Monozygotic twin rate among ART centers: a multicenter analysis of data from 18 Italian units

Giulia Scaravelli[1] · Valerio Pisaturo[2] · Paolo Emanuele Levi Setti[3,4] · Filippo Maria Ubaldi[5] · Claudia Livi[6] · Andrea Borini[7] · Ermanno Greco[8,9] · Maria Teresa Villani[10] · Maria Elisabetta Coccia[11] · Alberto Revelli[12] · Giuseppe Ricci[13,14] · Francesco Fusi[15] · Mauro Costa[16] · Emanuela Migliorati[17] · Roberto De Luca[1] · Vincenzo Vigiliano[1] · Simone Bolli[1] · Marco Reschini[2]

Abstract

Purpose The risk of monozygotic twins (MZTs) is increased in couples undergoing assisted reproductive technology (ART) treatments. Several systematic reviews have investigated the possible determinants linked to ART, but results obtained have not been conclusive. The study aims to investigate whether the incidence of MZT differed among ART centers.

Methods This is a multicenter retrospective cohort study using the Italian ART National Registry database and involving the centers reporting data from individual ART cycles from 2015 to 2019. To investigate the incidence of MZT, only single embryo transfer cycles were considered. Women who had sex-discordant deliveries were excluded. MZT rate was calculated as the number of multiple pregnancies (more than one gestational sac at first ultrasound) out of the total number of clinical pregnancies. A binomial distribution model was used to determine the 95% CI of the frequency of MZT.

Results Eighteen centers were included, and they provided data on 10,433 pregnancies. The total number of MZT was 162, corresponding to an incidence of 1.5% (95% CI: 1.3–1.8%). The rate of MZT among centers varied between 0% (95% CI: 0.0–25.9%) and 3.2% (95% CI: 1.3–8.1%). All the 95% CIs included 1.5%, rejecting the hypothesis that the MZT rate may significantly differ among centers.

Conclusions The rate of MZT did not significantly vary among ART centers. Local factors are unlikely to explain the increased rate of MZT in ART pregnancies.

Keywords Twin · Multiple pregnancy · Monozygotic · ART · IVF

Introduction

Assisted reproductive technology (ART) is associated to a 2–3 folds increase in the risk of monozygotic twin pregnancy (MZT), a neglected complication that is however receiving growing attention in recent years [1, 2]. MZT pregnancies are not only exposed to the well-known adverse obstetric outcome of multiple pregnancies but also to some peculiar additional risks [3]. In about 15% of cases, an imbalance in blood exchange occurs and pregnancies can develop frightful complications such as the twin-to-twin transfusion syndrome (TTTS), twin anemia-polycythemia sequence (TAPS), and twin reversed arterial perfusion (TRAP) [4].

A causal relation between ART and MZT remains however controversial. Two recent systematic reviews investigated possible determinants, but results were not definitive. They both showed an association with extended embryo culture up to the blastocyst stage and young female age [1, 2]. Conventional IVF compared to ICSI, and assisted hatching emerged as possible additional risk factors, but evidence is controversial [1, 2, 5, 6]. Conversely, embryo biopsy, embryo cryopreservation, and egg donation were not found to be associated [2]. Meta-analyses, however, cannot provide robust evidence because of the impossibility to perform multivariate analyses [5]. In addition, given the rarity of

Key message Our study failed to identify differences in the incidence of MZT among ART centers, suggesting that local factors may be unremarkable. However, our results cannot rule out a role for clinical or laboratory variables in the determinism of ART-associated MZT and further evidence is needed.

✉ Valerio Pisaturo
valerio.pisaturo@policlinico.mi.it

the event, all available investigations were retrospective and relatively small, hampering the quality of the collected data.

