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SPECIAL CONTRIBUTION

Cryopreserved embryos in the United States and their availability for research

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In association with The Society for Assisted Reproductive Technology (SART) and RAND

Objective: To determine the number of embryos stored at assisted reproductive technology (ART) clinics in the United States and their current disposition.

Design: A targeted survey instrument sent by the SART–RAND team to all medical practices providing in vitro fertilization services in the United States.

Result(s): The SART–RAND team surveyed all 430 ART practices in the United States. Of these practices, 340 returned surveys for analysis. The data from these surveys were merged with data taken from the 1999 SART dataset, which contains information about practice size and success rates. Responding clinics reported a total of 396,526 embryos in storage as of April 11, 2002. The vast majority of the embryos (88.2%) were targeted for patient use. Small numbers of embryos were available for research, donation, destruction, quality assurance, or other uses.

Conclusion(s): Nearly 400,000 embryos are stored in the United States, the majority of which (88.2%) are targeted for patient use. Few are available for research (2.8%), limiting possible conversion into embryonic stem cell lines. (Fertil Steril® 2003;79:1063–9. ©2003 by American Society for Reproductive Medicine.)

Key Words: IVF, ART, cryopreserved embryos, cryopreservation, stem cells

Recently, frozen human embryos have been the focus of considerable media attention. These human embryos, produced during fertility treatment, represent a potential source of the embryonic stem cells (ESCs) that might be used to grow replacement tissues for people suffering from cancer, Alzheimer's disease, diabetes, and other diseases. The capacity to create ESCs has touched off debates on embryo adoption, abortion rights, cloning, and the appropriateness of allowing federal funds to be used for human embryo research.

During the last 20 years, medical breakthroughs have allowed growing numbers of patients suffering from a variety of infertility-causing diseases to achieve healthy pregnancies through assisted reproductive technologies (ART). During ART clinical procedures, the number of human embryos produced is often in excess of the number that can be prudently transferred to the patient at one time. Patients can choose to store any embryos not transferred by cryopreserving them for future attempts at establishing pregnancies. For any number of reasons, the patient couple may decide to terminate their fertility treatment. At termination, the couple may reassign the frozen embryos from their own infertility treatment to one of several options. Couples may donate their embryos to another infertile couple (embryo donation), donate them to approved research projects such as those that generate ESCs, make them available for quality assurance activities, or ask that their embryos be destroyed.

The first live births from cryopreserved embryos were reported in Australia in 1984 and the United States in 1986 (1). Therefore, one can reasonably argue that embryos have been stored for future use since that time. In 1996, a British organization known as the Human Fertilization and Embryology Authority (HFEA) estimated that a total of 52,000 frozen embryos existed in the United Kingdom (2). In 2000 there were a total of 71,176 frozen embryos in Australia and New Zealand (3).

Despite the fact that embryos have been stored for decades, and not withstanding the

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recent widespread interest in their potential uses, the number of cryopreserved embryos in the United States is not known. Many of the proposed resolutions to the "embryonic stem cell debate" are based on the numbers of cryopreserved embryos that are actually available for research; however, this critical information is not available either. The media, legislators, and policy makers have been forced to rely on guesses about the number of cryopreserved embryos; they range from tens of thousands to several hundred thousand. The numbers of such embryos that are available for research, assumed to be a small fraction of these totals, are rarely even guessed.

This article presents data from a survey of all ART facilities in the United States regarding the numbers and disposition of cryopreserved embryos. The purpose of the research was to produce empirically derived estimates of the numbers of cryostored human embryos in the United States. In addition, we sought to determine the number of embryos that might possibly be available for donation for research, as a way of providing information that might help clarify the debates about stem cell research.

