

Efficiency of oocyte cryopreservation: a meta-analysis

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Objective: To determine the efficiency of oocyte cryopreservation relative to IVF with unfrozen oocytes.

Design: Meta-analysis.

Setting: Academic assisted reproduction center.

Patient(s): Results of all reports from January 1997 to June 2005 with the patients undergoing IVF-intracytoplasmic sperm injection (ICSI) with cryopreserved cycles between 1996 and 2004 were compared with those of patients who underwent IVF-ICSI with unfrozen oocytes in 2002 and 2003 in our program.

Intervention(s): Mean age and number of ET cycles originating from unfrozen oocytes was matched with those for thaw cycles originating from oocytes cryopreserved with a slow-freezing (SF) protocol. Vitrification (VF) reports were not included in the comparative analysis because of a small number of pregnancies (10) before June 2005.

Main Outcome Measure(s): The comparison of fertilization rate, clinical pregnancy, and live-birth rates per injected oocyte, clinical pregnancy and live-birth rates per transfer, and implantation rate between IVF-ICSI cycles with frozen and unfrozen oocytes.

Result(s): Live-birth rates per oocyte thawed were 1.9% and 2.0% for SF and VF, respectively, before June 2005. Live-birth rates per injected oocyte and ET, respectively, were 3.4% and 21.6% for SF and were 6.6% and 60.4% for IVF with unfrozen oocytes. Compared to women who underwent IVF after SF, IVF with unfrozen oocytes resulted in significantly better rates of fertilization (odds ratio [95% confidence interval]); 2.22 (1.80, 2.74), of live birth per injected oocyte; 1.5 (1.26, 1.79), and of implantation; 4.66 (3.93, 5.52). These odds ratios were lower when oocyte cryopreservation success rates from 2002–2004 were compared with those for IVF with unfrozen oocytes. When the reports after June 2005 were considered, this trend did not appear to continue. With the consideration of VF reports after June 2005, however, higher pregnancy rates were achieved.

Conclusion(s): In vitro fertilization success rates with slow-frozen oocytes are significantly lower when compared with the case of IVF with unfrozen oocytes. Although oocyte cryopreservation with the SF method appears to be justified for preserving fertility when a medical indication exists, its value for elective applications remains to be determined. Pregnancy rates with VF appear to have improved, but further studies will be needed to determine the efficiency and safety of this technique. (*Fertil Steril*® 2006;86:70–80. ©2006 by American Society for Reproductive Medicine.)

Key Words: Oocyte cryopreservation, slow freeze, vitrification, IVF, ICSI, fertility preservation

Oocyte cryopreservation undoubtedly has potential to improve and extend the current assisted reproductive technologies. First, this technique does not require partner's sperm and thus can be used to preserve fertility in single women. Second, some of the ethical quandaries relating to the storage and disposal of surplus embryos are avoided. The technique also can simplify the egg donation process for IVF; if frozen oocytes are fertilized as efficiently as the unfrozen ones, donor oocyte banks can be established. There no longer would be a need to synchronize donor and recipient cycles, and long waiting periods for appropriately matched donors could be avoided.

Even though the first pregnancies from oocyte cryopreservation were reported nearly 2 decades ago, the true efficiency of performing IVF with frozen banked mature (metaphase II) oocytes remains unknown. From 1986, when the first pregnancies with frozen-thawed oocytes were reported (1–6), until 1997, when intracytoplasmic sperm injection (ICSI) first was used to fertilize frozen-thawed oocytes (7), there were only five live births from frozen-thawed oocytes. After the use of ICSI to circumvent zona hardening associated with the cryopreservation process, numerous small reports have appeared in the medical literature. Nevertheless, because of the lack of a prospective study determining the effectiveness of IVF using frozen versus unfrozen oocytes, it remains difficult to properly counsel patients who consider oocyte cryopreservation.

Several reviews have attempted to address this deficiency, but they did not go beyond a crude summary of the literature. Although they tallied up the pregnancies from all previously

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published studies, these reports did not consider data overlap and did not have access to raw data on all oocyte cryopreservation reports (8–10). Moreover, in general, the pregnancy rates from frozen–thawed oocytes were summarized on a per-oocyte frozen or thawed basis that did not allow a direct comparison to standard IVF with unfrozen oocytes, because in the latter, success rates typically are reported per attempt (ET) to achieve pregnancy.