Local laboratory conditions and local clinical policies are an unexplored field in the context of risk factors for MZT pregnancies after ART. IVF laboratories significantly differ in terms of settings, procedures, and embryo culture conditions [7]. Clinical policies also vary widely among centers [8]. This variability may have a clinical impact. For instance, euploidy rates in donor oocyte cycles significantly differ among different fertility centers, indicating that the rate of embryonic chromosomal abnormalities may be partly iatrogenic [9]. Similar findings could be expected for the rate of MZT. To note, the evolution and changes of procedures have been proposed as a possible explanation for the variation in ART-related MZT frequency observed over time [10].

In this study, we hypothesized that, if local laboratory conditions or clinical policies could play a role in the determinism of MZT, one would expect significant variations in the rate of MZT rate among infertility centers. To test this hypothesis, we investigated the MZT rate among eighteen Italian centers regularly reporting their results per cycle (i.e., not in an aggregated manner) to Italian ART National Registry.

Materials and methods

In 2016, the Italian ART National Registry (National Health Institute, Rome, Italy) launched a new database to collect data from individual ART cycles. The aim was obtaining information on each single cycle instead of cumulative aggregate information per center. Participation of the centers to the initiative was on a voluntarily bases. This new platform initially included 9 ART clinics, both private and public. Subsequently, other centers progressively adhered to the data collection platform, to reach a total of 18 centers. Details of this program are reported elsewhere [11, 12]. All data were anonymized prior to be submitted to the Italian ART National Registry. No Institutional Review Board approval was requested for the purpose of this study because, according to Italian legislation, analysis of anonymous database does not require Ethics Committee approval.

The primary aim of the study was investigating whether the incidence of MZT differed among centers. For the calculation on these rates, the following inclusion criteria were used: (1) single embryo transfer and (2) clinical pregnancy as documented by the presence of one or more gestational sacs at ultrasound assessment done at 6–7 weeks' gestation. MZTs were defined as the presence of two or more gestational sacs at first trimester ultrasound. These cases were subsequently excluded if clinical data at birth revealed sexual discordant between the newborns. The rate of MZT was calculated as the number of MZT pregnancies out of the total number of clinical pregnancies. A binomial distribution model was used to determine the 95% confidence interval (95% CI) of the frequency of MZT. The rates among centers were deemed heterogeneous (thus rejecting the null hypothesis of no differences) if the 95% CI of in at least one center did not include the mean MZT rate. The mean MZT rate was calculated for the whole cohort.

To obtain informations on the different characteristics and policies of the centers, data from all cycles were used. The following information were extracted: mean number of oocyte retrieval per year, mean age, mean BMI, proportion of conventional IVF, proportion of frozen embryo transfer, proportion of multiple pregnancies, proportion of cycles with gametes/embryos donation, proportion of transfers at blastocyst stage, proportion of single embryo transfer, and proportion of preimplantation genetic testing (PGT) cycles. Statistically significant differences among centers were determined using one-way ANOVA, Kruskal–Wallis test, or chi-square test as appropriate. Spearman correlations were performed to identify any potential correlation between MZT rate and the characteristics of the centers. To rule out confounders associated to small sample sizes, these analyses were repeated including only the centers with higher burden of activity (at least 1,000 cycles per year).

Results

The 18 included ART centers reported on a total of 87,076 IVF cycles, corresponding to a mean number of 17,415 cycles per year. Nine centers reported data from 2015 to 2019, one from 2017 to 2019 and eight from 2018 to 2019. The number of cycles per center varied between 294 and 19,032 (median: 3,454). The main characteristics of the centers are summarized in Table 1. A remarkable and statistically significant variability emerged for all the items. The most relevant differences were observed for the mean number of oocyte retrieval (from 65 to 2,265), the proportion of cycles with gametes donation (from 0 to 31.6%), the use of conventional IVF rather than ICSI (from 0 to 54.1%), PGT (from 0 to 67.3%), transfer at blastocyst stage (from 0 to 99.7%), proportion of frozen transfers (from 18.9 to 89.6%), single embryo transfer rate (from 31.7 to 98.2%), and multiple pregnancy rate (from 1.7 to 23.1%).

