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OOCYTE BIOLOGY

ART results with frozen oocytes: data from the Italian ART registry (2005–2013)

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Abstract
Purpose This study is a retrospective collection of aggregated data from all the Italian ART centers reporting to the Italian National Register from cycles started between January 2005 and December 2013.
Methods Data from both slow freezing (SF) and vitrification (V) were assessed for the period 2007–2013, while during the years 2005–2006 cryopreservation was exclusively performed by SF.
Results In the study period, a total of 2,526,024 oocytes were retrieved (from 378,543 retrievals), of which 1,346,061 (53.3 %) were inseminated in fresh cycles and 214,481 (8.5 %) were cryopreserved. Cryopreserved oocytes were used in 24,173 cycles yielding 19,453 transfer cycles (80.5 % of the thawing/warming cycles) and 3043 clinical pregnancies (15.6 % per transfer). A significant difference in implantation (8.7 vs 12.9 % OR 1.30 CI 1.20–1.40) and pregnancy rates per transfer (12.2 vs 14.9 % OR 1.34 CI 1.23–1.46) was found between SF and V. Complete outcome data was available for 2708 pregnancies (89.8 %), leading to 1882 deliveries and 2152 live births. Neonatal major congenital anomalies were 0.9 % (20/2152).
Conclusions A wide variation in pregnancy rates were found among different centers and lower rates were reported in donor cycles and in centers with more experience.

Keywords Oocyte freezing · Safety in ART · Cumulative ART pregnancy rate · Congenital anomalies · ART registry

Capsule With oocyte freezing, Italian registry database confirms a significantly higher implantation and pregnancy rate through vitrification as compared to slow freezing. However, a wide range of performance among centers was found with better results in centers with large experience. This is most likely due to the larger register data on oocyte freezing published in literature in infertile couples and because congenital anomalies were reported at very low rates in pregnancy follow-up.

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Introduction

Oocytes are complex human cells and it took many years to perfect protocols for their cryopreservation [1, 2]. In Italy, during the years 2004–2009, by law, embryo cryopreservation was banned and thus there was an impetus to develop and refine oocyte cryopreservation (OC) methods, although oocyte cryopreservation was first offered as a means to preserve fertility in women at risk of premature menopause [3]. Later, it has been adopted as a successful alternative for storing the excess of oocytes produced during ART therapies, thus avoiding legal restrictions (in our context, Italy's ban on embryo freezing) as well as potential ethical and religious dilemmas (by reducing the number of embryos cryostored). The first report of a pregnancy from a frozen egg was described by Chen in 1986 [4]. A few other births were achieved shortly after [5, 6], but for many years, reports on oocyte freezing remained sporadic. Gook et al.

[7] were first to suggest that intracytoplasmic sperm injection (ICSI) could improve fertilization rates in frozen/thawed oocytes, by overcoming fertilization failures due to premature cortical granule release and zona hardening. However, in spite of several successes [8–12], there were still technical problems (low survival and few pregnancies) associated with oocyte freezing [8]. Comparison of success rates have been reported to be higher in vitrification (V) cycles than with slow freezing (SF) [13] and, in some centers, V gave the same pregnancy rate as fresh oocytes [14, 15]. Initial reports of deliveries and follow-up of babies born after using cryopreserved oocytes have demonstrated safety of the technique [16–18]. However, the majority of published outcome results have been obtained from the use of cryopreserved oocytes for donor cycles, while the literature is scarce about data on live births and the incidence of congenital malformations after using cryopreserved oocytes in a large cohort of infertile, non-donor patients. The aim of this report is to present the results of OC from a very large national data set to update the results obtained by SF and V and to report the outcome of pregnancies, live birth, and the rate of congenital malformations in OC cycles performed by Italian ART centers during the years 2005–2013.

Materials and methods

This retrospective study analyzed aggregated data of In vitro fertilization (IVF) cycles carried out with cryopreserved oocytes between 2005 and 2013 as reported to the Istituto Superiore di Sanità, Rome, Italy. Data were collected using the internet website www.iss.it/rpma, a resource which was set up by the ART National Registry at the National Center for Epidemiology, Surveillance and Health Promotion. Records were stored on a secure server, password protected, and anonymized. According to Law 40/2004, no more than three embryos could be generated after oocyte thawing/warming until May 2009. After this date, the Italian Constitutional Court modified the law and lifted the restriction on the maximum number of three embryos. Cycles from both SF and V were reported only for the period 2007–2013 since before (2005–2006) the only method of cryopreservation was SF. Implantation rates were calculated by dividing the number of gestational sacs by the number of embryos transferred according to ICMART and WHO [19, 20], and ectopic pregnancies were also included. A live birth was defined as a viable infant born at ≥24 weeks of gestation. Neonatal major congenital anomalies were classified according to the EUROCAT Coding Subgroups of Congenital Anomalies [21], the requested codes for all European Register. However, specific details of congenital anomalies are not a required field included in the registry data set. Since detailed patients characteristics are unknown, the data presented are not adjusted for potential confounders.

