% for fresh embryo transfer (Centers for Disease Control and Prevention *et al.*, 2002).

 

**Table I.** Percentage distribution of assisted reproductive technology procedures using thawed embryos, by patient age and other factors, USA 1999–2000

| | Age (years) | | | | |
|---|---|---|---|---|---|
| | 20–29 ($n$ = 3020) | 30–34 ($n$ = 8515) | 35–37 ($n$ = 5185) | 38–40 ($n$ = 3338) | 41–44 ($n$ = 1497) |
| Prior births[a] | | | | | |
| Yes | 21.5 | 32.8 | 44.7 | 47.1 | 45.2 |
| No | 78.5 | 67.3 | 55.3 | 52.9 | 54.8 |
| No. of prior assisted reproductive technology cycles[a] | | | | | |
| 1 | 60.2 | 53.4 | 47.7 | 44.8 | 39.0 |
| ≥2 | 39.8 | 46.6 | 52.3 | 55.2 | 61.0 |
| No. of embryos transferred[a] | | | | | |
| 1 | 7.9 | 8.0 | 10.1 | 9.8 | 10.4 |
| 2 | 27.5 | 27.6 | 26.7 | 24.1 | 21.0 |
| 3 | 37.9 | 36.0 | 31.9 | 29.6 | 28.5 |
| 4 | 18.4 | 20.3 | 22.5 | 23.3 | 21.4 |
| ≥5 | 8.3 | 8.1 | 8.8 | 13.2 | 18.7 |
| Use of assisted hatching[a] | | | | | |
| Yes | 34.2 | 36.0 | 38.5 | 41.9 | 52.4 |
| No | 65.8 | 64.0 | 61.5 | 58.1 | 47.6 |

[a]$P < 0.0001$, $\chi^2$ to test for differences in distributions by age. Sample size was reduced for some analyses due to missing values; maximum number of missing values was 4.8% for prior assisted reproductive technology cycles.

Few studies have evaluated factors associated with either the live-birth rate or multiple-birth risk for thawed embryo transfer. Research has shown that 30–40% of frozen embryos do not survive the thawing process (Wang *et al*., 1994; 2001; Mandelbaum, 2000); both the developmental stage of the frozen embryo and the apparent embryo quality have been associated with survival (Testart *et al*., 1987). Studies that examined implantation and pregnancy rates for thawed embryo cycles have been limited in their assessment of predictors of success and multiple-birth risk by insufficient sample sizes (Toner *et al*., 1991; Wang *et al*., 1994; 2001; Van den Abbeel *et al*., 1997; Kowalik *et al*., 1998; Hu *et al*., 1999; Guerif *et al*., 2002). We used a population-based sample of assisted reproductive technology cycles that used thawed embryos to examine both live-birth rates and multiple-birth risk with consideration for patient and treatment factors, such as patient age and the number of embryos transferred.

## Materials and methods

### *Study population*

The Fertility Clinic Success Rate and Certification Act (1992) requires every medical clinic in the USA that performs assisted reproductive technology to report information on each cycle initiated to the Centers for Disease Control and Prevention. An assisted reproductive technology cycle is initiated when a woman begins taking fertility drugs or starts ovarian monitoring with the intent of having embryos transferred. The Society for Assisted Reproductive Technology (SART) annually creates a database of assisted reproductive technology cycles performed in the USA based on data compiled from individual clinics and provides these data to the Centers for Disease Control and Prevention. A more detailed description of this database, which is estimated to capture >95% of all cycles performed in the USA, has been published elsewhere (Schieve *et al*., 1999). While we do not have complete information on reasons for non-reporting in some clinics, anecdotal data suggest that these clinics were smaller in size than average practices and many were practices that were closing or reorganizing by the time of data collection. We have no data suggesting that the lack of participation for these non-reporting clinics was related to practice patterns or multiple-birth risk.

For this study, our unit of analysis was assisted reproductive technology cycles, rather than individual patients, as the data file is organized as one record per cycle and multiple cycles for an individual woman are not linked. We selected assisted reproductive technology cycles performed in 1999 and 2000 that used thawed embryos derived from the patients' oocytes (i.e. non-donor oocytes) ($n$ = 25 088). Cycles that did not progress to embryo transfer ($n$ = 3324) and cycles in which embryos were transferred to both the uterus and the Fallopian tubes ($n$ = 7) were excluded. Because of insufficient sample size at the extremes of patient age, we further limited analyses to cycles in which the patient was 20–44 years old at the time of thawed embryo transfer. Our final sample consisted of 21 555 cycles.