METHODOLOGY

Sample Construction

The SART–RAND research team sought to determine the number of frozen embryos in cryopreservation in the United States as well as the disposition of these embryos. To achieve this objective, the team surveyed all 430 ART practices in the United States. The survey sample was comprised of all SART member practices as well as those not affiliated with SART. SART collects ART data under a contract with the Centers for Disease Control and Prevention, which is charged with the collection of these data under the Fertility Clinic Success Rate and Certification Act of 1992; the Act is referred to as the Wyden Act (P.L. 102-493, 42 U.S.C. 263-a-1 et seq.). This Act was passed to provide consumers with ART practice efficacy information. Presently, 90% (375) of the IVF programs in the United States are SART members. Nonmembers still report their data to SART for inclusion in the annual Assisted Reproductive Technology Success Rates report published by the Centers for Disease Control (CDC). A survey was sent to all practices known to SART.

Survey Instruments

SART and RAND codeveloped the 23-item main survey instrument as well as a 19-item supplemental survey used in this study. Each practice was asked to complete the main survey, which included questions about the facility's use of cryopreservation; policies with respect to informed consent; an accounting of the total number of embryos stored at its facility, plus any off-site storage facilities; the disposition or future plan for those embryos; and policies and procedures regarding cryostorage of embryos. Practices that do not cryopreserve were asked to indicate this, and to answer a single question about why they do not do so. They were then asked to return the otherwise blank survey.

Practices that stored embryos at off-site or at satellite storage facilities were asked to request that these off-site facilities complete a supplemental survey form in an effort to ensure that all embryos associated with the sampled practice would be counted. The supplemental survey asked auxiliary storage facilities to provide an inventory of stored embryos and their disposition; each facility also was asked to indicate whether it could report for specific clinics or whether it could only provide aggregate counts. In addition, the supplemental survey covered cryopreservation practices and policies.

In order to keep the survey brief, practices were *not* asked to describe basic parameters, including number of cycles attempted in the last year for women of all ages, those less than age 35, and live birth event rates. Those data elements were obtained from the 1999 SART database described previously and were merged with data reported by responding clinics using SART identification numbers (which are also assigned to non-SART practices in the SART dataset). Because the two different surveys were fielded at different times, however, the practices included in each did not completely overlap. In the interval between the surveys, some clinics closed or merged, and new practices were established. Consequently, we were not able to merge the two datasets perfectly. For 50 of the 340 surveys, we could not match embryo survey and parameter data. These practices are not included in the analyses that relate parameter data to embryo profiles, but those facilities that responded to the embryo profile survey are included in the embryo counts.

Fielding the Survey

The survey package was mailed to the 430 ART practices in the SART database in January 2002. A number of efforts were made to ensure as high a response rate as possible. First, broadcast faxes and e-mails were used to alert practices to the study and the imminent arrival of a survey in December 2001; SART uses this method routinely to communicate with its members and with nonmember practices. Shortly after the initial mailing was completed, all surveyed practices were sent a reminder about the survey and the due date of January 31, 2002. This mailing elicited a number of messages that respondents had not received a survey. Surveys were then re-mailed or faxed to these practices, depending on their preferences. In March 2002, a reminder e-mail or fax was sent to all practices that had not yet returned a survey. In April 2002, each practice that had still not responded was assigned to a member of the SART Working Group. Each working group member then made a personal phone call to each nonresponding practice, urging that a survey be returned. The field period was ended May 1, 2002, when it was determined that we would not be able to increase the response rate any further.

The final sample includes 340 ART clinics. Clinics that indicated they did not cryopreserve were excluded from the

denominator; therefore, the overall final response rate was 85% at the end of the data collection period.

Data Handling

Practices were asked to provide an overall count of embryos in storage and to account for stored embryos by disposition. Once this more differentiated count was done, responding practices were asked to arrive at a total embryo count by adding each of the disposition categories. In nearly all cases, the two embryo counts were identical or very similar. In a few instances, practices were unable to provide a full accounting of embryos by disposition. Unless noted otherwise, embryo counts reported here are based upon the aggregate count instead of the total derived by summing embryos by disposition.

