Comparative analysis of fetal and neonatal outcomes of pregnancies from fresh and cryopreserved/thawed oocytes in the same group of patients

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Objective: To analyze the fetal and neonatal outcomes of pregnancies achieved with fresh and/or frozen oocytes in the same group of patients.
Design: Observational study and comparative analysis.
Setting: Research unit of an academic medical center.
Patient(s): A group of 855 women with cryopreserved oocytes and their resulting 954 assisted reproductive technology clinical pregnancies were enrolled and followed up during the same time period and in the same clinical setting; the outcomes of 197 pregnancies from frozen/thawed oocytes were compared with 757 obtained from fresh sibling oocyte cycles.
Intervention(s): None.
Main Outcome Measure(s): Pregnancies were followed until delivery, and neonatal data (up to 28 days after delivery) were collected.
Result(s): No significant differences were found between the use of fresh and frozen oocytes in the rates of therapeutic abortions for fetal anomaly (1.5% vs. 0.8%) and ectopic pregnancies (3.6% vs. 2.9%), but a significantly higher rate of spontaneous abortions at $\leq$12 weeks (17.6% vs. 26.9%) was observed in the frozen/thawed oocytes group. No statistical differences were found in major anomalies at birth (2.8% vs. 4.6%). Despite no difference in gestational age at delivery, the mean birth weights were significantly lower with fresh oocyte pregnancies, both in singleton (2,725 $\pm$ 727 g) and twins (2,128 $\pm$ 555 g), than with frozen–thawed oocytes (3,231 $\pm$ 615 g and 2,418 $\pm$ 492 g, respectively). However, the analysis of the 63 patients who obtained pregnancies both in fresh and thawed cycles (138 pregnancies) showed no differences in the abortion rate and in the mean birth weight.
Conclusion(s): These results provide strong support to the notion that fetal and perinatal complications and congenital anomalies do not differ between pregnancies from frozen–thawed and fresh oocytes. The significantly lower mean birth weight observed with pregnancies from fresh oocytes supports similar observations reported for pregnancies from embryo cryopreservation and requires further prospective studies. (Fertil Steril® 2013;100: 396–401. ©2013 by American Society for Reproductive Medicine.)
Key Words: IVF pregnancy outcome, oocyte cryopreservation, slow-freezing, birth defects, fertility preservation

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For a long time cryopreservation has been used as a complement to IVF. Historically, in 1954 there was the first report of successful sperm cryopreservation (1), in 1984 the first report of births from embryo freezing (2, 3), followed by, in 1986, the first baby from oocyte cryopreservation (4). Technological developments have allowed embryo

and oocyte cryopreservation to become a much more common clinical strategy. In fact, today a growing proportion of assisted reproductive technology (ART) children are born after cryopreservation of either embryos or gametes (5). Contrary to embryo cryopreservation, the history of oocyte cryopreservation and its implementation is more recent, owing to the initial low success rates and the lack of reproducibility, which made the technique largely neglected for a long period.

A significant improvement in oocyte cryopreservation has been forced because of legal reasons in Italy, where no more than three oocytes were allowed (March 2004-May 2009) for insemination. Although even before the 2004 Italian law there were successful reports of births after oocyte cryopreservation (6–9), it was after the implementation of legal restrictions (10) that the interest in oocyte cryopreservation peaked and made Italy the experimental clinical setting to test this procedure (11–14).

Today several clinical indications call for oocyte cryopreservation (15–17). They include fertility preservation for young oncologic patients at risk of premature ovarian failure or premature ovarian impairment due to gonadotoxic treatments (18–22); personal concerns or ethical issues for embryo cryopreservation; "freezing for fertility postponement" owing to the tendency in today's society of delaying childbirth until a later age (23); and establishing oocyte banks as a source for oocyte donation (24–26).