Given the current state of confusion in medical literature regarding the success rates of IVF with cryopreserved oocytes, we performed a meta-analysis with the aim of providing the best estimate of success for patients undergoing this procedure. Moreover, we determined the relative success rates of women undergoing IVF-ICSI with slow-frozen oocytes compared with women who underwent IVF with unfrozen oocytes in a large academic assisted reproduction program.

MATERIALS AND METHODS

Data Collection

MEDLINE was searched for the period from January 1986, when the first pregnancy from oocyte cryopreservation was reported, until June 2005. We used *oocyte*, *cryopreservation*, *freezing*, and *vitrification* as key words and limited the search to human studies with pregnancy as the outcome measure. Reference lists of authoritative reviews on oocyte cryopreservation also were examined for published articles or abstracts. In addition, the online database of Fertility, Sterility, and Human Reproduction also was searched for the same period. Abstract books of the European Society of Human Reproduction and Embryology and the American Society for Reproductive Medicine annual meetings from 1986–2004 were searched manually. We also included unpublished data as provided by three investigators.

We contacted investigators to obtain the raw data for age, clinical pregnancy and live-birth outcomes, number of frozen and thawed oocytes, and number of embryos transferred if these data were not already reported in the publication and, when an investigator published more than one article on oocyte freezing, to rule out data overlap. We obtained three sets of unpublished data from three investigators; one updated the published data (11), another sent the results of an ongoing study (Donaldson MJ, Quintans CJ, unpublished data), and the third investigator sent data from an article that was in press and that since has been published (12).

Institutional review board approval was not needed because this was a meta-analysis and because the summary data for the control group were identified retrospectively from a clinical embryology database without patient identifiers.

Definitions

A clinical pregnancy was defined as a pregnancy that had ≥ 1 gestational sac by ultrasound evaluation. A live birth was defined as a delivery resulting in ≥ 1 live infant, regardless of

whether the gestation was singleton or multiple. Implantation rates were calculated by dividing the number of gestational sacs by the number of embryos transferred. A spontaneous abortion was defined as the loss of pregnancy prior to 20 weeks of gestation, including ectopic pregnancies. If the pregnancy persisted beyond the 24th week of gestation but delivery had not occurred, it was considered an ongoing pregnancy.

Exclusion Criteria

The articles that have missing raw data, used immature oocytes for freezing, or did not use ICSI as the IVF method were excluded from the comparison but were used for calculating the total number of children born and for calculating the overall pregnancy and live-birth rates from oocyte cryopreservation.

Selection of the Studies for Comparative Analysis

Excluding the reports that did not have pregnancy as the main outcome, the search provided a total of 54 reports, of which 40 were manuscripts (including 1 updated manuscript), 12 were abstracts, and 2 were unpublished data sets (Donaldson MJ, Quintans CJ; Kyono K, now published as Kyono et al. [12]). All reports were in English, with the exception of two, written in German and Czech, respectively.

Of the 54 reports, 47 used the slow-freezing (SF) technique (1–7, 11, 13–50), whereas in 7, vitrification (VF) was used to freeze oocytes (12, 51–56). Two reports with VF (51, 52) and 11 reports with SF (13–23) were not included because they contained overlapping data, reducing the number of manuscripts to 41. Of the 36 SF reports, 6 were non-ICSI (1–6), 3 used immature oocytes (47–49), 1 did not have age data available (48), and the authors of 1 could not be reached (50). In one of the five VF reports, immature oocytes were used (53).

Because there were too few pregnancies and live births with VF, only the studies in which SF was used to cryopreserve oocytes were used for the comparative analysis. Thus, after excluding reports that used vitrification, had immature oocytes, were non-ICSI, or were missing data, 26 SF reports were used for comparative analysis (Table 1). Those that were not included in comparative analysis and those that were published after June 2005 are summarized in Table 2.

Comparison Group

The comparison (control) group was selected from patients who underwent IVF-ICSI with unfrozen oocytes in our program in 2002 and 2003 and whose delivery information was available. The number of oocyte thaw cycles was matched with the number of ET cycles originating from unfrozen oocytes. We compared the similar number of cycles in which the pregnancy was intended; in patients with frozen oocytes, this represented each thaw attempt, whereas in women un-

TABLE 1

Summary of oocyte cryopreservation pregnancies with slow freeze that were included in the comparative analysis.