The total number of clinical pregnancies fulfilling our inclusion criteria was 10,440. Data on birth was available for 8,052 of them. Of these latter cases, 7 were found to be twin pregnancies with discordant gender and were excluded, leaving 10,433 for data analyses. The number of cases included per center varied between 11 and 2,823 (median 336).

Overall, 162 pregnancies were found to be multiple: 48 ended into a miscarriage, 28 spontaneously reduced to singletons, and 86 delivered two or more newborns.

Table 1 Main characteristics of the 18 included centers

Characteristics	Median	Range	*p*
Number of oocytes retrievals per year	546	65–2,265	< 0.001
Number of embryo transfers per year	683	107–2,914	< 0.001
Mean age (years)	36.6	35.5–38.2	< 0.001
Mean BMI (kg/m^2)	22.6	22.0–24.3	< 0.001
Proportion of conventional IVF (%)	16.9	0–54.1	< 0.001
Proportion of frozen embryo transfer (%)	43.2	18.9–89.6	< 0.001
Proportion of multiple pregnancies (%)	11.3	1.7–23.1	< 0.001
Proportion of cycles gametes/embryos donation (%)	4.9	0–31.6	< 0.001
Proportion of blastocyst embryo transfer (%)	46.3	0–99.7	< 0.001
Proportion of single embryo transfer (%)	58.0	31.7–98.2	< 0.001
Proportion of PGT cycles (%)	1.5	0–61.3	< 0.001

IVF, in vitro fertilization. *BMI*, body mass index. *PGT*, preimplantation genetic testing

The vast majority were twins ($n = 160$), one was triplet, and one was quadruplet. Considering the denominator of 10,433 pregnancies, the rate of MZT was thus 1.5% (95% CI: 1.3–1.8%). The MZT rate in the different centers is represented in Fig. 1. This rate varied between 0% (95% CI: 0.0–25.9%) and 3.2% (95% CI: 1.3–8.1%). All the 95% CIs of the rates of MZT include the 1.5% common rate, thus rejecting the hypothesis that this incidence significantly differed among centers.

Spearman correlations between the rate of MZT and the variables illustrating the policies of the centers are reported in Table 2. None was found to be significantly associated. Even when restricting the analyses only to the largest centers, no association emerged (Table 2).

Discussion

In this large multicenter observational study of IVF pregnancies, the rate of MZT was 1.5% (95% CI: 1.3–1.8%), in line with previous evidence [2]. No significant differences emerged among the participating centers: even if the incidence varied between 0 and 3.2%, the 95% CIs of the MZT rates included 1.5% for all centers. The lack of any significant correlations between the MZT rates and the main characteristics of the centers further supports this conclusion. Overall, our findings do not support the hypothesis that the increased incidence of MZT pregnancies in ART could be related to differences in local laboratory conditions or local clinical policies. Our observation also contrasts with a previous meta-analysis showing a wild difference in the rate of MZT among studies [6]. The included studies,

