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Full length article

Multiple births from medically assisted reproduction: contribution of different types of procedures and trends over time

Giovanna Esposito [a,*], Fabio Parazzini [a], Paola Viganò [b], Anna Cantarutti [c,d], Matteo Franchi [c,d], Giovanni Corrao [c,d], Carlo La Vecchia [a], Edgardo Somigliana [a,b]

[a] Department of Clinical Sciences and Community Health, Dipartimento di Eccellenza 2023-2027, University of Milan, Milan, Italy
[b] Infertility Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[c] Unit of Biostatistics, Epidemiology and Public Health, Department of Statistics and Quantitative Methods, University of Milano-Bicocca, Milan, Italy
[d] National Centre for Healthcare Research and Pharmacoepidemiology, Milan, Italy

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ABSTRACT

Objective: To evaluate the relative impact of different strategies of medically assisted reproduction (MAR), i.e. first line treatment (ovarian stimulation with or without intrauterine insemination) and in vitro fertilization (IVF) procedures (conventional IVF or intracytoplasmic sperm injection), on the risk of multiple births.
Study design: We utilized the health care utilization databases of the Lombardy region to identify births resulting from MAR between 2007 and 2022. We gathered data on the total number of multiple births and calculated the prevalence rate by dividing the number of multiples by the total number of births. To examine the temporal trend in the proportion of multiple births after MAR over time, a linear regression model was employed separately for different types of techniques and in strata of maternal age.
Results: A total of 30,900 births after MAR were included; 4485 (14.5 %) first line treatments and 26,415 (85.5 %) IVF techniques. Overall, 4823 (15.6 %) multiple births were identified. The frequency of multiple births over the study period decreased from 22.0 % in 2007 to 8.7 % in 2022 ($p < 0.01$). Multiple births from first line treatments were stable ranging from 13.5 % in 2007–2008 to 12.0 % in 2021–2022 ($p = 0.29$). Multiple births from IVF procedures decreased from 23.8 % in 2007–2008 to 8.4 % in 2021–2022 ($p < 0.01$). Stratifying for maternal age (i.e. < 35 and ≥ 35 years), the trends remained consistent.
Conclusions: The reduction in multiple births has been influenced by changes in IVF strategy and procedures. The decline has been gradual but steady since 2009, when a law restricting embryo freezing was repealed in Italy. In contrast, the proportion of multiple births resulting from first line treatments has remained constant over time. Despite declining, multiple births from MAR remained about one order of magnitude higher than those from spontaneous pregnancies.

Introduction

The use of medically assisted reproduction (MAR) has been associated with a higher rate of multiple pregnancies [1]. This is because multiple embryos may be transferred to increase the likelihood of a successful pregnancy after in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) cycles [2,3]. Additionally, fertility medications used to stimulate the ovaries to facilitate conception in vivo may result in the release of multiple oocytes during a single menstrual cycle [4]. This issue is a public health priority, as multiple pregnancies are related to a markedly higher incidence of perinatal mortality and severe neonatal morbidity. Developing strategies to reduce the incidence of multiple pregnancies is a key focus of MAR clinicians.

Several efforts have been made to reduce the number of multiple pregnancies resulting from MAR. One important approach is promoting and implementing elective single embryo transfer (eSET), especially in those patients at better prognosis and most likely to have twins [5,6]. Clinics and fertility specialists are promoting eSET as a safer alternative, highlighting its potential to mitigate the potential risks associated with multiple pregnancies without affecting the cumulative chance of success

* Corresponding author at: Department of Clinical Sciences and Community Health, Dipartimento di Eccellenza 2023-2027, University of Milan, Via Celoria 22, Milan, Italy.
E-mail address: giovanna.esposito@unimi.it (G. Esposito).

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per oocyte retrieval.

Although eSET is associated with a lower live birth rate than double embryo transfer in a single fresh IVF cycle, no substantial difference in cumulative live birth rate was observed when comparing a single cycle of double embryo transfer with repeated eSET [7]. A cumulative success rate at least equal to and possibly higher than that of double embryo transfer can be achieved with a policy of repeated eSET with an effective cryopreservation programme [8,9].

In Italy, from 2004 to 2009, the creation of more embryos than strictly necessary for a single and simultaneous transfer, and in any case not more than three, was prohibited (Law 90/2004). The cryopreservation of embryos was therefore an exception: it was allowed only if the transfer of the embryos into the uterus was not possible for serious and documented reasons of *force majeure* relating to the woman's state of health, unforeseeable at the time of fertilization. As a result, all the embryos obtained were concomitantly transferred to increase the chances of success, causing an increase in multiple pregnancies.

Multiple pregnancies may also result from ovarian stimulation with or without intrauterine insemination (IUI) rather than from multiple embryo transfer after IVF and ICSI [10]. While transferring a single embryo during an IVF cycle can reduce the rate of multiple pregnancies, controlling the number of ovulated eggs when using fertility drugs that stimulate the ovaries poses a greater challenge. While appreciable improvements have been made in reducing the risk of multiple pregnancies in IVF or ICSI cycles [11], no stringent guidelines for reducing twinning in the context of ovarian induction with or without IUI are available [12–14].

To evaluate the relative impact of first line treatments (ovarian stimulation with or without IUI) and IVF procedures (conventional IVF or ICSI) on the risk of multiple pregnancies, we analysed temporal trends of MAR-mediated multiple births in the period 2007–2022 in Lombardy (Italy) using regional administrative healthcare data.