First author, year (reference citation no.)	Report type	Patients	Clinical pregnancies	Abortions	Children born	Live births
Porcu, 1997 (7)	Case report	1	1	0	1	1
Young, 1998 (24)	Case report	1	1	1	0	0
Polak de Fried, 1998 (25)	Case report	1	1	0	1	1
Nawroth, 1998 (26)	Case report	1	0 ^a	0	0	0
Allan, 1998 (27)	Retrospective	12	0 ^a	0	0	0
Wurfel, 1999 (28)	Case report	1	1	0	2	1
Porcu, 1999 (29)	Prospective	96	16	3	16	13
Porcu, 1999 (30)	Case report	1	1	0	2	1
Porcu, 1999 (31)	Case report	1	1	0	2	1
Porcu, 2000 (32)	Prospective ^b	23	3	0	3	3
	Case report	1	1	0	1	1
Chia, 2000 (33)	Case report	1	1	1	0	0
Winslow, 2001 (34)	Prospective	33	12	2	16	10
Kyono, 2001 (35)	Case report	1	1	0	1	1
Chen, 2002 (36)	Case report	1	1	0	2	1
Quintans, 2002 (37)	Retrospective	12	6	4	2	2
Fsoas, 2003 (38)	Prospective	7	4	0	5	4
Borini, 2004 (39)	Retrospective	68	15	3	14	12
Huttelová, 2004 (40)	Case report	3	1	0	1	1
Miller, 2004 (41)	Case report	1	1	0	3	1
Notrica, 2004 (42)	Case report	1	1	0	1	1
Chen, 2004 (43)	Case report	1	1	0	1	1
Chen, 2005 (44)	Prospective	21	7	0	8	7
Levi Setti, 2005 (45)	Case report	1	1	0	1	1
Tjer, 2005 (46)	Case report	1	1	0	1	1
Donaldson, unpublished data	Unpublished	16	3	1	2	2
Boldt, 2003 (11)	Prospective ^c	47	13	4	11	9
Total		354	95	19	97	76

Note: All data are n.

^a Biochemical pregnancy.

^b Review containing a prospective study and a case report.

^c Updated report.

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dergoing IVF with unfrozen oocytes, this represented IVF-ET attempts. Because these were ICSI cycles, they also represented a comparable number of retrievals in which ≥ 1 mature oocyte was available. This approach also enabled us to account for the negative effect of thawing efficiency when comparing the total numbers of children born from frozen versus unfrozen oocytes and the per-thaw pregnancy rates between frozen oocytes and embryos. For comparing pregnancy rates per oocyte injected and total number of live births and infants, we also considered the pregnancies that resulted from the transfer of frozen-thawed surplus embryos in these IVF cycles versus unfrozen oocytes.

To ensure that the difference in IVF success rates with frozen versus unfrozen oocytes was not a result of later

overall improved success with IVF, we also compared our rates for mean live births per ET and for implantation from 1995–2004 with the success rates for oocyte cryopreservation from the same period. Moreover, oocyte-cryopreservation success rates from 2002–2004 (reported from 2003 to June 2005) were compared separately with those of unfrozen IVF-ICSI cycles from 2002–2003 and with age-matched Society for Assisted Reproductive Technology (SART) data from 2003 (57).

Statistical Methods

Summary statistics were used to describe pregnancy rates per oocyte thawed. We did not permute success rates per frozen oocyte as a significant proportion of those that re-

TABLE 2

Studies that were excluded from the initial analysis or published after June 2005.

First author, year (reference citation no.)	Patients	Clinical pregnancies	Abortion	Children born	Live births	Reasons to exclude
Chen, 1986 (1)	7	2	0	2	2	Non-ICSI
Chen, 1988 (2)	1	1	0	1	1	Non-ICSI
Al-Hasani, 1986 (3)	22	1	1	0	0	Non-ICSI
Al-Hasani, 1987 (4)	NA	2	2	0	0	Non-ICSI
Van Uem, 1987 (5)	2	1	0	1	1	Non-ICSI
Siebzehnuebl, 1989 (6)	10	1	0	1	1	Non-ICSI
Kan, A 2004 (49)	1	1	1	0	0	Immature oocytes
Tucker, 1998 (47)	1	1	0	1	1	Immature oocytes
Tucker, 1998 (part B) ^a (48)	5	1	0	1	1	Immature oocytes
Tucker, 1998 (part A) ^a (48)	22	5	3	3	2	No age data
Vidali, 1998 (50)	48	6	NA	NA	NA	Author not reached
Wu, 2001 (53)	36	0 ^b	0	0	0	VF and immature oocytes
Kuleshova, 1999 (55)	4	1	0	1	1	VF
Yoon, 2003 (56)	34	6	0	7	6	VF
Katayama, 2003 (54)	2	2	0	2	2	VF
Kyono, 2005 (12)	1	1	0	1	1	VF
Chian, 2005 (59) updated data	25	11	2	19	9	VF, after June 2005
Kim, 2005 (60)	13	7	2	3	2 + 3 ^c	VF, after June 2005
Ruvalcaba, 2005 (61)	NA	8	4	NA	4 ^c	VF, after June 2005
Okimura, 2005 (62)	NA	12	2	7	7 + 3 ^c	VF, after June 2005
Lucena, 2006 (63)	73	13	NA	NA	13 ^c	VF, after June 2005
Azambuja, 2005 (64)	1	1	0	1	1	SF, after June 2005
Azambuja, 2005 (65)	32	8	NA	NA	NA	SF, after June 2005
Jain, 2005 (66)	6	3	NA	NA	NA	SF, after June 2005
Levi Setti, 2006 (67)	120	18	6	13	12	SF, after June 2005
Montag, 2006 (68)	1	1	0	1	1	SF, after June 2005
Borini, 2006 (69)	146	18	3	4	4 + 11 ^c	SF, after June 2005
Li, 2005 (70)	8	4	1	3	3	SF, after June 2005