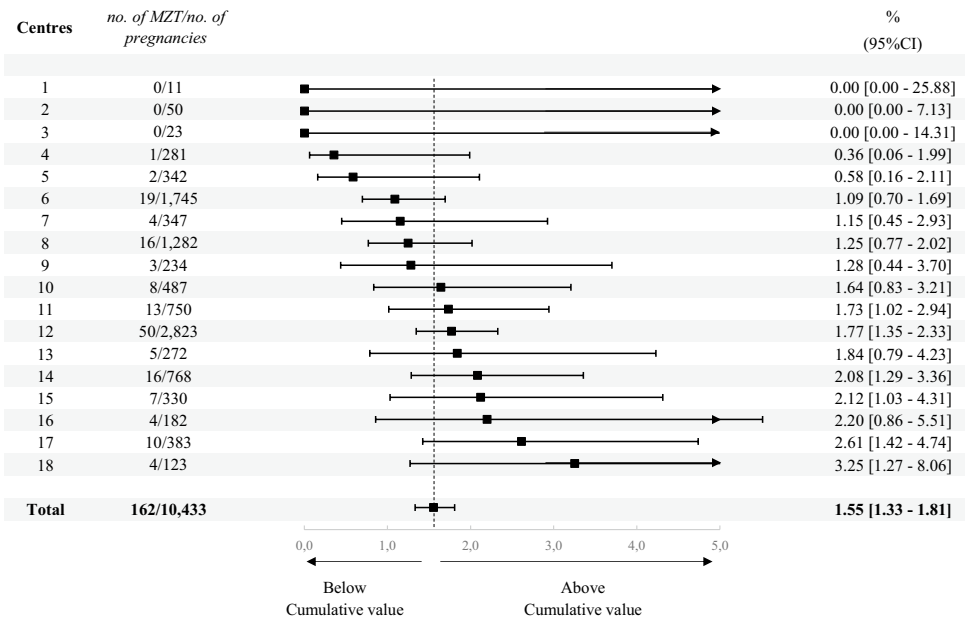

Fig. 1 Rate of MZT among the participating centers. The crude numbers, the percentages, and the 95% CIs are reported. The latter were calculated using a binomial distribution model. The vertical dotted red line represents the common rate of MZT (1.5%). All 95% CIs cross this line, rejecting the hypothesis of significant differences among the centers

Table 2 Spearman correlations between the rate of MZT and the main characteristics of the centers

Characteristics	All cohort ($n=18$)		Larger centers ($n=9$)	
	ρ	p	ρ	p
Mean age	−0.24	0.34	0.20	0.61
Mean BMI	−0.07	0.78	0.22	0.58
Proportion of conventional IVF	0.05	0.85	0.19	0.62
Proportion of frozen embryo transfer	−0.28	0.26	−0.55	0.13
Proportion of multiple pregnancies	0.36	0.14	0.38	0.31
Proportion of cycles gametes/embryos donation	−0.33	0.19	−0.10	0.79
Proportion of blastocyst embryo transfer	−0.25	0.33	−0.65	0.10
Proportion of single embryo transfer	−0.16	0.53	−0.57	0.11
Proportion of PGT cycles	−0.17	0.51	−0.24	0.54

ρ: Sperman correlation factor

IVF, in vitro fertilization. *BMI*, body mass index. *PGT*, preimplantation genetic testing

however, varied widely in study design and did not exclusively focus on single embryo transfer, thus introducing potential confounders.

The rationale supporting a role of laboratory factors in the risk of MZT was based on theories related to the mechanical effect induced on the zona pellucida that undergoes changes through laboratory procedures (ICSI, assisted hatching, or cryopreservation) or exposure to non-physiological culture conditions [2]. As mentioned earlier, however, the two recent meta-analyses on the risk factors for MZT failed to provide robust and definite evidence [1, 2]. Only young age and extended embryo culture emerged as possible risk factors. Noteworthy, these two factors typically overlap, and meta-analyses cannot disentangle the pure effect of each of them. Moreover, in our analyses, the correlation between incidence of MZT and age and rate of blastocyst transfer failed to highlight any statistical significance. We also failed to highlight an increased risk in centers performing PGT. This contrasts with a recent study based on national data from the Human Fertilisation and Embryology Authority (HFEA), reported an increased risk of monozygotic splitting with embryo biopsy also adjusting for potential confounders [12]. The morphological characteristics of blastocysts have also been associated with an increased risk of MZ twins. Shi et al. [13] reported an increased risk of MZT in blastocysts with low grading inner cell mass (ICM) and high grading trophectoderm (TE). In contrast, the same study did not show a difference in the day of development (day 5 vs day 6). These results support the hypothesis proposed by Otsuki and coworkers [14] that the loose ICM is susceptible in splitting into two ICMs. Unfortunately, the data from the Italian ART National Registry does not collect this information, so we cannot confirm these results.