Of the original sample, 90 practices did not respond to the main survey. Size parameter variables were available for 58 of these 90 nonresponding clinics. For these 58 clinics, four parameter variables—total annual cycles attempted, total annual cycles attempted for women under 35, total live births to women under 35, and the corresponding live birth rates—were used to impute the total number of embryos in storage.

In order to estimate total counts for the nonresponding clinics, we ran an ordinary least-squares regression. In this regression, the dependent variable was the total embryo count derived in the main survey. The independent variables used were the four-parameter variables (e.g., total annual cycles attempted, total annual cycles attempted for women under 35, and the corresponding live birth rates).

Statistics

SAS for PC version 8.0 (SAS Institute, Inc., Cary, NC) was used to analyze data. Means, medians, standard deviations, maximums, and minimums were computed for all survey items. Correlations between the SART parameter data and survey responses were computed using Pearson's R statistic.

Considerable care was taken to ensure that the embryo counts were accurate. To do so, data from the supplemental survey had to be carefully matched to data from the practice survey. This matching task was complicated by the fact that the practice survey data were reported at the clinic level (one record per clinic), while the supplemental data were reported at the facility level (because several supplemental facilities might store embryos for a single practice, or one supplemental facility might store embryos for several practices, some of which might not have returned a survey). Indeed, some off-site facilities were unable to account for stored embryos separately by practice. (We were told that they are typically stored by patient name, so that aggregating to each practice would be extremely time-consuming.) Because an accurate total number of embryos was extremely important, we forced a merge by including only those supplemental facilities that said they were reporting for just one clinic. By doing this, we eliminated the possibility of double counting of any embryo. Of the 17 facilities that submitted a supplemental survey, 12 indicated that they were reporting for only one practice. Two practices had two supplemental facilities submit supplemental surveys. This meant that the 12 supplemental facilities that could report for one clinic could be linked to 10 practices for which we could cleanly merge supplemental data.

To obtain overall totals of embryos in storage for practices using auxiliary storage sites, embryo counts assigned to specific practices from the supplemental survey were added to the embryo totals for those practices in the main survey. In the next section, we also provide the number of embryos that could not be associated with a specific practice. When we rely on this larger figure, we make this very clear.

RESULTS

In this section, we first describe the clinics that responded to the survey. Then, we present the embryo counts and dispositions. We conclude this section with a discussion of the relationship between clinic characteristics and embryo storage and disposition.

Clinic Characteristics

Responding Practices

The 290 responding clinics for which we were able to merge parameter data varied dramatically in size, measured by the number of cycles attempted in the previous year on women of any age. The smallest clinic had made eight attempts; the largest clinic made 3,204. The median number of cycles attempted was 151.

Substantial variation also occurred in the number of cycles attempted in the previous year on women less than 35 years old. The smallest clinic had attempted one such cycle; the largest clinic attempted 1,020. The median number of cycles attempted in the previous year on women less than 35 was 57.

These clinics also varied substantially in their rates of success. Success rate was defined as the number of live birth events to women under 35 at the time of oocyte retrieval, divided by the number of fresh, nondonor cycles attempted in women under 35 in the last year. The measure of live birth events assigns a value of "1" to every live birth, even if it is a multiple birth. The clinics in our sample reported live birth event rates that varied from 0%–67%. The median live birth event rate for patients under 35 years of age was 31%.

We also examined parameter data for the 80 clinics for which we had no merged survey. Of these 80, 50 are practices for which we had a survey, but a merge was not possible. The remaining 30 are nonresponding practices.

Parameter data for the 80 nonmerged practices indicate that they are considerably smaller as a group, measured by the number of cycles attempted in the previous year on women of any age. The smallest clinic had made six at-

TABLE 1

Number of stored embryos by location and embryo disposition.