Very recently the American Society for Reproductive Medicine has removed the label of "experimental" from oocyte cryopreservation for fertility preservation (27); however, at the same time it stressed the importance of accumulating more evidence on the health of children born after the use of cryopreserved–thawed oocytes. Assisted reproductive technology outcomes (rates of congenital anomalies, birth weights, prematurity, pregnancy losses, and epigenetic problems) have always been a matter of concern (28–33). Reassuring recent publications indicate that pregnancy rates achieved with frozen oocytes and frozen embryos are comparable to those achieved in fresh cycles, and multiple studies addressing oocyte and embryo physiology during cryopreservation have been recently published (34–43). Nevertheless, they mostly focused on efficacy of cryopreservation techniques, whereas larger studies are needed to verify the safety in terms of newborns' health.

This issue is even more relevant in pregnancies obtained with the use of cryopreserved oocytes. Recent literature has been reassuring for births obtained after the use of cryopreserved oocytes for donation from egg banks (35); however, it is particularly important to gather outcome data from births obtained with the patient's own oocytes.

The aim of the present study was to review the obstetric and neonatal outcomes of pregnancies obtained from fresh and cryopreserved/thawed oocytes in the same group of patients that cryopreserved oocytes and had pregnancies in the fresh or thawing cycles or with both procedures.

MATERIALS AND METHODS

The same group of patients (n = 855) that obtained pregnancies from fresh and/or cryopreserved–thawed oocytes was identified from our database, and their fetal and neonatal outcomes were compared.

A maximum of three oocytes were used in fresh cycles, as established by an Italian law regulating ART (2004–2009), and the surplus sibling oocytes were cryopreserved for later use. As for fresh cycles, a maximum of three thawed oocytes were used per frozen cycles. The cryopreservation technique used in this series consisted of slow freezing and rapid thawing, and it has been detailed in previous reports (12, 14). All oocytes (fresh and those that survived the cryo/thaw process) were inseminated by intracytoplasmic sperm injection.

In transfers with embryos from frozen oocytes, the endometrial preparation was obtained with sequential estrogen/P therapy, with GnRH down-regulation used only in the first 18 pregnancies.

All the oocytes used for the study came from the same group of patients that obtained a pregnancy with fresh oocytes only, or with their frozen oocytes only, or with both fresh and frozen oocytes and thus served as their own control group.

A group of clinicians and psychologists was dedicated to the follow-up through structured forms and direct contact with family doctors, obstetricians, and pediatricians. The data presented are up to the end of the perinatal period (28 days after delivery). Children are still in follow-up on a yearly basis, and these data will be reported at a later time.

Pregnancies $\geq$24 weeks were considered deliveries, pregnancies ending in spontaneous abortions were divided into miscarriages $\leq$12 weeks and >12 weeks. Pregnancies concluded with a therapeutic abortion for fetal anomaly and ectopic pregnancies were also considered in the study, as well as fetal anomalies diagnosed in the perinatal and postnatal period. Pregnancies and neonatal outcomes obtained after oocyte thawing were compared with pregnancies obtained during the same period with the transfer of embryos obtained in fresh IVF/ICSI cycles.

Anomalies were considered according to the European Surveillance of Congenital Anomalies classification (44).

Data were described as number and percentage or mean and standard deviation. Statistical analysis was performed with Stata11 (45), and $P<.05$ was considered statistically significant.

The study was approved by our institutional review board, and this investigation has been supported by a finalized grant from the National Center for Epidemiology, Surveillance and Health Promotion, National Health Institute, Rome, Italy.

RESULTS

A total of 855 couples and 954 clinical pregnancies were evaluated, of which 197 were obtained with frozen oocytes and 757 from fresh oocyte cycles. In detail, 672 women had 687 pregnancies and 649 babies born only from fresh cycle (Table 1); 120 women had 129 pregnancies with 102 babies born only from frozen oocytes; 63 couples had 138 pregnancies (total of 86 babies born) from both fresh and frozen oocytes cycles (70 pregnancies with 43 babies delivered in fresh cycles and 68 with 43 babies delivered in thawed cycles). The mean woman's age (calculated at the time of oocyte retrieval) was 34.0 ± 3.7 years for those pregnant in fresh cycles vs. 33.8 ± 3.8 in those pregnant with frozen oocytes

TABLE 1

Obstetric outcomes after oocyte thawing and with fresh oocyte cycles.