Our study did not provide significant clues to reveal the cause of the increased rate of MZT in ART. Even if indirect, evidence showing significant differences among centers would have strongly argued in favor of an iatrogenic cause. At the same time, one cannot rule out this possibility based on our findings. It may be speculated that the causes could be identified among laboratory procedures or conditions that are common to all centers. Culture conditions are far from in vivo situations, regardless of the specific center considered. In vitro, embryos are exposed to significant environmental stressors, including higher and less stable temperature than in the female genital tract, different oxygen tension, mechanical stress during oocytes aspiration, and light exposure [15]. This is common to all centers and could account for the increase in MZT pregnancies.

Several studies have shown that the above factors can influence cell fate by acting at the epigenetic level by modifying the expression of genes involved in the embryonic development [16]. The MZ twinning event occurs at an early stage of development during which major epigenetic reprogramming occurs. It has been shown that MZTs exhibit a strong epigenetic signature and one hypothesis proposed is that methylation status is established in early zygotes prior to separation leading to MZ twins, and subsequently inherited through mitosis [16]. If the epigenetic modification involved genes implicated in cell adhesion, this alteration could account for tendency of an embryo to dissociate. Moreover, it has been theorized that methylation defect and twinning are closely related [17]. This correlation could justify why epigenetic disorders such as Beckwith-Wiedemann and Silver-Russell syndrome and monozygotic twins have an increased incidence in the ART pregnancies [17, 18].

Another element resulting in the increase in MZT pregnancies could take place into the uterus, at the time of embryo-endometrium crosstalk (the culture medium used for the transfer does not reflect conditions within the uterus and may interfere with this crucial interaction). Finally, the intrinsic factor of couples with subfertility could contribute to an increased rate of and cannot be discarded. Further large studies in an unexplained subfertile population conceiving naturally are required to elucidate this issue.

This is the first study aimed to analyze the association between center-specific ART treatment practices and the incidence of monozygotic pregnancies. However, some limitations should be recognized. First, our study was limited by its retrospective nature, larger prospective studies are needed to confirm our results. Second, some centers provided less than 100 cases for data analyses and the confidence intervals are wide, with a larger margin of error. External confirmation from large-scale studies directly involving ART centers is therefore required. Third, the incidence of MZ twins is very low and, therefore, comparisons from a statistical point of view are limited, drawing misleading conclusions. Moreover, data related to laboratory aspects that might play a role in the determinism of MZ twins (e.g., in vitro culture time, type of media used, assisted hatching, and embryo grading) are not collected by the Italian ART National Registry. Finally, albeit data from national registries are commonly verified, incorrect entry of some data cannot be excluded, and this may affect the outcome.

In conclusions, our study did not show a difference in incidence among centers performing ART: the existence of differences could have supported a potential role of the methods in the pathogenesis of MZ twinning. Further studies are required to identify the causes of the increased risk of MZT in ART.

Funding This work was technically and financially supported by Ministero della Salute—Direzione Generale della Prevenzione Sanitaria/CCM and (partially) funded by Italian Ministry of Health—current research IRCCS.

Declarations

Conflict of interest The authors declare no competing interests.