Note: NA = not available.

^a Two-part report; age data of part A can't be obtained, pregnancy in part B derived from immature oocytes.

^b Biochemical pregnancy.

^c Ongoing pregnancy.

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mained thawed in the SF group. Because the outcome data of interest were binary, we were able to reproduce raw data from the summary statistics we obtained. To evaluate the relative efficiency of IVF-ICSI using frozen oocytes compared with that using unfrozen oocytes, odds ratios (ORs) with 95% confidence intervals (CIs) were calculated for fertilization and pregnancy rates. To this end, we used generalized estimating equations to account for within-study correlation, which is expected to be moderate to high (58). Compound symmetry (i.e., equicorrelation) was assumed for correlation structure, and robust empirical standard error was used for constructing confidence limits. Because we did not have patient-specific information such as a patient identifier (only for further clustering within each study), other than the

ages in the published and raw data, we could not adjust for factors other than the mean age that might have impacted the outcomes in the analysis.

RESULTS

Overall Number of Pregnancies Resulting From SF and VF Oocytes

After the correction for overlapping studies but before other exclusions, there were a total of 117 and 10 clinical pregnancies and of 85 and 10 live births resulting from SF and VF, respectively, until June 2005. These resulted in the birth of 107 and 11 children, respectively, for SF and VF. Because of the small number of pregnancies with VF, the compara-

TABLE 3**Fertilization and pregnancy results after vitrification.**

Variable	Reports before June 2005	Reports after June 2005	All VF reports
Age, mean \pm SE (y) ^a	32.3 \pm 0.85	32.3	32.3
Fertilization rate	70.6 (156/221)	75.4 (481/638)	74.2 (637/859)
Clinical pregnancies per thawed oocytes	2 (10/503)	6 (51/851)	4.5 (61/1354)
Live births per thawed oocytes	2 (10/503)	4.6 (39 [7]/851)	3.6 (49 [7]/1354)
Clinical pregnancies per transfer	29.4 (10/34)	51 (51/100)	45.5 (61/134)
Live births per transfer	29.4 (10/34)	39 (39 [7]/100)	36.6 (49 [7]/134)
Implantation rate	8.8 (12/137)	20.5 (69/336)	17.1 (81/473)

Note: Data are % (n) unless otherwise indicated. Data in square brackets are number of ongoing pregnancies.

^a Because raw data were not available for studies published after June 2005, SE of the mean could not be calculated.

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tive analysis only was performed with SF. With the inclusion of reports after June 2005 (59–70) (Table 2), the total numbers of clinical pregnancies were 170 and 61 for SF and VF, respectively, resulting in 106 live births and 11 ongoing pregnancies and in 42 live births and 7 ongoing pregnancies for SF and VF, respectively.

Vitrification Success Rates

Mean clinical pregnancy rates per thawed oocyte after exclusions for VF are shown in Table 3. Fertilization rate, live-birth rate per injected oocyte, and live-birth rate per ET for VF, after excluding one report (53) that used immature oocytes and resulted in a biochemical pregnancy, were 70.6%, 4.5%, and 29.4%, respectively. For the same group, the mean live-birth rate per thaw cycle was 24.4%, whereas the implantation rate was 8.8%. Of the 10 live births from VF, 1 involved delivery of twins, whereas others were singletons. There was no pregnancy loss reported.

