Review of currently available publications addressing the efficacy of egg freezing by vitrification

The first pregnancy resulting from IVF with frozen eggs was reported in 1986 (1), followed by reports of the first birth the following year (2). Since then numerous publications from fertility centers around the world have reported successful pregnancies and births resulting from IVF using frozen eggs (3-34). To date nearly 1,000 live births resulting from frozen eggs have been reported, with no apparent increase in congenital abnormalities (35). Despite this experience, the most recent practice committee opinions of both the American College of Obstetricians and Gynecologists and the American Society for Reproductive Medicine still consider egg freezing to be an investigational procedure (36, 37).

Results of egg freezing have been mixed. Many early studies of egg freezing reported relatively poor survival (3, 5, 7, 9, 12, 13, 15, 19-21, 27, 38-43), while in later studies survival rates of 90% or greater were achieved by many different groups working independently (10, 11, 16, 18, 22, 24, 29, 30, 44, 45). A few studies reported reduced fertilization or limited developmental capacity (21, 46-48), while most have demonstrated fertilization rates (7, 9-13, 15, 16, 18-20, 22, 24, 29, 30, 34, 39, 42, 45, 47), chromosomal status (16, 39, 42), embryonic development (7, 16, 20, 22, 30, 41, 42, 45, 49), and pregnancy rates (10, 15, 16, 18, 22, 24, 29, 30, 34, 45) similar to that achievable with non-frozen eggs.

The two basic methods that have been used to freeze eggs are slow cooling or rapid cooling (vitrification). While slow cooling is the more conventional and commonly used method of freezing eggs, many studies have demonstrated that egg survival (11, 16, 22, 24, 30, 44, 50-53) and subsequent embryonic development and pregnancy outcomes (16, 22, 23, 30, 44, 54) are considerably better when the vitrification method is used.

A comprehensive summary of published reports of survival and fertilization rates following human egg vitrification is provided in Table 1. Collectively, these studies report outcomes for 7,811 vitrified and thawed human eggs, of which 6,757 (86.5%) survived the vitrification and thawing procedures. A total of 5,502 of these vitrified and thawed eggs were subsequently inseminated by intracytoplasmic sperm injection (ICSI), resulting in the successful fertilization of 4,239 eggs (77.0%). Closer examination of these reports suggests that the efficacy of egg vitrification has improved over time as the technique has been refined. Rates of survival and fertilization for reports published through 2006 were 79.7% (2120/2660) and 71.1% (744/1047) respectively. Among reports published in 2007 or later, rates of egg survival increased significantly ($p<0.0001$) to 90.0% (4637/5151, 95% confidence interval = 89.2-90.8). Fertilization rates also increased significantly ($p<0.0001$) to 78.5% (3495/4455, 95% confidence interval = 77.2-79.7) during this time, a success rate that is at least as high as can typically be achieved with conventional insemination of freshly retrieved eggs. The available literature thus indicates that the best estimates for egg survival and fertilization for vitrification as

currently practiced are 90% and 78% respectively. These reports also demonstrate that there is no apparent reduction in fertilization potential following vitrification.

Table 1. Published reports of human egg survival and fertilization rates following vitrification.

Author	Year	Eggs thawed	Eggs surviving	Percent survival	Eggs insem.	Eggs fertilized	Percent fertilized
Hunter *et al.* (55)	1995	20	13	65%	13	9	69%
Hong *et al.* (54)	1999	106	98	92%	90	59	66%
Kuleshova *et al.* (6)	1999	17	11	65%	11	5	45%
Chen *et al.* (51)	2000	152	145	95%	145	73	50%
Katayama *et al.* (11)	2003	46	43	93%	43	39	91%
Liebermann *et al.* (52)	2003	1120	904	81%			
Yoon *et al.* (12)	2003	474	325	69%	198	142	72%
Chian *et al.* (56)	2005	180	169	94%	169	126	75%
Kim *et al.* (57)	2005	233	165	71%	131	77	59%
Kuwayama *et al.* (16)	2005	64	58	91%	58	52	90%
Kyono *et al.* (17)	2005	5	5	100%	5	5	100%
Ruvalcaba *et al.* (58)	2005	60	46	77%	46	38	83%
Lucena *et al.* (22)	2006	159	120	75%	120	105	88%
Selman *et al.* (59)	2006	24	18	75%	18	14	78%
Antinori *et al.* (24)	2007	330	328	99%	328	305	93%
Yoon *et al.* (60)	2007	364	302	83%	218	168	77%
Chang *et al.* (61)	2008	200	168	84%	168	140	83%
Cobo *et al.* (29)	2008	693	666	96%	666	487	73%
Cobo *et al.* (62)	2008	16	14	88%	14	10	71%
Cao *et al.* (63)	2009	292	268	92%	268	182	68%
Fadini *et al.* (64)	2009	285	225	79%	162	118	73%
Keskintepe *et al.* (65)	2009	690	665	96%	665	612	92%
Nagy *et al.* (66)	2009	153	134	88%	134	117	87%
Schoolcraft *et al.* (67)	2009	160	123	77%	123	97	79%
Cobo *et al.* (68)	2010	937	889	95%	889	643	72%
Grifo *et al.* (69)	2010	163	155	95%	155	115	74%
Kim *et al.* (70)	2010	395	320	81%	285	206	72%
Rienzi *et al.* (71)	2010	124	120	97%	120	95	79%
Smith *et al.* (72)	2010	349	260	74%	260	200	77%
Total		**7811**	**6757**	**87%**	**5502**	**4239**	**77%**