Location	Total	Embryo disposition					
		Patient's treatment	Destruction	Research	Quality assurance	Donation to another patient	Other
Clinic	391,661[a]	340,762	8,698	11,158	744	9,104	13,878
Off-site facility	4,865[b]	4,522[c]	38[c]	44[c]	8[c]	40[c]	0[c]
Total	396,526	349,830	8,840	11,283	752	9,225	13,878

[a] No. of embryos.

[b] Represents embryos enumerated in off-site facilities; excludes those that could not be assigned to a specific clinic. When the possibility of double counting is allowed, the total number is 9,677.

[c] Based on unduplicated totals. The disposition totals may not sum to the overall total because some embryos could not be assigned a disposition, but were part of the total count.

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tempts; the largest clinic made 100. The median number of cycles attempted was 81. Using number of cycles attempted on women under 35 in the last year, the same pattern emerged.

Success rates varied from 0%–100%, with the median live birth rate at 30%. These figures are comparable with those for the practices for which we were able to merge surveys.

Nonresponding Practices

Of the 90 practices that did not respond to the main survey, parameter data were available for 58 clinics. These 58 practices did not vary as greatly as the responding practices. For example, with respect to practice size (as reflected by the total number of cycles attempted for all women for the previous year), the smallest practice attempted seven cycles while the largest attempted 1,095. The median number of cycles attempted for these nonresponding clinics was 80. Similarly, with respect to all cycles attempted with women under 35 for the previous year, there was less variation for these practices than those captured in the main survey. The minimum number of cycles attempted for this age group was zero, and maximum number of cycles was 316, with a median of 25.

With respect to birth outcome parameter variables, nonresponding clinics exhibited less variance than responding clinics. For example, the live birth rate varied from a minimum of 13% to a maximum of 31% (median value was 30%). As noted above, we are unable to provide any insight into the characteristics of the 30 nonresponding practices for which we have no parameter data.

Embryo Counts and Dispositions

Responding Clinics

Our data reveal that virtually all the ART clinics responding to our survey do indeed cryopreserve embryos. The few that do not cryopreserve indicated that it is because of lack of capability or lack of demand.

Virtually all embryos are stored at clinic facilities. Only 17 of the 340 responding clinics reported using an off-site storage facility to store any frozen embryos. Such storage represents less than 2% of the total number of embryos (unduplicated count) identified by responding clinics.

Responding clinics reported a total of 391,661 embryos in storage at clinic facilities at the time of the survey. The supplemental survey revealed an additional 9,677 embryos stored off-site. When the number off-site is limited to facilities that can report for each clinic for which they store embryos separately (ensuring no double counts), embryos in storage totaled 4,865. Together, these two sources, using the more conservative off-site estimate, reveal a total of 396,526 embryos in storage as of April 11, 2002.

Most clinics were able to tell us why embryos were in storage. Most embryos (87%) that were in storage in clinic facilities (not off-site) were being held for patient treatment (340,762 embryos). Of those stored in clinics for other reasons, less than 3% are in storage awaiting donation to research. Just over 2% are in storage awaiting destruction per patient request; an equal percentage is in storage awaiting donation to another patient. Well under 1% of these embryos are being stored for use in quality assurance activities. See Table 1 for detail.

The preceding distribution of stored embryos presumably reflects patient preferences. In 338 of the 340 responding practices, the patient or couple must sign a consent form before their embryos are frozen. In nearly all cases, that consent form asks the patient to indicate what is to be done with the frozen embryos in the event that no one is able to make a decision. Virtually all consent forms allow patients the options of donating unwanted embryos to another patient or couple, donating them to research, making them available

TABLE 2

Imputation results for nonresponding practices in comparison with results obtained for responding practices.

Nonresponding clinics		Responding clinics	
Total embryo count	Average embryo count per practice	Total embryo count	Average embryo count per practice
51,753	892	396,526	1,166

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for quality assurance activities, or having them destroyed.