Outcome	Thawed oocytes	Fresh oocytes	*P* value
All deliveries[a]			
Deliveries	134	568	
Babies born	145	692[a]	
Gestational age (wk)	37.9 ± 2.7	37.4 ± 3.0	.020[b]
Birth weight (g)	3,107 ± 664	2,725 ± 727	<.001[b]
Females	85 (58.6)	348 (50.4)	.070
Singleton			
Babies delivered	123	444	
Gestational age (wk)	38.4 ± 2.6	38.5 ± 2.7	.747
Birth weight (g)	3,231 ± 615	3,012 ± 659	.001[b]
Females	74 (60.2)	235 (53.1)	.161
Twins			
Babies delivered	22	248	
Gestational age (wk)	35.4 ± 1.7	35.4 ± 2.7	.425
Birth weight (g)	2,418 ± 492	2,212 ± 537	.045[b]
Females	11 (50.0)	135 (54.4)	.689

Note: Values are mean ± SD or number (percentage).
[a] Triplets (n = 16, all in fresh cycles) were excluded.
[b] Statistically significant.

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cycles (nonsignificant). The mean number of transferred embryos was 2.6 ± 0.6 in fresh cycles vs. 2.4 ± 0.7 in frozen oocyte cycles (*P*=.001).

Among the 197 pregnancies from frozen oocytes, 134 (68.0%) resulted in deliveries, 53 (26.9%) ended in spontaneous abortions (all before 12 weeks), 3 in (1.5%) therapeutic abortions for fetal anomaly, 7 (3.5%) were ectopic pregnancies, and no pregnancies were lost to follow-up. Of the 757 pregnancies from fresh oocyte cycles, 584 (77.2%) resulted in deliveries, 133 (17.6%) ended in spontaneous abortions ≤12 weeks, 12 (1.6%) ended in abortions >12 weeks, 6 (0.8%) in therapeutic abortions for fetal anomaly, 22 (2.9%) were ectopic pregnancies, and no pregnancies were lost to follow-up.

In the fresh oocytes group a significantly higher number of pregnancies reached delivery (77.1% vs. 68.0%, *P*=.008), and fewer embryonic losses ≤12 weeks were observed (17.6% vs. 26.9%, *P*=.003). However, in the fresh pregnancies there were losses after 12 weeks, not seen with pregnancies from frozen oocytes. There were no significant differences in ectopic pregnancies and therapeutic abortion rates. There was one stillbirth at 31 weeks for placental abruption in the fresh and none in the frozen group.

The vanishing twin rate, defined as loss of an embryo with cardiac activity before 12 gestational weeks, was 2.81% (n = 16) in the fresh group and 2.23% (n = 3) in the thawed group (nonsignificant).

In the frozen/thawed oocyte pregnancies, the reasons for the therapeutic abortions were Arnold-Chiari syndrome (n = 1), Turner's syndrome (45,X0 karyotype) with fetal anasarca (n = 1), and a twin pregnancy with both fetuses affected by trisomy 18 (n = 1), related to a balanced translocation in the father.

In the fresh oocyte pregnancies, the therapeutic abortions were for genitourinary malformations (n = 1), gastroenteric malformation (n = 1), respiratory system malformation (n = 1), and for chromosomal anomalies (n = 3).

Of the 134 deliveries in the cryopreserved oocytes group, 123 (91.8%) were singleton and 11 (8.2%) were twins, for a total of 145 babies born. No triplets were observed in this group. In the fresh oocyte ET group 584 deliveries were recorded: 444 (76.0%) were singletons, 124 (21.2%) twins, and 16 (2.7%) triplets, for a total of 740 babies born.