With the addition of cases reported after June 2005, pregnancy rates were significantly increased (Table 3). Among the five recent studies, mean age was reported in only two, and one of the other three authors sent age data, and this was comparable to the mean age of patients previously reported (32.3 \pm 0.85 years [mean \pm SE] in previous reports vs. 32.4 years in recent studies). But because mean age was not available in three of five new reports, an effect of age on recently improved pregnancy rates could not be ruled out. Mean number of embryos transferred was similar between the reports published before June 2005 (4 embryos per transfer) and those published afterward (3.5 per transfer).

Comparative Meta-Analysis Between SF and Unfrozen Oocytes

After the exclusions, there were 397 oocyte thaw cycles, resulting in 95 clinical pregnancies and 76 live births in the

SF group. This amounted to 4,564 frozen and 4,000 thawed oocytes. Clinical pregnancy and live-birth rates per thawed oocyte were 2% and 1.9%, respectively. Mean number of embryos transferred was similar between SF and unfrozen IVF cycles (respectively, 2.6 vs. 2.8). Results from the most recent studies that were not included in this comparative analysis were similar, with a 2.2% rate of clinical pregnancy per oocyte thawed after the transfer of 2.3 embryos. Including these studies, the overall clinical pregnancy rate per thawed oocyte for the period of 1996–2005 was 2.3%.

In the control group, there were 126 cycles, resulting in 415 surplus embryos that were frozen. Sixty-four patients returned to use these embryos. Of the 181 embryos that were thawed, 141 were transferred in 62 ET cycles. The frozen ET cycles resulted in a 51.6% delivery rate per ET, 39 clinical pregnancies, and 32 viable pregnancies (24 live births as well as 8 ongoing at the third trimester).

The results of the comparative analysis are shown in Table 4. In comparison to IVF with unfrozen oocytes, IVF with frozen–thawed oocytes resulted in significantly lower fertilization, clinical pregnancy, live-birth, and implantation rates ($P < .005$). Per-ET delivery and implantation rates were similarly lower when compared with our mean rates from 1996–2004 for the same age group: respectively, 21.6% and 13.1% with SF versus 54.1% and 32.9% with unfrozen oocytes ($P < .0001$, Fisher's exact test). When pregnancy rates were calculated per oocyte thaw cycle, these rates were even lower; the clinical pregnancy and live-birth rates per thaw cycle in the SF group were 23.9% and 19.1%, respectively.

To rule out the possibility of overall improvement in oocyte cryopreservation with time, we compared the SF success rates from 2002–2004 separately with our comparison group that underwent IVF with unfrozen oocytes. Valid statistical analyses still had to account for age differences because the mean age of patients undergoing SF during

TABLE 4

Comparison of IVF-ICSI success rates between frozen-thawed and unfrozen oocyte cycles.

Variable	SF 1996–2004 (group a)	SF 2002–2004 (group b)	IVF with unfrozen oocytes (group c)	IVF with frozen oocytes (group d)	Group c vs. group a, OR (95% CI)	Group d vs. group a, OR (95% CI)	Group c vs. group b, OR (95% CI)
Age, mean \pm SE (y)	33 \pm 0.24	31.1 \pm 0.6	33.6 \pm 0.22	33.5 \pm 0.45			
Fertilization rate	61 (1,346/2,217)	64.9 (309/476)	76.7 (2,788/3,637)	NA	2.22 (1.80, 2.74)	NA	2.13 (1.24, 3.64)
Clinical preg per injected oocyte	4.3 (95/2,217)	5.9 (28/476)	7.5 (272/3,637)	NA	1.69 (1.44, 1.98)	NA	1.30 (0.94, 1.81)
Live births per injected oocyte	3.4 (76/2,217)	5 (24/476)	6.6 (240/3,637)	NA	1.50 (1.26, 1.79)	NA	1.42 (0.95, 2.14)
Clinical preg per transfer	27.1 (95/351)	37.8 (28/74)	68.5 (272/397)	62.9 (39/62)	7.05 (5.73, 8.09)	5.50 (4.47, 6.76)	4.95 (3.41, 7.19)
Live births per transfer	21.6 (76/351)	32.4 (24/74)	60.4 (240/397)	51.6 (32/62)	6.83 (5.76, 8.09)	4.76 (4.02, 5.65)	4.73 (2.63, 8.51)
Implantation rate	13.1 (122/929)	15.3 (34/222)	39.8 (436/1,095)	36.9 (52/141)	4.66 (3.93, 5.52)	4.11 (3.47, 4.88)	4.49 (3.44, 5.88)

Note: Data are % (n) unless otherwise marked.