### *Outcome definitions*

We defined pregnancy as the presence of one or more gestational sacs observed on ultrasound. A live-birth delivery was defined as the delivery of one or more liveborn infants. Thus, the live-birth delivery of triplets was counted as one live birth rather than three. A pregnancy was considered to be a multiple gestation if two or more fetal hearts were noted on an early ultrasound. If less than two fetal hearts were noted, but two or more infants were subsequently born, we also coded the pregnancy as a multiple gestation. A live-birth delivery was classified as a multiple birth if two or more fetuses were delivered and at least one was liveborn.

### *Data analysis*

Six outcome measures were assessed: the percentage of transfer procedures that resulted in a pregnancy (pregnancy rate); the percentage of transfer procedures that resulted in a live-birth delivery (live-birth rate); the proportion of pregnancies that were multiple gestations (multiple gestation rate); the proportion of live births that were multiple births (multiple-birth rate); the proportion of pregnancies that were triplet or higher-order gestations (triplet or higher-order gestation rate); and the proportion of live-birth deliveries that were triplet or higher-order births (triplet or higher-order birth rate). Because the patterns of our findings were similar for pregnancy and

**Table II.** Pregnancy, live-birth and multiple-birth rates for assisted reproductive technology procedures using thawed embryos, by patient age, USA 1999–2000

| Age (years) | Pregnancies per transfer procedure | | Live births per transfer procedure | | Multiple births per LBD[c] | | Triplet or higher-order gestations per pregnancy[d] | | Triplet or higher-order births per LBD[d] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No. of transfers | % | No. of transfers | % | No. of LBD | % | No. of pregnancies | % | No. of LBD | % |
| 20–29 | 3020 | 26.6 | 3020 | 21.6 | 632 | 28.2 | 581 | 7.1 | 482 | 4.4 |
| 30–34 | 8515 | 25.8 | 8515 | 20.7 | 1707 | 28.9 | 1636 | 7.6 | 1321 | 4.2 |
| 35–37 | 5185 | 25.3 | 5185 | 20.0 | 996 | 27.4 | 955 | 7.0 | 758 | 4.2 |
| 38–40 | 3338 | 22.6[a] | 3338 | 16.0[a] | 509 | 23.4 | 568 | 6.3 | 410 | 2.9 |
| 41–44 | 1497 | 19.8[b] | 1497 | 14.0 | 200 | 22.0 | 240 | 5.0 | 166 | 2.4 |
| $\chi^2$-test for trend | | $P < 0.01$ | | $P < 0.01$ | | $P < 0.01$ | | NS | | NS |

LBD = live-birth delivery; NS = not significant.
[a]$P < 0.01$, [b]$P < 0.05$ (comparison between the proportion in a given age group and the proportion in the preceding age group within the same data column).
[c]Excludes cycles in which only one embryo was transferred.
[d]Excludes cycles in which only one or two embryos were transferred.

live-birth rates, we focused our presentation on live-birth rates. Likewise, we focused our presentation on multiple-birth rates. However, both triplet and higher-order gestations and births are presented here because the pattern of results was not completely analogous between these two measures.

We stratified all measures according to patient age at the time of thawed embryo transfer (20–29, 30–34, 35–37, 38–40, or 41–44 years) and the number of embryos transferred (1, 2, 3, 4, or 5 or more). In addition, to assess potential effect modification and confounding, we stratified live-birth and multiple-birth rates by patient age, embryos transferred, and each of the following: prior births, prior assisted reproductive technology cycles, and use of assisted hatching techniques (in the current treatment cycle). Data on the stage of embryo development at cryopreservation or whether there was a period of post-thaw culture were not collected. Statistical significance for bivariate associations and analyses of trends were evaluated using $\chi^2$-tests.