Statistical analyses

Data were analyzed with SPSS Statistics 17.0 (SPSS Inc.). Percentages of transferred embryos per inseminated oocytes, implantation rates, pregnancy rates (for started cycle and transfer), delivery rates, negative outcomes (spontaneous, therapeutic abortions, and ectopic pregnancies), and incidence of malformed babies per live birth were calculated. These parameters were compared between thawed (slow freezing protocol) and warmed (vitrification protocol) oocyte cycles using crude odds ratios (OR) and 95 % confidence intervals (CI). $P < 0.05$ was considered statistically significant.

Results

A total of 2,526,024 oocytes were retrieved, of which 1,346,061 (53.3 %) were either conventionally inseminated (IVF) or injected (ICSI) and 214,481 (8.5 %) were cryopreserved. A number of mature oocytes are unaccounted for because they were discarded due to the limits imposed by the law (no more than 3 oocytes usable for insemination) and by many centers lacking sufficient expertise to provide oocyte cryopreservation. Out of 378,543 oocyte retrievals, 34,239 (9.0 %) were cycles where oocyte cryopreservation was used (Table 1).

A progressively higher total number of retrievals with frozen oocyte (FO) procedures were reported during the period 2005–2009 while a reduction was observed in 2010–2013 in comparison to 2009, explained by the abolition of the law banning embryo cryopreservation. The percentages of retrievals with FO were 12.2 % in 2009 and 4.7 % in 2013 ($p < 0.001$). Accordingly, a higher number of oocytes were used for insemination in the fresh cycles (36.8 % in 2005 and 66.7 % in 2013) ($P < 0.001$). Differences in FO procedures were available from 2007 onward when a progressive switch from SF to V began to take place. In 2007, oocytes were cryopreserved in 82.2 % by SF and by V in 17.8 %. In 2013, SF was applied in 14.4 % of the cycles and V in 85.6 % of the cycles (Table 1). A significant difference was found ($P < 0.001$) among oocytes cryopreserved by SF and V in 2007 and 2013.

During the 9 years of analysis, a progressively higher number of oocytes survived the cryopreservation/thawing process (Table 2). However, it must be remembered that in the period 2005–2009, only 3 oocytes could be used even if more had survived the thawing/warming process. The higher total oocyte survival rate was related to a switch in the cryopreservation methodology with increasing number of cycles using vitrification as opposed to slow freezing technique [13]. The FO survival rate was 55.2 % in 2005 and 67.2 % in 2013 and the

Table 1 Retrievals with frozen oocytes, inseminated fresh oocytes, and frozen oocytes divided by type of technique 2005–2013

Year	Retrievals with frozen oocytes per retrieval (%)	Inseminated Fresh oocytes per retrieved oocytes (%)	Frozen oocytes per retrieved oocytes (%)	Frozen oocytes with slow freezing per frozen oocytes (%)	Frozen oocytes with vitrification per frozen oocytes (%)
2005	3919/29,345 (13.4)	76,914/209,236 (36.8)	25,489/209,236 (12.2)	ND	ND
2006	4541/32,821 (13.8)	86,743/223,359 (38.8)	28,784/223,359 (12.9)	ND	ND
2007	4443/35,645 (12.5)	89,645/234,004 (38.3)	27,513/234,004 (11.8)	22,612/27,513 (82.2)	4901/27,513 (17.8)
2008	4753/39,434 (12.1)	98,423/256,293 (38.4)	30,420/256,293 (11.9)	23,777/30,420 (78.2)	6643/30,420 (21.8)
2009	4301/43,243 (9.9)	140,864/285,042 (49.4)	25,705/285,042 (9.0)	12,761/25,705 (49.6)	12,944/25,705 (50.4)
2010	3853/47,499 (8.1)	192,244/312,481 (61.5)	21,865/312,481 (7.0)	7405/21,865 (33.9)	14,460/21,865 (66.1)
2011	3216/50,286 (6.4)	216,514/333,618 (64.9)	20,391/333,618 (6.1)	5928/20,391 (29.1)	14,463/20,391 (70.9)
2012	2875/50,096 (5.7)	219,363/334,339 (65.6)	18,478/334,339 (5.5)	4694/18,478 (25.4)	13,784/18,478 (74.6)
2013	2335/50,174 (4.7)	225,351/337,652 (66.7)	15,836/337,652 (4.7)	2287/15,836 (14.4)	13,549/15,836 (85.6)
Overall	34,236/378,543 (9.0)	1,346,061/2,526,024 (53.3)	214,481/2,526,024 (8.5)	79,464/160,208 (49.6)	80,744/160,208 (50.4)