Clinical preg = clinical pregnancies, NA = not available.

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2002–2004 was lower than that of the comparison group. This comparison yielded lower ORs with 95% CI, and the ORs with 95% CI for clinical and live pregnancy rates per injected oocyte crossed unity (Table 4). The latter finding suggests that the success rates with oocyte cryopreservation technology are improving. In all models that we implemented, age was negatively associated with the outcome.

However, inclusion of the most recent reports with SF (after June 2005) did not support the assumption that the SF success rates are improving, because rates of clinical pregnancy per transfer were lower than during the previous periods (Table 5). The mean age was specified in six of the seven recent SF reports, and it was higher than that reported previously (34.7 vs. 33.3 years). The greater mean age could account partially for the lower success rates in recent reports.

Although obviously the raw data from the SART report on overall success rates were not available, we performed a naive χ^2 analysis to compare the 2003 per-ET live-birth rates in 31-year-old women with the per-ET live-birth rates from oocyte cryopreservation cycles (SF) during 2002–2004 (data reported 2003 to June 2005). This comparison also revealed that the live-birth rates using unfrozen oocytes were significantly higher compared with those using frozen oocytes (Table 6). Likewise, the comparison of SF pregnancy rates from 1996–2004 (data reported before June 2005) to mean SART data for the similar time period confirmed that the IVF success rates with SF oocytes were lower compared with those with unfrozen oocytes (Table 6).

Inclusion of pregnancies from frozen embryos resulted in a larger difference in live births between frozen and unfrozen oocytes (Table 4). Although there were 76 live births with 97 newborns after IVF-ICSI of frozen-thawed oocytes, these numbers were 240 and 335 and 260 and 359 for IVF-ICSI with unfrozen oocytes without and with accounting for frozen embryos, respectively. In addition, there were eight ongoing pregnancies from frozen embryos. Nevertheless, because ≥ 500 oocytes and 297 embryos remained frozen, the final number of infants that could result from each treatment remains to be determined. However, because pregnancy per thaw and transfer cycle as well as implantation rates from frozen oocytes also were significantly lower compared with those using frozen-thawed embryos (Table 4), the usage of all remaining oocytes and embryos is more likely to increase the number of newborns in favor of IVF with unfrozen oocytes.

In the SF, 25% of all pregnancies were multiples and 20% resulted in pregnancy loss, whereas in the group that received IVF with unfrozen oocytes, the corresponding rates were 38.3% and 12.5%.

DISCUSSION

We performed a meta-analysis of all oocyte cryopreservation cycles reported since 1986. Moreover, we performed a comparative analysis of all IVF cycles that were performed with

SF oocytes and IVF cycles that used unfrozen oocytes. Our comparison of the pregnancy outcomes to the results of one of the largest IVF programs in the United States revealed that the efficiency of IVF-ICSI with frozen oocytes is significantly lower. Estimated ORs of success by various indices of pregnancy ranged from 1.5 to 7 when IVF using unfrozen oocytes was compared with IVF-ICSI using frozen-thawed oocytes. We also found that the mean age of the patient in all reported oocyte cryopreservation cases was approximately 33 years, an age at which IVF with unfrozen oocytes is expected to be highly successful. For example, although the live-birth rate with the SF technique was approximately 21%, the corresponding rate was 60% for patients undergoing IVF with unfrozen oocytes in our program.

Vitrification is a relatively new approach to oocyte freezing, and there had been only 10 live births from this technique as of June 2005. Because of this, we were unable to perform a comparative analysis. Since the first preparation of this article, there have been five new reports on vitrification, with 51 clinical pregnancies. Considering these, overall per-thaw and ET pregnancy rates appear to have improved significantly and have well exceeded those with SF oocytes. In fact, the current success rates with VF appear to be better than the rates reported by SART in 2003.

There are two caveats to the latter observation, however. First, raw data and mean age were not available for the majority of studies reported after June 2005, and thus it currently is not possible to directly compare these rates with those achieved previously from frozen and unfrozen oocytes. Second, a higher mean number of embryos was transferred in VF compared with SF (3.7 vs. 2.5 for all years), suggesting that supernumerary ET may be responsible at least partially for improved success rates. It will be worthwhile to find out the incidence of multiple births resulting from VF oocytes as the details of these recent abstracts become available. Thus, although VF currently is associated with higher rates of pregnancy per ET, judgment should be reserved until all confounders are known.