Almost 4% of the embryos that were declared by responding practices are in storage for other reasons. We asked respondents to describe reasons why embryos were in storage when they checked "other" at the bottom of the disposition list. A total of 13 other dispositions were specified by 50 clinics. These reasons included: lost contact with the patient; abandoned; patient deaths; waiting 7 years to discard; waiting for shipment out; undecided about transfer to another state; waiting for a disposition decision; donate to research or to another couple; embryology training; wishes not specified on permit/no permit; divorce case—awaiting final decision; and awaiting transfer to long-term storage.

The disposition profile for embryos stored in off-site facilities is quite similar to those stored in clinics. The vast majority (93%) is earmarked for patient treatment. Less than 2.5% are being stored awaiting donation to research, awaiting destruction, or awaiting donation to another patient. Only 0.16% is being stored for use in quality assurance activities. There were no embryos being stored for "other" reasons in off-site facilities. See Table 1 for detail.

Imputation of Total Embryo Counts for Nonresponding Practices

As described previously, we attempted to provide estimates of total embryo count through an imputation procedure that made use of the merged dataset of respondent surveys and parameter values. The R squared obtained for the regression on total embryo count on the four-parameter variables was 0.64.

In Table 2, we provide a summary of the imputation results for the 58 nonresponding clinics for which we have parameter data. The estimate of the total number of embryos for the 58 clinics was 51,753. This suggests an average of 892 embryos in storage per clinic.

Table 2 also presents the total embryo count for responding clinics as well as the average number of embryos stored per clinic. The average number of embryos stored per nonresponding practice was somewhat smaller at 892 compared with 1,166 for the responding practices.

Estimates of Total Embryo Count for Nonresponding Practices without Parameter Data

For the remaining 32 practices for which we have neither survey data nor parameter data, there is no reliable way of producing empirically sound estimates of the total number of embryos stored at these facilities. Although we could employ the average number of embryos for the respondent clinics or the imputed average for the 58 nonresponding practices with parameter variables, there is little justification for arguing that these 32 practices resemble either of these two categories of practices in the absence of any descriptive data for these clinics. Therefore, we provide no estimate because we know nothing about these practices.

The Relationship between Clinic Characteristics and Embryo Storage Profiles

We explored whether any association existed between clinic characteristics and embryo storage profiles. Correlations between clinic characteristics and embryo storage profiles were few. The strongest correlations were also the most obvious. For example, we found strong correlations between the total number of embryos stored and the number of cycles attempted in the last year. Because the number of cycles attempted approximates clinic size, this .77 correlation confirmed that larger clinics store more embryos. Of slightly more interest was the finding that the relationship between clinic size and number of embryos stored for patient use was higher (.70) than correlations between clinic size and other dispositions. For example, the correlation between clinic size and storage awaiting destruction or awaiting donation to research were both .32. This suggests that, as clinics become bigger, the majority of additional embryos that are stored are allocated to patient treatment.

Not surprisingly, little relationship existed between rate of live birth events—a measure of success—and embryo disposition; however, a significant correlation existed between total number stored and rate of live birth events. Clinics with more stored embryos had a higher live birth event rate. This correlation most likely approximates the relationship, already known in the literature (Centers for Disease Control and Prevention, 2001), between a facility's experience, which is measured in terms of number of cycles

TABLE 3

Relationship between practice characteristics and embryo storage profiles.

		Embryo disposition					
	Total	Patient's treatment	Destruction	Research	Quality assurance	Donation to another patient	Other
Practice characteristics							
No. of cycles attempted	0.77[a]	0.70[a]	0.32[a]	0.32[a]	0.25[a]	0.20[b]	0.51[a]
No. of cycles attempted, <35 years	0.68[a]	0.61[a]	0.27[a]	0.27[a]	0.13[c]	0.13[c]	0.50[a]
Live birth event rate, <35 years	0.15[c]	0.16[b]	—[d]	—[d]	—[d]	—[d]	—[d]

[a] $P<.0001$

[b] $P<.01$

[c] $P<.05$

[d] Correlations with $P>.05$ are not included in the table.