Multivariable logistic regression analyses were also conducted for each outcome measure. The first regression model used live-birth delivery (yes/no) as the dependent variable and included cycles in which at least one embryo was transferred ($n$ = 20 247). The second model used multiple birth (yes/no) as the dependent variable and was limited to cycles in which at least two embryos were transferred and a live birth resulted ($n$ = 3808). The third model used triplet and higher-order gestation pregnancy (yes/no) as the dependent variable and was limited to cycles in which at least three embryos were transferred and a pregnancy resulted ($n$ = 3747). The fourth model used triplet and higher-order birth (yes/no) as the dependent variable and was limited to cycles in which at least three embryos were transferred and a live birth resulted ($n$ = 2954). All models included patient age, number of embryos transferred, prior births, prior assisted reproductive technology cycles, and use of assisted hatching techniques as independent variables. Since stratified analyses did not reveal any effect modification, interaction terms were not included in any model. All analyses were performed using Statistical Analysis Software (SAS) Version 8.2.

The institutional review board at the Centers for Disease Control and Prevention approved this study.

## Results

Both patient and procedure characteristics varied according to age (Table I). The proportion of women who had a prior birth, had at least two prior assisted reproductive technology cycles, and used assisted hatching techniques each increased with age. Number of embryos transferred was similar across age groups; however, the width of the distribution increased with age. For example, women 41–44 years of age were more likely to transfer a single embryo but were also more likely to transfer five or more embryos than younger women.

Pregnancy rates declined with age, from 26.6% among women 20–29 years to 19.8% among women 41–44 years of age (Table II). Live-birth rates declined with age, from 21.6% among women 20–29 years of age to 14.0% among women 41–44 years of age. Multiple-birth rates rose slightly from 28.2 to 28.9% among women 20–29 and 30–34 years respectively, and then declined, reaching 22.0% among women 41–44 years of age. A similar pattern emerged for triplet and higher-order gestation rates, as they increased from 7.1% to 7.6% among women 20–29 and 30–34 years of age respectively, and then declined, reaching 5.0% among women 41–44 years of age. Triplet and higher-order birth rates were comparable for women 20–37 years of age (4.2 to 4.4%), but lower for women 38–40 years of age (2.9%), and lowest for women 41–44 years of age (2.4%). All five measures showed an inverse trend with age; the trends for pregnancy, live-birth and multiple-birth rates were statistically significant.

Stratification by both patient age and the number of embryos transferred is presented in Table III. In general, the live-birth rate increased with each embryo transferred up to five or more for women aged 20–40 years and up to four for women aged 41–44 years. However in all age groups, the increase was greatest as the number of embryos increased from one to three and slight when the number increased from three to four or to five or more. Tests for trend were statistically significant for all age groups. Similar patterns were observed for pregnancy rates (data not shown).

Multiple-birth rates in all age groups generally increased with each additional embryo transferred up to five with the exception of 30–34 year olds, who had a slight decline in multiples from four to five embryos (Table III). Although the individual $\chi^2$-tests for increases in multiple births between successive embryo groups were not all statistically significant, the tests for trend were statistically significant for all five age

**Table III.** Live-birth and multiple-birth rates for assisted reproductive technology procedures using thawed embryos, by the number of embryos transferred and patient age, USA 1999–2000

| Age (years) | No. of embryos transferred | | | | | $\chi^2$-test for trend |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | ⩾5 | *P* value |
| Live births per transfer procedure (%) | | | | | | |
| 20–29 | 8.4 | 18.0[a] | 24.4[a] | 24.5 | 26.7 | < 0.05 |
| 30–34 | 8.5 | 16.4[a] | 23.9[a] | 24.3 | 24.6 | < 0.05 |
| 35–37 | 7.5 | 17.2[a] | 20.9[a] | 24.9[b] | 26.7 | < 0.05 |
| 38–40 | 7.4 | 12.3[b] | 16.9[a] | 19.0 | 21.5 | < 0.05 |
| 41–44 | 5.8 | 10.8 | 14.8 | 19.3 | 14.7 | < 0.05 |
| Multiple births per live birth (%) | | | | | | |
| 20–29 | | 16.0 | 29.0[a] | 35.3 | 37.3 | < 0.05 |
| 30–34 | | 17.1 | 29.4[a] | 36.3[b] | 35.3 | < 0.05 |
| 35–37 | | 16.0 | 28.3[a] | 32.4 | 35.2 | < 0.05 |
| 38–40 | | 16.2 | 20.4 | 25.7 | 32.6 | < 0.05 |
| 41–44 | | [c] | 15.9 | 22.6 | 34.1 | < 0.05 |
| Triplet or higher-order gestations per pregnancy (%) | | | | | | |
| 20–29 | | | 4.2 | 9.7[b] | 13.6 | < 0.05 |
| 30–34 | | | 5.5 | 9.0[b] | 13.2 | < 0.05 |
| 35–37 | | | 4.2 | 9.2[a] | 10.2 | < 0.05 |
| 38–40 | | | 4.5 | 4.3 | 12.4[a] | < 0.05 |
| 41–44 | | | 5.4 | 4.8 | 4.7 | NS |
| Triplet or higher-order births per live birth (%) | | | | | | |
| 20–29 | | | 3.6 | 5.9 | 4.5 | NS |
| 30–34 | | | 3.3 | 5.2 | 5.9 | NS |
| 35–37 | | | 2.9 | 5.5 | 4.9 | NS |
| 38–40 | | | 1.8 | 0.7 | 8.4[a] | < 0.05 |
| 41–44 | | | 4.8 | 1.6 | 0.0 | NS |