It can be questioned whether the comparison of oocyte-freezing success rates from numerous programs to the results of a highly successful IVF program reflects the current state of success with oocyte cryopreservation. Although the ideal comparison would be to the IVF success rates of the programs reporting oocyte-freezing studies that are included in this meta-analysis, we chose this approach, aside from practical reasons, in order to determine the gap between the full potential of IVF-ICSI with unfrozen and frozen oocytes. Because our program has one of the highest success rates in the United States, the difference in success rates would have represented the progress that needed to be made to equalize the efficiency of performing IVF-ICSI on frozen and unfrozen oocytes. Moreover, oocyte cryopreservation is performed by a select group of programs around the world that usually are considered to be the leading centers in their areas. We did, however, attempt to address this potential pitfall by

TABLE 5 Success rates of IVF with slow-freezing oocytes over three consecutive time periods. ^a				
Variable	SF 1996–2004 (Reported before June 2005)	SF 2002–2004 (Reported 2003–June 2005)	SF 1999–2005 (Reported after June 2005)	SF 1996–2005 (All cycles reported as of March 2006)
Age, mean \pm SE (y) ^b	33 \pm 0.24	31.1 \pm 0.6	34.7	33.7
Fertilization rate	61 (1,346/2,217)	64.9 (309/476)	71.7 (1,094/1,526)	64.9 (2,478/3,818)
Clinical pregnancies per thawed oocyte	2.4 (95/4,000)	4.1 (28/688)	2.2 (53/2,409)	2.3 (153/6,720)
Clinical pregnancies per injected oocyte	4.3 (95/2,217)	5.9 (28/476)	3.5 (53/1,526)	4.0 (153/3,818)
Clinical pregnancies per transfer	27.1 (95/351)	37.8 (28/74)	14.1 (53/376)	20.6 (153/742)
Implantation rate	13.1 (122/929)	15.3 (34/222)	6.5 (57/871)	10.1 (185/1,828)
Note: Data are % (n) unless otherwise indicated. ^a To compare reports over three consecutive time periods, all reports regardless of age data were included, but non-ICSI and immature oocyte cycles were excluded. ^b Because raw data were not available for studies published after June 2005, SE of the mean could not be calculated.				
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TABLE 6						
Comparison of slow-freeze success rates with SART data from similar time periods.						
Variable	SF 1996–2004 (group a; 33 ± 0.24) ^b	SART (1997–2003) success rates for age 33 y (group b)	P values, group a vs. group b ^a	SF 2002–2004 (group c; 31.1 ± 0.6) ^b	SART 2003 success rates for age 31 y (group d)	P values, group c vs. group d ^a
Clinical pregnancies per transfer	27.1 (95/351)	46.6 (14,037/30,109)	<.0001	37.8 (28/74)	54.5 (2,317/4,250)	.004
Live births per transfer	21.7 (76/351)	38.4 (11,552/30,109)	<.0001	32.4 (24/74)	47.8 (2,031/4,250)	.009
Note: Data are % (n) unless otherwise indicated.						
^a Conducted by χ^2 test.						
^b Age, mean ± SE (y).						
Okay. Efficiency of oocyte cryopreservation. Fertil Steril 2006.						

comparing pregnancy rates from frozen–thawed oocytes with the national data in the United States. Nevertheless, the data presented here also can be used by each IVF program for comparison with its own age-matched data and to counsel patients considering oocyte freezing for various reasons.

Our main comparison group that underwent IVF with unfrozen oocytes was from 2002–2003, whereas the oocyte cryopreservation cycles were from 1996–2004 (reported by June 2005). To rule out the possibility that the higher success rates with unfrozen oocytes were a result of overall improvement in IVF techniques in recent years, we also compared the mean live-birth rate per ET and implantation rates from our program during 1995–2004 with those from frozen–thawed oocytes and confirmed the higher success rates with unfrozen oocytes. Furthermore, live-birth rate per ET using unfrozen oocytes from all reporting clinics in the United States ranged from 27% in 1997 to 37% in 2003 for the same age group (57), rates consistently higher than those using frozen–thawed oocytes (Table 6). Even though the difference from the national live-birth rates appears to be smaller than with our own data, the SART data do not take into account the pregnancies resulting from surplus embryos. However, when we compared oocyte cryopreservation success rates from 2002–2004 (published from 2003 to June 2005) with those with unfrozen oocytes, we found weaker associations. In fact, when clinical pregnancy and live-birth rates per oocyte injected were compared between the two groups, the OR estimates were not differentiable from the null value. However, the ORs for per-ET pregnancy rates still were >4 when the success rates for IVF with unfrozen oocytes were compared with the success rates with frozen–thawed oocytes from 2002–2004. Moreover, pregnancy rates from the most recently reported cycles with SF (after June 2005) were lower than from those for 2002–2004. Overall, these findings further support our conclusion that IVF success rates are lower with frozen-banked oocytes compared with unfrozen oocytes.