total SF survival rate was 52.2 % and 65.3 % (Table 2). In the study period, a mean of 176 Italian reproductive units reported their data to the National ART Register with 228 total reporting centers during the period. 177 (77.6 %) performed at least one cycle of oocyte cryopreservation and 171 (75.0 %) centers offered at least one thawing/warming FO cycle (Table 3), no mixed cycles, either fresh or frozen/thawed.

The overall pregnancy rate for started cycle was 12.6 % (9.5 % in 2005 and 15.2 % in 2013) and the pregnancy rate for transfer was 15.6 % (11.4 % in 2005 and 20.1 % in 2013). The overall delivery rate per transfer was 9.7 % (5.9 % in 2005 and 13.5 % in 2013). Of the 24, 174 thaw cycles, 19,453 (80.5 %) reached the transfer of at least one embryo, 3043 pregnancies were obtained and a complete obstetrical/perinatal outcome was available for 2708 (89 %) pregnancies and for 1882 live birth deliveries (Table 3). Differences between SF and V for the period 2007–2011 have been analyzed in a previous publication [13] and the complete register results for the period 2005–2013 confirm our previous conclusions. A significant difference ($P<0.001$) in the pregnancy rate for started cycle and transfer cycle was found between SF and V. The pregnancy rate was 12.2 and 14.9 % for started cycle and transfer in SF and 14.9 and 19.0 % in V. The odd ratio (OR) (95 % CI) was 1.26 (1.16–1.37) for cycle and 1.34 (1.23–1.46) for transfer. The implantation rate in SF was 8.2 % (1414/17,274) and 1470/14,204 for V ($P<0.001$) with an OR of 1.30 (1.20–1.40). However, in centers performing less than 200 fresh cycles/year, the pregnancy rate in frozen embryos replacement (FER) cycles was 19.9 and 13.5 % with oocyte vitrification ($p=0.0008$); in centers performing between 200–500 fresh cycles/year, the FER pregnancy rate was 18.8 vs 11.7 %

Table 2 Number of frozen cycles, oocytes thawed/warmed and used during 2005-2013

Year	FO thawing/ warming cycles		Slow freezing		Vitrification	
	Frozen oocytes cycles	Inseminated oocytes per thawed or warmed oocytes (%)	Frozen Oocytes Cycles	Inseminated Oocytes per Thawed Oocytes (%)	Frozen oocytes cycles	Inseminated oocytes per warmed oocytes (%)
2005	2711	7005 / 12,689 (55.2)	ND	ND	ND	ND
2006	2977	7622 / 15,338 (49.7)	ND	ND	ND	ND
2007	2994	7378 / 14,890 (49.6)	2426	6008 / 12,753 (47.8)	568	1370 / 2317 (59.1)
2008	3284	8128 / 16,541 (49.1)	2625	6445 / 13,592 (47.4)	659	1683 / 2949 (57.1)
2009	3102	9011 / 16,528 (54.5)	1916	5523 / 10,821 (51.0)	1186	3488 / 5707 (61.1)
2010	2441	8063 / 12,974 (62.1)	1097	3633 / 6068 (59.9)	1344	4430 / 6906 (64.1)
2011	2507	8615 / 13,485 (63.9)	863	2873 / 4860 (59.1)	1644	5742 / 8625 (66.6)
2012	2189	8251/12,437 (66.3)	614	2283/3788 (60.3)	1575	5968/8649 (69,0)
2013	1969	7301/10,868 (67.2)	351	1318/2130 (61.9)	1618	5983/8738 (68.5)
Overall	24,174	71,374/125,750 (56.8)	9892	28,083/53,832 (52.2)	8594	28,664/43,891 (65.3)