References

1. Hviid KVR, Malchau SS, Pinborg A, Nielsen HS. Determinants of monozygotic twinning in ART: a systematic review and a meta-analysis. Hum Reprod Update. 2018;24:468–83. https://doi.org/10.1093/humupd/dmy006.
2. Busnelli A, Dallagiovanna C, Reschini M, Paffoni A, Fedele L, Somigliana E. Risk factors for monozygotic twinning after in vitro fertilization: a systematic review and meta-analysis. Fertil Steril. 2019;111:302–17. https://doi.org/10.1016/j.fertnstert.2018.10.025.
3. Marleen S, Dias C, Nandasena R, MacGregor R, Allotey J, Aquilina J, Khalil A, Thangaratinam S. Association between chorionicity and preterm birth in twin pregnancies: a systematic review involving 29 864 twin pregnancies. BJOG. 2021;128:788–96. https://doi.org/10.1111/1471-0528.16479.
4. Lewi L. Monochorionic diamniotic twins: what do I tell the prospective parents? Prenat Diagn. 2020;40:766–75. https://doi.org/10.1002/pd.5705.
5. Dallagiovanna C, Vanni VS, Somigliana E, Busnelli A, Papaleo E, Villanacci R, Candiani M, Reschini M. Risk factors for monozygotic twins in IVF-ICSI cycles: a case-control study. Reprod Sci. 2021;28:1421–7. https://doi.org/10.1007/s43032-020-00406-0.
6. Vitthala S, Gelbaya TA, Brison DR, Fitzgerald CT, Nardo LG. The risk of monozygotic twins after assisted reproductive technology: a systematic review and meta-analysis. Hum Reprod Update. 2009;15:45–55. https://doi.org/10.1093/humupd/dmn045.
7. Knudtson JF, Robinson RD, Sparks AE, Hill MJ, Chang TA, Van Voorhis BJ. Common practices among consistently high-performing in vitro fertilization programs in the United States: 10-year update. Fertil Steril. 2022;117:42–50. https://doi.org/10.1016/j.fertnstert.2021.09.010.
8. Lintsen AM, Braat DD, Habbema JD, Kremer JA, Eijkemans MJ. Can differences in IVF success rates between centres be explained by patient characteristics and sample size? Hum Reprod. 2010;25:110–7. https://doi.org/10.1093/humrep/dep358.
9. Munné S, Alikani M, Ribustello L, Colls P, Martínez-Ortiz PA, McCulloh DH, Referring Physician Group. Euploidy rates in donor egg cycles significantly differ between fertility centers. Hum Reprod. 2017;32:743–9. https://doi.org/10.1093/humrep/dex031.
10. Esposito G, Somigliana E, Franchi M, Dallagiovanna C, Pisaturo V, Corrao G, Parazzini F. Trend of medically induced monozygotic twin deliveries according to age, parity, and type of assisted reproductive technique during the period 2007–2017 in Lombardy Region, Northern Italy: a population-based study. J Assist Reprod Genet. 2021;38:2341–7. https://doi.org/10.1007/s10815-021-02268-0.
11. Scaravelli G, Zacà C, Levi Setti PE, Livi C, Ubaldi FM, Villani MT, Greco E, Coccia ME, Revelli A, Ricci G, Fusi F, Vigiliano V, De Luca R, Bolli S, Borini A. Fertilization rate as a novel indicator for cumulative live birth rate: a multicenter retrospective cohort study of 9,394 complete in vitro fertilization cycles. Fertil Steril. 2021;116:766–73. https://doi.org/10.1016/j.fertnstert.2021.04.006.
12. Scaravelli G, Levi-Setti PE, Livi C, et al. Contribution of cryopreservation to the cumulative live birth rate: a large multicentric cycle-based data analysis from the Italian National Registry. J Assist Reprod Genet. 2019;36:2287–95. https://doi.org/10.1007/s10815-019-01566-y.
13. Shi W, Jin L, Liu J, Zhang C, Mi Y, Shi J, Wang H, Liang X. Blastocyst morphology is associated with the incidence of monozygotic twinning in assisted reproductive technology. Am J Obstet Gynecol. 2021;225:654.e1-654.e16. https://doi.org/10.1016/j.ajog.2021.06.101.
14. Otsuki J, Iwasaki T, Katada Y, Sato H, Furuhashi K, Tsuji Y, Matsumoto Y, Shiotani M. Grade and looseness of the inner cell mass may lead to the development of monochorionic diamniotic twins. Fertil Steril. 2016;106:640–4. https://doi.org/10.1016/j.fertnstert.2016.05.007.
15. Mortimer D, Cohen J, Mortimer ST, Fawzy M, McCulloh DH, Morbeck DE, Pollet-Villard X, Mansour RT, Brison DR, Doshi A, Harper JC, Swain JE, Gilligan AV. Cairo consensus on the IVF laboratory environment and air quality: report of an expert meeting. Reprod Biomed Online. 2018;36:658–74. https://doi.org/10.1016/j.rbmo.2018.02.005.
16. van Dongen J, Gordon SD, McRae AF, Odintsova VV, Mbarek H, Breeze CE, Sugden K, Lundgren S, Castillo-Fernandez JE, Hannon E, Moffitt TE, Hagenbeek FA, van Beijsterveldt CEM, Jan Hottenga J, Tsai PC, BIOS Consortium, Genetics of DNA Methylation Consortium, Min JL, Hemani G, Ehli EA, Paul F, Stern CD, Heijmans BT, Slagboom PE, Daxinger L, Maarel van der SM, Geus de EJC, Willemsen G, Montgomery GW, Reversade B, Ollikainen M, Kaprio J, Spector TD, Bell JT, Mill J, Caspi A, Martin NG, Boomsma DI. Identical twins carry a persistent