There are, however, several potential explanations for the drop in success rates with SF in reports after June 2005. First, pregnancy rates from North American centers appear to be higher than those reported from elsewhere in the world (albeit the mean age of patients was lower in the United States; Table 7), and there were fewer reports from North America after June 2005 compared with the previous period. Second, patients reported after June 2005 were older compared with those reported before that date (34.7 vs. 33 years).

A number of reports have been published or presented on oocyte cryopreservation with SF and VF since the first preparation of this article. Those reports were incorporated into this article during the revision stage, but because the raw data and live-birth rates were not available for these recent data, we could not include them in the comparative analysis.

Another speculative question regarding oocyte freezing is whether it is efficient enough to be used electively to delay childbearing. Although a few commercial programs already

TABLE 7

Comparison of North American and other centers' results after slow freezing.

Variable	North American studies	Other studies
Age, mean \pm SE (y) ^a	31.3	34
Clinical pregnancies per thawed oocyte	3.2 (34/1,065)	2.1 (119/5,655)
Clinical pregnancies per injected oocyte	6.1 (34/556)	3.6 (119/3,262)
Clinical pregnancies per transfer	32.4 (34/105)	18.7 (119/637)
Implantation rate	15.9 (47/295)	7.8 (119/1,533)

Note: All studies are reported as of March 2006. Data are % (n) unless otherwise indicated.

^a Because raw data were not available for studies published after June 2005, SE of the mean could not be calculated.

Oktaç. Efficiency of oocyte cryopreservation. Fertil Steril 2006.

have begun doing this, the usefulness of this approach has not yet been validated. On the basis of this meta-analysis, we found that implantation rates using frozen-thawed oocytes for a 33-year-old woman matched those for a 41- to 42-year-old woman for the same time period in our program. Even when the implantation rates from 2002–2004 or those only from the United States are considered, these correspond to those of a 40-year-old woman undergoing IVF with unfrozen oocytes in our program. This indicates that, at least in our program, if a woman will not be delaying childbearing for >7 years, elective oocyte freezing currently is not feasible with SF.

For VF, even though the current success rates appear to be much more acceptable, direct comparisons to SF and standard IVF cannot yet be performed because of reasons already discussed. Further comparative studies with careful attention to the possible toxic effects of high gradients of cryoprotectants will be needed to render a judgment on this technique.

Like many meta-analyses and comparative studies, our investigation was subject to limitations in study design. Because we did not have access to individual-level information other than patient age, we could not adjust for other potential confounders in the statistical modeling. However, we successfully controlled for dependence within each study, age (as an additional covariate), and year of the study (by subgroup analysis restricting to recent years), and kept statistical estimation and inference methods conservative to ensure valid and fair evaluation. Because no prior publications that we identified were based on the controlled design and the data were limited, we could not conduct the standard meta-analysis for two-group comparisons, homogeneity test of ORs across studies, and multivariate modeling.

On the basis of our meta-analysis, the efficiency of IVF-ICSI with frozen-thawed oocytes appears to justify its use to preserve fertility in young women who will be receiving medical treatments that have high probabilities of ovarian failure and infertility. The current take-home-infant rate using SF oocytes may be acceptable for a 33-year old woman

who otherwise faces a significant risk of losing her fertility as a result of medical treatments. However, the efficiency of this procedure does not appear to have caught up sufficiently with the efficiency of IVF using unfrozen oocytes to justify its use to develop donor egg banks or replace embryo freezing, nor its routine use for elective deferral of childbearing. Further prospective controlled studies in which patients are randomized to IVF treatment with unfrozen versus SF versus VF oocytes are needed to determine the current comparative efficiency of oocyte freezing. Moreover, more laboratory research will be needed to improve the freezing protocols and success rates with oocyte freezing. Further follow-up data will be needed on the children born after oocyte cryopreservation, especially with the VF technique. Until then, the data presented herein can be used to counsel patients in deciding whether to resort to this strategy under various clinical and social scenarios.

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