In the period 2005–2009 (up to May) only 3 oocytes could be used for ICSI even if more than 3 had survived the cryopreservation/thawing process

Table 3 ART outcomes using frozen oocytes (FO) 2005–2013

Year	Centers with FO cycles / all centers (%)	Transfers per started cycles (%)	Pregnancies per started cycles (%)	Pregnancies per transfer (%)	Deliveries per transfer (%)
2005	74/169 (43.8)	2261 / 2711 (83.4)	257 / 2711 (9.5)	257 / 2261 (11.4)	133 / 2261 (5.9)
2006	98/184 (53.3)	2366 / 2977 (79.5)	298 / 2977 (10.0)	298 / 2366 (12.6)	159 / 2366 (6.7)
2007	93/181 (51.4)	2428 / 2994 (81.1)	327 / 2994 (10.9)	327 / 2428 (13.5)	213 / 2428 (8.8)
2008	104/185 (56.2)	2662 / 3284 (81.1)	402 / 3284 (12.2)	402 / 2662 (15.1)	253 / 2662 (9.5)
2009	114/180 (63.3)	2535 / 3102 (81.7)	434 / 3102 (14.0)	434 / 2535 (17.1)	255 / 2535 (10.1)
2010	109/174 (62.6)	1962 / 2441 (80.4)	335 / 2441 (13.7)	335 / 1962 (17.1)	221 / 1962 (11.3)
2011	120/179 (67.0)	2012 / 2507 (80.3)	352 / 2507 (14.0)	352 / 2012 (17.5)	226 / 2012 (11.2)
2012	116/182 (63.7)	1736/2189 (79.3)	338/2189 (15.4)	338/1736 (19.5)	221/1736 (12.7)
2013	116/178 (65.2)	1491/1969 (75.7)	300/1969 (15.2)	300/1491 (20.1)	201/1491 (13.5)
Overall	171/228 (75.0)	19,453/24,174 (80.5)	3043/24,174 (12.6)	3043/19,453 (15.6)	1882/19,453 (9.7)

Complete follow up for pregnancy outcome was available for 2708 out of 3043 pregnancies (89 %)

with oocyte vitrification ($P<0.001$), while in centers performing more than 500 fresh cycles/year, there was no difference between FER and oocyte vitrification results, 23 vs 22.9 % ($P=0.8648$), respectively.

Of all the pregnancies, 28.5 % were spontaneous abortions, 0.4 % were therapeutic terminations, and 1.8 % were ectopic pregnancies, while the live birth delivery rate was 69.5 % (Table 4).

A total of five stillbirths out of 1882 deliveries were reported (0.3 %). A total of 407 pregnancies (13.4 %) were twins and 36 (1.2 %) were triplets. In total, 548 (25.4 %) babies were born from multiple pregnancies (in detail, 1609 from singleton, 500 from twin, and 48 from triplet pregnancies). Reassuringly, from the total of 2252 live babies available for full analysis, only 20 (0.9 %) had congenital malformations reported to the registry according to the EUROCAT classification criteria for being major or minor, but the specific details were not a required field (Table 5).

Discussion

This study provides the most comprehensive assessment of results and safety from using oocyte cryopreservation in infertile, non-donor patients as reported to the Italian ART registry during the years 2005 through 2013. Concerns that oocyte cryopreservation may be harmful have not been shown in our neonatal data across Italy and the findings of a very low incidence of congenital anomalies (0.9 %) are very reassuring. Our data are in agreement with a recent publication showing similar low incidence of congenital anomalies, but in a smaller data set [22] and with another publication [23], but in a diverse patient population (users of oocytes from cryopreserved donor

Table 4 Pregnancies and delivery outcomes using FO (years 2005–2013)

Year	Pregnancies	Pregnancy outcome available		Deliveries		Negative outcomes[a]	
		N	% pregnancies	*N*	% pregnancies monitored	*N*	% pregnancies monitored
2005	257	182	70.8	133	73.1	49	26.9
2006	298	243	81.5	159	65.4	84	34.6
2007	327	300	91.7	213	71.0	87	29.0
2008	402	364	90.5	253	69.5	111	30.5
2009	434	384	88.5	255	66.4	129	33.6
2010	335	323	96.4	221	68.4	102	31.6
2011	352	315	89.5	226	71.7	89	28.3
2012	338	315	93.2	221	70.2	94	29.8
2013	300	282	94.0	201	71.3	81	28.7
Total	3043	2708	89.0	1882	69.5	826	30.5