[a]$P < 0.01$, [b]$P < 0.05$ for comparison between the proportion in a given embryo category to the proportion in the preceding embryo category within the same age group.
[c]Insufficient sample size.

groups. For women 20–37 years of age, trends in triplet and higher-order gestation rates were similar to those observed for multiple-birth rates. Trends were less pronounced or not significant for women in the oldest age groups. For all age groups, trends in triplet and higher-order birth rates were attenuated, compared with the triplet and higher-order gestation rates. Moreover, the triplet and higher-order birth rates were difficult to interpret in the oldest two age groups because of insufficient sample sizes.

The results of our multivariable logistic regression analyses substantiate an age association with live births, multiple births, and triplet and higher-order gestations and deliveries (Table IV). Although categorical odds ratios were not statistically significant for the two triplet outcomes, when we entered age in the models as an ordinal variable, results indicated significant trends for live birth, multiple birth, and triplet and higher-order gestation pregnancies. The number of embryos transferred continued to be significantly associated with all four outcomes. Prior births and use of assisted hatching techniques were positively associated with live births and multiple births. Two or more prior assisted reproductive technology cycles were negatively associated with live birth.

## Discussion

We examined the live-birth rates and multiple-birth risk among women who underwent assisted reproductive technology using thawed embryos that had been created using their own oocytes. The use of thawed embryos is on the rise in the USA: the number of thawed embryo transfers rose 30% from 1996 ($n$ = 8661) to 2000 ($n$ = 11 394) (Centers for Disease Control and Prevention *et al*., 1998; 2002). Embryo cryopreservation could possibly have a favourable impact on the multiple-birth risk in fresh cycles by relieving some pressure that patients and providers may feel to transfer high numbers of embryos in a single cycle. Single embryo transfer in fresh cycles with cryopreservation of extra embryos has been an increasing focus among researchers in this field (Vilska *et al*., 1999; Martikainen *et al*., 2001; Tiitinen *et al*., 2001; Hogue, 2002). However, in our study we found that even among thawed cycles in the USA, two or more embryos were transferred in 90% of cycles and three or more embryos were transferred in ~65% of cycles. Thus, thawed cycles also posed a risk for multiple births. Studies to date present few or no data on the elective transfer of a single thawed embryo.

We found that both patient age and the number of embryos transferred were independent predictors of live birth. Even among the youngest women, the transfer of three or more embryos in a thawed cycle resulted in an increase in the live-birth rate, compared with cycles with one or two embryos transferred. However, the increase in success was offset by the multiple-birth risk, which also increased with the number of embryos transferred. In all age groups, the transfer of just two embryos resulted in a multiple-birth risk of 16–17%. The multiple-birth risk increased with the number of embryos transferred and was particularly marked for women aged <37 years, approaching 30% with three embryos transferred and well over 30% with more than three embryos transferred.