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per year, and improved ART outcomes. See Table 3 for correlations and significance levels.

DISCUSSION

The numbers of embryos identified by this survey reflect the state of cryostorage in the United States as of April 2002. We believe that the high survey response rate (85% of all ART programs) resulted in an accurate enumeration of embryos in storage at that time. The small number of nonresponding ART clinics represents a very small portion of the total ART treatments. Only a small number of embryos are not accounted for, and their omission does not significantly alter the count.

Previous estimates of the number of human embryos in storage have ranged from as few as 30,000 (4) to as many as 100,000 to 200,000 (5). These reports also imply that most of the embryos in storage are no longer needed for patient therapy and are therefore available for research. Among the surprising outcomes of the survey was that neither of these estimates was accurate. The results indicate that the actual number of embryos in storage is slightly less than 400,000, or about twice the previously estimated number. In addition, the number of stored embryos available for research was a very small fraction of the total, 2.8%. The vast majority (88.2%) of the embryos in frozen storage are designated for patient treatment. Patients are holding these embryos in storage for future utilization in their clinical efforts to establish a family. Only about 11,000 embryos have actually been designated for research.

Although 11,000 embryos is a seemingly large number, these embryos may not have the highest developmental potential for a variety of reasons. First, ART clinics generally transfer the best-quality embryos to the patient during the fresh treatment cycle. Consequently, the remaining embryos available to be frozen are not always the highest quality. Second, some of these cryostored embryos may have been in storage for a number of years. At the time they were created, culture conditions used in ART laboratories may not have been as optimized as they are today. Finally, some loss of embryos is inherent in the actual freeze-thaw process. For these reasons, the cryopreserved embryos are less likely to produce viable blastocysts than nonfrozen embryos.

What this means in terms of the number of embryos available for stem cell production is illustrated in the following series of calculations. We conservatively estimate that about 65% of these embryos will survive the freeze-thaw process, resulting in about 7,334 embryos actually available for research ($11,283 \times 65\% = 7,334$) (6, 7). Additionally, embryo development to the blastocyst stage is a necessary step to the production of ESCs. It can be expected that only about 25% of these embryos will be able to develop to the blastocyst stage ($7,334 \times 25\% = 1,834$) (8). Finally, production from the blastocyst is also very inefficient. Researchers at the University of Wisconsin needed 18 inner-cell masses from blastocysts to create five human cell lines, while workers at The Jones Institute used 40 blastocysts to develop three lines (efficiencies of 27% and 7.5%, respectively) (9, 10). If we use a conservative estimate between these two stem cell conversion rates (7.5%–27%), for example, 15%, there could be as many as 275 embryonic stem cell lines created ($1,834 \times 15\% = 275$); however, this number is only possible if all of the embryos donated to research in the United States are used exclusively to create stem cells, which is highly unlikely to occur.

Although our survey estimates almost twice as many frozen embryos as the most generous previous speculation, very few of these embryos are available for research. These findings have significant implications for current debates on stem cell research.

DESCRIPTION OF SART AND RAND

SART was established in 1987 in an effort to provide support and direction for the IVF programs in the United States. Since that time, SART has provided education, practice guidelines, oversight, and, most important, data collection for IVF programs. The data collection function has resulted in the publication of clinic-specific data since 1989 and has been published in conjunction with the CDC since 1995.

RAND, a private nonprofit research institution was established in 1948 to conduct independent, objective research and analysis. A partnership between RAND and SART was a logical fit for this project. Jointly, the two organizations provide the necessary tools to collect and analyze the data: SART with access to and knowledge of IVF programs, procedures, and science and RAND in design, implementation, and analyses of surveys and survey data.

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