epigenetic signature of early genome programming. Nat Commun. 2021;12:5618. https://doi.org/10.1038/s41467-021-25583-7.
17. Cohen JL, Duffy KA, Sajorda BJ, Hathaway ER, Gonzalez-Gandolfi CX, Richards-Yutz J, Gunter AT, Ganguly A, Kaplan J, Deardorff MA, Kalish JM. Diagnosis and management of the phenotypic spectrum of twins with Beckwith-Wiedemann syndrome. Am J Med Genet A. 2019;179:1139–47. https://doi.org/10.1002/ajmg.a.61164.
18. Riess A, Binder G, Ziegler J, Begemann M, Soellner L, Eggermann T. First report on concordant monozygotic twins with Silver-Russell syndrome and ICR1 hypomethylation. Eur J Med Genet. 2016;59:1–4. https://doi.org/10.1016/j.ejmg.2015.12.003.

Authors and Affiliations

Giulia Scaravelli[1] · Valerio Pisaturo[2] · Paolo Emanuele Levi Setti[3,4] · Filippo Maria Ubaldi[5] · Claudia Livi[6] · Andrea Borini[7] · Ermanno Greco[8,9] · Maria Teresa Villani[10] · Maria Elisabetta Coccia[11] · Alberto Revelli[12] · Giuseppe Ricci[13,14] · Francesco Fusi[15] · Mauro Costa[16] · Emanuela Migliorati[17] · Roberto De Luca[1] · Vincenzo Vigiliano[1] · Simone Bolli[1] · Marco Reschini[2]

1 ART Italian National Register, National Center for Diseases Prevention and Health Promotion, National Health Institute, Rome, Italy

2 Infertility Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via M. Fanti 6, 20122 Milan, Italy

3 Department of Gynecology, Division of Gynecology and Reproductive Medicine, Fertility Center, IRCCS Humanitas Research Hospital, Rozzano (Milan), Italy

4 Department of Biomedical Sciences, Humanitas University, Milan, Italy

5 Clinica Valle Giulia, GeneraLife IVF, Rome, Italy

6 Demetra GeneraLife Assisted Procreation Center, Florence, Italy

7 9.baby - GeneraLife, Bologna, Italy

8 Villa Mafalda, Rome, Italy

9 UniCamillus, International Medical University, Rome, Italy

10 Department of Obstetrics and Gynaecology, Fertility Centre, Arcispedale Santa Maria Nuova, Azienda Unità Sanitaria Locale-IRCCS di Reggio Emilia, Reggio Emilia, Italy

11 Assisted Reproductive Center, Careggi Hospital, University of Florence, Florence, Italy

12 Gynecology and Obstetrics 1U/2U, Physiopathology of Reproduction and IVF Unit, Sant'Anna Hospital, University of Torino, Turin, Italy

13 Department of Obstetrics and Gynecology, Institute for Maternal and Child Health, IRCCS Burlo Garofolo, Trieste, Italy

14 Department of Medicine, Surgery and Health Sciences, University of Trieste, Trieste, Italy

15 Department of Maternal Fetal and Pediatric Medicine, ASST, Papa Giovanni XXIII, Bergamo, Italy

16 Department of Reproductive Medicine, Ospedale Evangelico Internazionale, Genoa, Italy

17 Surgery for Gynecology and Obstetrics, Genera Umbria S.R.L, Umbertide, Perugia, Italy