**Table IV.** Adjusted odds ratios (OR)[a] and 95% confidence intervals (CI) for live birth and multiple birth among assisted reproductive technology procedures using thawed embryos, USA 1999–2000

| | Live-birth delivery | | Multiple-birth delivery[b] | | Triplet and higher-order gestation pregnancy[c] | | Triplet and higher-order birth delivery[c] | |
|---|---|---|---|---|---|---|---|---|
| | OR[d] | 95% CI | OR[d] | 95% CI | OR[d] | 95% CI | OR | 95% CI |
| Age (years) | | | | | | | | |
| 20–29 (ref.) | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| 30–34 | 0.92 | 0.82, 1.02 | 0.97 | 0.79, 1.20 | 1.00 | 0.68, 1.47 | 0.87 | 0.51, 1.49 |
| 35–37 | 0.85 | 0.76, 0.96 | 0.87 | 0.68, 1.10 | 0.90 | 0.59, 1.38 | 0.87 | 0.48, 1.57 |
| 38–40 | 0.64 | 0.56, 0.73 | 0.66 | 0.49, 0.87 | 0.74 | 0.46, 1.21 | 0.60 | 0.29, 1.26 |
| 41–44 | 0.53 | 0.44, 0.63 | 0.56 | 0.38, 0.84 | 0.49 | 0.24, 1.01 | 0.49 | 0.16, 1.49 |
| No. of embryos transferred | | | | | | | | |
| 1 | 0.31 | 0.26, 0.38 | | | | | | |
| 2 | 0.69 | 0.63, 0.76 | 0.50 | 0.40, 0.62 | | | | |
| 3 (ref.) | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| 4 | 1.11 | 1.01, 1.21 | 1.33 | 1.12, 1.59 | 1.72 | 1.28, 2.31 | 1.51 | 0.98, 2.32 |
| ⩾5 | 1.22 | 1.08, 1.37 | 1.50 | 1.20, >1.87 | 2.74 | 1.97, 3.81 | 2.05 | 1.25, 3.36 |
| Prior birth | | | | | | | | |
| No (ref.) | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| Yes | 1.41 | 1.31, 1.52 | 1.27 | 1.09, 1.47 | 1.22 | 0.94, 1.58 | 1.18 | 0.80, 1.74 |
| No. of prior assisted reproductive technology cycles | | | | | | | | |
| 1 (ref.) | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| ⩾2 | 0.86 | 0.80, 0.92 | 0.96 | 0.83, >1.11 | 1.05 | 0.81, 1.36 | 0.99 | 0.68, 1.45 |
| Use of assisted hatching | | | | | | | | |
| No (ref.) | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| Yes | 1.19 | 1.11, 1.28 | 1.20 | 1.04, 1.40 | 1.00 | 0.78, 1.29 | 1.00 | 0.69, 1.45 |

[a]Adjusted for all variables shown in this table.
[b]Procedures in which only one embryo was transferred were excluded from the model.
[c]Procedures in which only one or two embryos were transferred were excluded from the model.
[d]Analyses were repeated with age as an ordinal variable for all four models. Results indicated a significant trend of declining live birth, multiple birth, and triplet and higher-order gestation pregnancies with increasing age ($P < 0.05$ for $\chi^2$-test for trend for age). The trend for triplet and higher-order births was not significant.

Moreover, the risk for having a triplet or higher-order gestation pregnancy was near 5% when three embryos were transferred and likewise increased with additional embryos. Trends in triplet birth were less pronounced than the trends in triplet pregnancy. This difference reflects reductions in the number of fetuses (both spontaneous and medical) between conception and birth. We did not have data to distinguish between spontaneous and medical reductions.

The pregnancy and live-birth rates reported here for thawed embryo transfer in the USA are higher than those reported from most international assisted reproductive technology registries (Nygren and Andersen, 2002). Similarly, the multiple-birth rates associated with thawed embryos were generally higher in the USA. From the data presented in registry reports, we could not determine how much these differences across countries might be explained by differences in the distributions of patient age and number of embryos transferred. The average number of thawed embryos transferred in the USA was 3.03 in 1999 and 3.00 in 2000, which was slightly higher than most other countries (generally fewer than three).

Although several studies of thawed embryo transfer from clinical settings have been reported (Wada *et al*., 1994; Wang *et al*., 1994; 2001; Frederick *et al*., 1995; Wennerholm *et al*., 1997; Mandelbaum *et al*., 1998), evaluation of predictive factors for success or risk factors for multiple birth appeared to have been hampered by low statistical power within strata. One of the largest clinical studies (Wang *et al*., 2001) noted that pregnancy rates increased among younger women, which is consistent with our findings. However, these researchers did not further evaluate age according to the number of embryos transferred.