The mean gestational age at delivery (Table 1) was 37.9 ± 2.7 weeks in the frozen oocyte cycles vs. 37.4 ± 3.0 weeks (excluding triplets) in the fresh cycles (*P*=.020), whereas the mean birth weight was 3,107 ± 664 g vs. 2,725 ± 727 g in the frozen vs. fresh groups, respectively (*P*<.001). There were 58.6% females in the frozen oocyte group and 50.4% in the fresh oocyte group (*P*=.070). The singleton deliveries of the frozen–thawed oocyte group had a mean gestational age at delivery of 38.4 ± 2.6 weeks and mean birth weight of 3,231 ± 615 g. In the twins deliveries the mean gestational age was 35.4 ± 1.7 weeks, and mean birth weight was 2,418 ± 492 g (Table 1).

The singleton deliveries of fresh cycles had a mean gestational age at delivery of 38.5 ± 2.7 weeks and a mean birth weight of 3,012 ± 659 g. The twin deliveries had a mean gestational age of 35.4 ± 2.7 weeks and a mean birth weight of 2,212 ± 537 g (Table 1).

Despite no differences in gestational age at delivery, the mean birth weights were significantly lower with fresh oocyte pregnancies, both in singletons and twins, than with frozen–thawed oocytes (Table 1).

Triplet deliveries were only obtained with fresh oocytes and had a mean gestational age of 33.0 ± 2.6 weeks and a mean weight of 1,691 ± 430 g. The mean gestational age was statistically different between the two groups only when all cases (singletons and twins) were considered (Table 1). Babies born from frozen–thawed oocytes had a significantly higher mean birth weight both in singleton (*P*=.001) and in twin deliveries (*P*=.045).

Of the 145 babies born from frozen–thawed oocytes, 4 (2.8%) had major anomalies diagnosed at birth or during the postnatal period (Table 2): one gastroenteric, one limb, and two genitourinary anomalies.

Of the 740 babies delivered after fresh embryo transfer, 34 (4.6%) had major anomalies (Table 2): 1 nervous system, 17 cardiovascular, 2 gastroenteric, 7 genitourinary, 1 musculoskeletal, 4 limb malformation, and 3 chromosomal anomalies. In triplets only a limb malformation was observed.

No statistically significant differences for congenital anomalies were found between newborns from fresh and frozen oocytes (*P*=.499).

The analysis of the 63 couples having pregnancies in both fresh and frozen cycles (Table 3) showed no statistically significant differences in any of the variables analyzed.

DISCUSSION

The data presented in this study represent one of the largest experiences in obstetric outcomes with oocyte cryopreservation by a single center and with the same patient population using their own oocytes for fresh and/or frozen oocyte

TABLE 2

Congenital anomalies after oocyte thawing and fresh cycle ET in the same group of patients.

Parameter	Oocyte thawing	Fresh cycles
Babies born	145	740[a]
All anomalies	4 (2.8)[b]	34 (4.6)[b]
Nervous system	0	1 (2.9)
Cardiovascular	0	17 (48.6)
Gastroenteric	1 (25.0)	2 (5.7)
Genitourinary	2 (50.0)	7 (20.0)
Muscle skeletal	1 (25.0)	1 (2.9)
Limb	0	4 (11.4)
Chromosomal	0	3 (8.6)

Note: Values are number (percentage).
[a] Includes triplets (n = 16 cases), 48 babies total.
[b] P = nonsignificant.