Four studies have directly compared outcomes of IVF using vitrified versus fresh eggs retrieved from the same women, and performed at the same facilities using the same embryo culture protocols. Antinori *et al.* (24) compared results of IVF using freshly retrieved eggs to a subset of the same patients who returned for another IVF cycle using vitrified excess eggs after failing to achieve pregnancy in their fresh cycles. They found that vitrification was associated with an only slightly lower fertilization rate (93% vs 97%) and comparable rates of cleavage, pregnancy per embryo transfer, pregnancy loss, and implantation per egg inseminated compared to fresh eggs. These results are especially impressive given that all patients included in the vitrification group had failed to achieve pregnancy in their fresh cycle, suggesting that they had a poorer prognosis. Nagy *et al.* (66) compared results of IVF with vitrified donor eggs to the results of previous fresh donor IVF cycles by the same donors. Even though the numbers of eggs available to each recipient were much lower in the frozen versus the fresh cycles, fertilization rates, implantation rates, pregnancy rates per fresh transfer, and cumulative pregnancy rates (combining both fresh transfers and subsequent transfers of frozen embryos) were all as high in the vitrified egg cycles as in the prior fresh cycles. In a particularly well-controlled study Cobo *et al.* (30) compared fertilization rates and embryonic development between sibling eggs by prospective randomization of donor egg cohorts into fresh IVF or post-vitrification IVF treatment groups. They found no significant differences in fertilization rates, day 2 cleavage, day 3 cleavage, day 3 embryo quality, blastocyst formation, or blastocyst quality. In a similarly well-designed study, Rienzi *et al.* (71) prospectively randomized sibling eggs within cohorts retrieved from infertility patients to either fresh IVF or post-vitrification IVF. They found comparable fertilization rates, embryonic development, and embryo quality between the fresh and post-vitrification treatment groups. All four of these comparative studies collectively and consistently demonstrate that fertilization and embryonic development of vitrified eggs appear to be comparable to freshly retrieved eggs, and the two studies that compared implantation and pregnancy rates indicate that these most clinically important outcomes are also comparable.

A recent review estimated that approximately 460-510 babies have been born worldwide after egg vitrification (73). The most recent SART/ASRM Practice Committee opinion recommends estimating the live birth rate per vitrified and thawed egg at 4% (74), based entirely on a meta-analysis of egg freezing published in 2006 (23). This meta-analysis noted an apparent increase in the efficacy of egg vitrifcation over time as the procedure was refined. Publications of egg vitrification after June 2005 reported 39 live births resulting from 851 vitrified and thawed eggs (4.6%), a significantly higher success rate than prior publications reporting 10 births from 503 vitrified and thawed eggs (2.0%). Since the publication of this meta-analysis, reports of clinical use of vitrified eggs have increased substantially (Table 1), and demonstrate continuing improvement in vitrification efficacy. Five of these later studies included complete birth outcomes (29, 65-67, 70), collectively reporting 97 babies resulting from 1,461 vitrified and thawed eggs, a birth per egg rate significantly higher than that of the latter studies included in the 2006 meta-analysis (6.6% vs 4.6% $p = 0.04$). The best estimate of the baby per egg rate for vitrification as currently practiced, based on the available literature, is therefore 6.6%.

Direct comparison of these birth outcomes with outcomes of IVF using fresh eggs is difficult, because outcomes of fresh cycles are generally reported per treatment cycle rather than per egg retrieved. The only exception is a 2009 study that evaluated egg-to-baby efficiency rates among fresh IVF treatment cycles (75). Following collection of 1,705 eggs in 87 donor egg cycles, 80 babies were born following fresh embryo transfer. In addition to fresh transfers, 372 embryos were frozen. Based on outcomes of frozen embryo transfers during the same time period, it was estimated that an additional 37 babies would be expected if all 372 frozen embryos were thawed and transferred. Thus the expected egg-to-baby efficiency if all viable embryos were utilized in fresh and subsequent frozen embryo transfers was 6.9% (117 babies from 1,705 eggs). Equivalently derived egg-to-baby efficiencies for patients using their own eggs were 4.4% (181/4152) among women <38 years, 3.0% (25/843) among women 38-40 years, and 1.0% (4/383) among women 41-42 years.

In order to supplement this limited published information, and to provide statistics more directly applicable to our center, we conducted a similar analysis of egg-to-baby efficiency of fresh IVF using our own clinical data. Over the five year period of 2004-2008, our center collected a total of 192,991 eggs in 14,324 retrieval procedures among infertility patients using their own eggs. During the same period, a total of 28,722 eggs collected from presumably fertile donors were used in 1,983 donor egg recipient cycles. Using the same methodology as used in the published report, we estimated the maximum number of babies that could have resulted from the use of all of these eggs by adding the estimated the number of babies that could potentially result from use of all frozen embryos (based on our observed rates of survival and birth among thawed and transferred frozen embryos) to the actual number of babies resulting from fresh embryo transfers. Our results indicated that approximately 6.5% of all eggs retrieved from donors were capable of producing a live baby. Among infertility patients using their own eggs, estimated maximum egg-to-baby efficiency rates were lower and declined with patient age: 5.2% for women <35 years; 4.4% for women 35-37 years; 3.3% for women 38-40 years; 1.8% for women 41-42 years; and 1.2% for women >42 years. Thus our results are in close agreement with the results of the published report. The results of our analysis and of the published report also demonstrate that the egg-to-baby efficiency rate of 6.6% estimated for egg vitrification as currently practiced is very similar to egg-to-baby efficiency rates calculated for conventional fresh IVF among the highest prognosis patients (i.e. those using donor eggs).

We believe this emerging literature and data on egg freezing, addressing outcomes including post-thaw survival, fertilization, embryo development, pregnancy and birth rates demonstrate great promise for its use in a variety of clinical situations. Early studies on the health of children conceived after transfer of embryos derived following the thawing of previously frozen eggs are also encouraging.

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