[a] 766 (28.3 %) were spontaneous abortions (before 12 weeks), 11 (0.4 %) therapeutic abortions for fetal anomalies and 49 (1.8 %) ectopic pregnancies

Table 5 Births and congenital malformations from frozen oocytes 2005-2013

Year	Babies Born	Live Births	Congenital Malformations	
			N	% live births
2005	139	139	3	2.2
2006	193	193	1	0.5
2007	249	249	4	1.6
2008	301	300	3	1.0
2009	289	289	2	0.7
2010	245	242	1	0.4
2011	258	258	1	0.4
2012	250	250	4	1.6
2013	233	232	1	0.4
Total	2157	2152	20	0.9

EUROCAT classification used to define major congenital malformations

egg banking). The rate of neonatal congenital malformations is low, perhaps reflecting a percentage of underreporting and lost to follow up, not significantly different from other large registers [24].

Since data from all the Italian centers are collected in an aggregated form due to the limits imposed by the National Privacy Authority, the details of congenital anomalies are not yet a required field included in the registry dataset. Therefore, a comparison with natural conception cannot be performed and it will be possible only when single case collection will be allowed. Nonetheless, the overall rate of reported congenital malformations at birth is low and comparable to the 0.9 % reported in 2013 by the national register for births from ART transfer cycles (91/10,217).

The low incidence of anomalies found in this analysis support the data reporting 12/936 anomalies (1.3 %) [17]. There was no significant increase in the risk of congenital malformations between births resulting from IVF and ICSI (combined) and frozen embryo cycles as compared with births to fertile women that did not involve assisted conception [25]. In a recent paper comparing neonatal anomalies in the same group of patients that delivered after an ART cycle with fresh or frozen oocytes, the number of malformations was 4.6 % in the fresh cycles and 2.8 % in the frozen oocyte transfers [22].

Safety is also inferred by the evidence that comparable aneuploidy frequencies were observed in embryos obtained from fresh or frozen oocytes (28 % and 26 %, respectively), by performing a FISH analysis, and employing specific probes for chromosomes 13,18,21, X, and Y [26] and by 24-chromosome PGS from fresh and vitrified oocytes not showing a significant difference (44.5 vs 47.6 %) in percentage of euploid embryo blastocysts [27].

The analysis of oocyte to baby rate revealed that a high number of oocytes were needed to result in a live birth. This can be explained by a selection bias favoring better outcome with fresh oocytes since the frozen ones were the supernumerary out of the best 3 used for fresh cycles during the period 2005–2009. In addition, after May 2009 the number of mature oocyte available for cryopreservation was reduced since embryo cryopreservation was reinstated [28, 29]. The reduced overall success rates in comparison with other reports [14] in infertile patients is also related to the great variability in pregnancy rates among reporting centers, each with different experience, number of cycles performed and use of slow freezing protocol [13].

This study however provides the historical foundation for the development of the technique and for its acceptance in the routine of clinical ART services. Without a doubt, the development of efficient methods of oocyte cryopreservation has brought about a major breakthrough in human IVF. To this effect, oocyte cryostorage has the potential not only to circumvent several ethical, legal, and storage problems associated with embryo freezing but is also a remarkable technology to preserve female fertility in oncological patients, for women at risk of premature ovarian failure or for women who are postponing their plans for motherhood.

Various studies from around the world have shown that young people (men and women alike) lack knowledge about the natural limits of human fertility and display an optimistic bias. In addition, a recent survey on the attitudes towards nonmedical egg freezing in Belgium shows that a third of the respondents (women aged 21–40 years) consider themselves potential users of this new technology for nonmedical uses [30–32].

The establishment of oocyte banks could improve the safety of fertility treatments for women using oocyte donors by allowing improved screening of donors for potential transmittable diseases. A recent prospective randomized controlled clinical trial of egg banking efficiency for recipients of ovum donation confirmed not only safety but also the efficiency of oocyte vitrification [2]. In summary, this report adds to a growing literature proving that cryopreservation of oocytes is as safe as embryo cryopreservation. These data are useful on counseling for the success rates and live birth in infertile, non donor patients. Finally, the data show a direct relationship between volume of procedures performed, experience, and training of the centers and the results of oocyte cryopreservation.

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