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pregnancies. The data confirm and reinforce the concept of safety of oocyte cryopreservation by showing no difference in the incidence of fetal and neonatal complications when using embryos from fresh or frozen oocytes. In addition, there were no differences in the rate of preterm birth or birth defects compared with children born after transfer of fresh embryos. In 2012 Davies et al. (43) reported 8.3% birth defects in pregnancies involving assisted conception compared with 5.8% in pregnancies not involving assisted conception, with a multivariate adjusted odd ratio of 1.28 (95% confidence interval 1.16–1.41), but births from spontaneous conception in fertile women and births from spontaneous conception in women who had had a previous birth with assisted conception were also associated with an increased overall risk of any birth defect, even after adjustment for confounders (adjusted odds ratio 1.25; 95% confidence interval 1.01–1.56). They reported birth defects in 7.8% (147 of 1,865) of the fresh cycles and 6.4% (49 of 705) of the frozen cycles. When their ET cycles were subdivided according to whether the embryos

TABLE 3

Outcomes of pregnancies in 63 couples having pregnancies from both fresh and frozen oocytes.

Outcome	Oocyte thawing	Fresh cycles	*P* value
Pregnancies	68	70	
Deliveries	40	37	
Abortions ≤12 wk	20 (29.41)	25 (35.71)	.430
Abortions >12 wk	0	3 (4.29)	.245
Ectopic pregnancies	6 (8.82)	3 (4.29)	.322
Therapeutic abortions	2 (2.94)	2 (2.86)	1.000
Babies delivered	43	43	
Singles	37	31	
Gestational age (wk)	38.1 ± 2.7	38.7 ± 1.6	.441
Birth weight (g)	3,210 ± 682	3,161 ± 635	.406
Females	21 (56.76)	15 (48.39)	.491
Twins	6	12	
Gestational age	35.8 ± 1.0	33.4 ± 4.7	.707
Birth weight (g)	2,442 ± 486	1,825 ± 772	.111
Females	4 (66.67)	9 (75.00)	1.000
Neonatal anomalies	0	3 (6.98)	.241

Note: Values are mean ± SD or number (percentage).

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were fresh or frozen, the relative risks for fresh embryo cycles vs. frozen embryo cycles, for IVF and ICSI combined or for either procedure individually, showed no significant differences (43). The largest report on pregnancy outcome after oocyte thawing or warming of oocytes reported in total four minor and eight major birth anomalies. The minor anomalies included three cases of clubfoot and one skin hemangioma; all other anomalies were major. The overall anomaly rate was 1.3%. The incidence in slow-freeze births was 6 of 532 (1.1%) and in vitrification births 6 of 392 (1.5%) (41). Comparing our data with the recent literature, it is important to note that our results are from the use of a slow freeze–thaw methodology and not from vitrification, thus proving that even with this procedure the outcomes are similarly safe and reassuring.

The results are even more robust because they were not obtained from cycles of egg donation, which are the most abundant in literature, but were obtained from infertile patients using their own frozen–thawed oocytes (46). Slow freezing of embryos has been used for 25 years, and data concerning infant outcomes seem reassuring, with even higher birth weights and lower rates of preterm and low birth weights than in children born after fresh IVF/ICSI (41–43). The better clinical outcome for children born after embryo cryopreservation could be due to an adverse effect of controlled ovarian hyperstimulation in fresh cycles; furthermore, embryos surviving freezing–thawing stress might be qualitatively better than fresh embryos, with a later positive influence on child outcome (47). These concepts of temporal separation from controlled ovarian hyperstimulation and of a sort of selection of the best embryo surviving to the thawing process could also be extrapolated to oocytes frozen and later thawed.

The successful use of oocyte cryopreservation also has important ethical benefits and fewer legal implications compared with embryo storage, especially in those countries where the legal environment is particularly restrictive.

In addition, it cannot be left unmentioned the enormous advantage of adopting oocyte freezing as an effective strategy for fertility preservation in cancer patients or for other medical conditions requiring aggressive gonadotoxic therapies (20, 21).