Our study, drawn from a large population-based registry of assisted reproductive technology procedures, had a sufficient sample size for in-depth analyses of several key patient and treatment factors. A number of methodological limitations must also be considered, however. These data are based on transfer cycles rather than patients and we were unable to link cycles from the same woman. Thus, women who underwent more than one transfer using thawed embryos during 1999–2000 would be represented more than once in this sample. Women who underwent multiple cycles in a 2 year period probably failed at least one cycle, so the live-birth rates presented here are likely lower than the true ‘per patient’ rates.

Treatment options, such as the number of embryos transferred, were based on patient and provider choice and practice patterns in individual assisted reproductive technology clinics rather than by randomization. Furthermore, information on the survival rate or the quality of thawed embryos, as measured by specific laboratory indicators, was not collected within the assisted reproductive technology registry. Previous studies estimate that 30–40% of embryos do not survive the thawing process (Mandelbaum, 2000; Wang *et al*., 1994; 2001).

**Table V.** Live-birth rates per transfer (%) for thawed cycles, by patient age and whether embryos were refrozen, USA 1999–2000

| Age (years) | Embryos refrozen Yes | No | *P* value |
|---|---|---|---|
| 20–29 | 31.3 | 21.4 | < 0.05 |
| 30–34 | 38.5 | 20.4 | < 0.01 |
| 35–37 | 32.4 | 19.8 | < 0.01 |
| 38–40 | 27.9 | 15.8 | < 0.05 |
| 41–44 | [a] | 14.0 | |

[a]Insufficient sample size.

Research on the viability of embryos that do survive thaw suggests that the transfer of fully intact thawed embryos (100% blastomere survival) results in higher implantation rates than do embryos with 50–99% blastomere survival (Van den Abbeel *et al*., 1997; Guerif *et al*., 2002).

Although we lacked specific data on embryo quality, we did have data on the total number of embryos thawed, number of embryos transferred, and number of embryos refrozen. In our study population, all thawed embryos were transferred in 41% of cycles, fewer embryos were transferred than thawed and no embryos were refrozen in 57% of cycles, and fewer embryos were transferred than thawed but one or more embryos were refrozen in 2% of cycles. The latter group with refrozen embryos could represent a subset with higher quality embryos; that is, a choice was made to set aside some embryos. We examined this small subset separately: in each age group up to age 40 years, cycles in which one or more embryos were refrozen had notably higher success rates than the remaining cycles did (Table V). These results support previous research on embryo quality as an important predictor of success. Unfortunately, our sample size was insufficient to examine the multiple birth risk for this subset. Additionally, we were unable to further divide the 98% of cycles in which no embryos were refrozen by presumed embryo quality. For example, for the cycles in which not all embryos that were thawed were transferred, we were not able to discern whether some embryos were not transferred because only the highest quality embryos were chosen for transfer or because some embryos did not survive the thawing process. We also lacked data on the total number of embryos that were originally cryopreserved.

Another limitation to our study is that patient age was based on age at transfer; data on age at embryo fertilization were not available. However, for a portion of our study population ($n$ = 8500), we did have information on the duration of infertility, defined as the number of months the couple has been sexually active together without using contraception. Separate analyses of this subset revealed that among women who reported a short duration of infertility (≤1 year), the effect of decreasing live-birth rates with increasing age was even more pronounced than in our total sample (data not shown). Presumably, women with a short duration of infertility were most likely to have had similar ages at fertilization and transfer. Thus, these findings suggest that the total age effects we documented might have been even stronger had we been able to adjust for differential age at fertilization.

Previous studies suggested that the outcome of the patient's prior fresh cycle is predictive of the outcome of the subsequent thawed cycle (Toner *et al*., 1991; Wang *et al*., 2001). Although information was collected on the number of prior assisted reproductive technology (fresh and thawed) cycles for each assisted reproductive technology transfer, we did not have specific data pertaining to the outcome of the fresh cycle in which the frozen embryos used in the current study were created. Yet we were able to compare the success rates for women who reported a prior birth with those who did not and found that the success rates were indeed higher for those who reported a prior birth (data not shown). Unfortunately, we were not able to distinguish which prior births were assisted and which were naturally conceived.

As the number of assisted reproductive techniques using thawed embryos increases, it becomes critical to monitor and evaluate the success of these procedures on the general population. Further analyses of live-birth, multiple-birth, and triplet and higher-order multiple birth rates which consider a more in-depth and specific examination of embryo quality, patient age at embryo fertilization, and the outcome of the fresh cycle from which the thawed embryos were derived, are needed in elucidating factors that are predictive of various outcome measures.

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