Additionally, in today's society, women are delaying motherhood well into their 30s and 40s, and by doing so they are forced to face the well-documented natural limits of their own reproductive system (23, 48). It is anticipated that with the rising number of women who choose to delay pregnancy until an advanced age, the demand for oocyte cryopreservation and ART will increase accordingly. The effective and, as documented here, reassuring method of oocyte cryopreservation can be considered an act of preventive medicine: it avoids egg donation (still prohibited in many nations) and the burden of ineffective fertility treatment at older ages (23). Moreover, it maintains women's own reproductive autonomy and increases their chances of genetic motherhood at a more advanced age.

Previous studies on follow-up of children born after oocyte freezing have been case reports from several countries, as soon as a healthy baby was born from the technique

(49–52). A recent review reporting perinatal or child outcomes after oocyte cryopreservation identified only 22 studies fulfilling the inclusion criteria for completeness of follow-up (53). Neonatal information on a total of 148 children born after oocyte slow freezing and 221 children born after oocyte vitrification were recorded, and no adverse outcomes emerged in that study population compared with naturally conceived newborns. Nevertheless, most studies are small, the only description given about children is "healthy," and frequently there is no control group. Another recent publication focused on health of newborns, with cumulative data on 308 babies born from slow freezing, 289 from vitrification, and 12 from a combination of both methods (41). No epigenetic malformations were reported, no increased incidence of major or minor malformations, when data were compared with the Centers for Disease Control and Prevention statistics for the general US population, even when analyzed for specific birth defects (41).

Important advantages of this study were [1] the clinical setting (patients were treated in only one center); [2] the same patients experienced pregnancies either with fresh or thawed oocytes, or with both; and [3] the cryopreservation protocol was the same (slow-freezing) throughout the study. These patients were all undergoing infertility treatment with their own oocytes (no biases from oocyte donation cycles), and all had supernumerary oocytes for cryopreservation. Another strength of the present study is the meticulous and complete follow-up of all pregnancies and deliveries. There are many shortcomings when analyzing the current literature on healthiness of ART babies in general and from oocyte cryopreservation in particular: [1] difficulty in comparing results across publications because freezing cycles are performed after different indications (oocyte donations, fertility preservation programmes, and for legal restrictions); [2] difficulty due to the use of different cryopreservation protocols and different vitrification methods and solutions; [3] data collection across centers could introduce recall bias; [4] limited access to perinatal data; and [5] lack of reporting of adverse outcome, and this is the reason why case reports cannot be considered a strong demonstration of the procedure's safety, until extended well-designed studies are published.

These difficulties are in part overcome in our study. Indications and clinical setting were in fact the same for all recorded data. Furthermore the risk of recall bias was the same for study and control populations, because follow-up data were recorded by the same person in charge of it. A possible limitation is still the relatively small sample size to detect differences in rare outcome measures, such as epigenetic birth defects, and the need for a prolonged follow-up of this cohort of children.

It is also our hope that in the near future more oocyte cryopreservation cycles would be performed by other ART centers and our data be confirmed.

In conclusion, cryopreservation of oocytes has gained increased importance in recent years, concomitant with the introduction of single embryo transfer, legal restrictive policy in some countries, and the increased demand to preserve oocytes for future use. The potential limits and safety of oocyte freezing need to be further explored. Continuous collection of obstetric and perinatal outcomes after transfer of embryos derived from frozen–thawed oocytes is of outmost importance owing to the limited number of reports on this subject (41, 53). Our follow-up data are reassuring because embryos derived from slow-cooling and thawed oocytes did not have an increased adverse effect on neonatal outcome. Oocyte cryopreservation, whether with slow-freeze or vitrification technology, can thus be performed with reproducible success, leading to normal and healthy offspring. Scientific publications on the safety of oocyte freezing when applied to an infertile patient's own oocytes, as in the present study, could help the American Society for Reproductive Medicine to completely remove the experimental designation from the procedure, because at this time it is not encouraged for healthy women wishing to postpone their fertility options and for egg donor banks (27).

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