Materials and methods

The data were obtained from the health databases of Lombardy, the largest region in Italy with about 10 milions inhabitans. These include information on services provided to beneficiaries of the National Health Service (NHS), a public health system that provides coverage and free or low-cost access to medical services for all citizens and residents in Italy. The certificate of delivery assistance (CeDAP) and hospital discharge form (scheda di dimissione ospedaliera, SDO) databases were considered. We linked the two databases using the unique identification code, which identifies each selected subject across all databases.

We identified all MAR-mediated births from 1st January 2007 to 31st December 2022 from mothers aged 18 to 45 years who delivered between 22 and 42 gestational weeks. Births lacking information regarding the modality of conception were excluded. MAR procedures included ovarian induction or IUI and conventional IVF or ICSI.

The total number of multiple births was acquired, and the prevalence rate was calculated by dividing the obtained number of multiples by the total number of births according to calendar period (i.e., biennia), type of procedures, and maternal age. In the main analysis, we did not distinguish between twin and higher-order multiple pregnancies since the latter represented a tiny minority; however, we provided the number of twins and higher order multiple pregnancies separately. Absolute numbers and percentages of multiple births were presented for different strata of maternal socio-demographic characteristics, such as age at birth (i.e. < 35 and ≥ 35) and parity (i.e. nulliparous and multiparous), and type of procedure. The Chi-square test was used to test differences in the proportions of singleton and multiple births based on the above variables.

The time trend in the proportion of MAR-mediated multiple births over time was tested using a linear regression model separately in the subsets of types of procedures. The same analysis was repeated, also considering the maternal age, stratifying the two subsets in mothers aged less than 35 years and mothers aged 35 years or more.

All data were treated anonymously. Ethical approval for studies based on administrative and anonymous data is therefore not required.

Results

A total of 30,900 MAR-mediated births occurred in Lombardy during the study period: 1726 (5.6 %) were achieved with ovulation stimulation alone, 2759 (8.9 %) following IUI, 13,162 (42.6 %) following conventional IVF, and 13,253 (42.9 %) following ICSI. The use of IVF procedures increased from 70.9 % of all MAR in 2007–2008 to 89.8 % in 2021–2022 (p for trend < 0.01). Overall, 4823 (15.6 %) multiple births were identified, of which 4600 (95.4 %) were twin pregnancies and 223 (4.6 %) were higher order multiple pregnancies. The frequency of multiple births decreased from 22.0 % in 2007 to 8.7 % in 2022 ($p < 0.01$). The overall proportion of multiple births after MAR over the study period is shown in the supplementary material (Figure S1).

Table 1 provides maternal characteristics and type of MAR procedure according to the type of pregnancy (i.e., singleton or multiple). Multiple births were more common among younger (<35 years, $p < 0.01$), nulliparous ($p < 0.01$) women, and those who underwent ICSI ($p < 0.01$). The frequency of higher order multiple births was 1.1 % for women who had first level treatment and 0.7 % for women who had IVF.

Fig. 1 shows the proportion of multiple births according to the MAR procedure (first line treatment *versus* IVF procedures) over the study period. Multiple births from first line treatments were stable ranging from 13.5 % in 2007–2008 to 12.0 % in 2021–2022 ($p = 0.29$). On the other hand, the proportion of multiple births from IVF procedures decreased from 23.8 % in 2007–2008 to 8.4 % in 2021–2022 with a linear trend ($p < 0.01$).

Table 2 reports the frequency of multiple births according to type of MAR procedure and calendar period in strata of maternal age. For both type of procedures, during the entire study period, multiple births were more common among women aged < 35. For first line treatments, the proportion of multiple births was smaller in women ≥ 35 *versus* < 35 and no marked trend emerged in both strata of age ($p = 0.35$ and $p = 0.58$, respectively for women aged < 35 and ≥ 35). For IVF procedures, the frequency of multiple births decreased 3-fold (from 28.8 % in 2007–2008 to 10.2 % in 2021–2022, $p < 0.01$) in women aged < 35 and halved in women aged ≥ 35 (from 20.3 % to 7.7 %, $p < 0.01$).

The relative impact of the different MAR procedures on the rate of multiple births over time is depicted in Fig. 2. Despite the reduction in multiple births with conventional IVF or ICSI and the unchanged scenario with first line treatments, the impact of the former still prevails because of their use is higher and even increased over time.

Table 1
Maternal characteristics and type of medically assisted reproduction procedures according to the type of pregnancy (i.e., singleton or multiple). Lombardy (Italy), 2007–2022.

	Singleton		Multiple		p-value*
	N	%	N	%	
Maternal age					
<35	8803	82.6	1855	17.4	
≥35	17,287	95.4	2955	14.6	<0.01
Mean (sd)	36.2	(4.3)	35.7	(4.3)	<0.01**
Parity					
Nulliparae	21,549	83.7	4185	16.3	
Multiparae	4528	87.7	638	12.4	<0.01
Type of technique					
Ovulation induction	1519	88.0	207	12.0	
IUI	2385	86.4	374	13.6	
Conventional IVF	11,216	85.2	1946	14.8	
ICSI	10,957	82.7	2296	17.3	<0.01

* Chi-square test; ** t-test for independent samples.

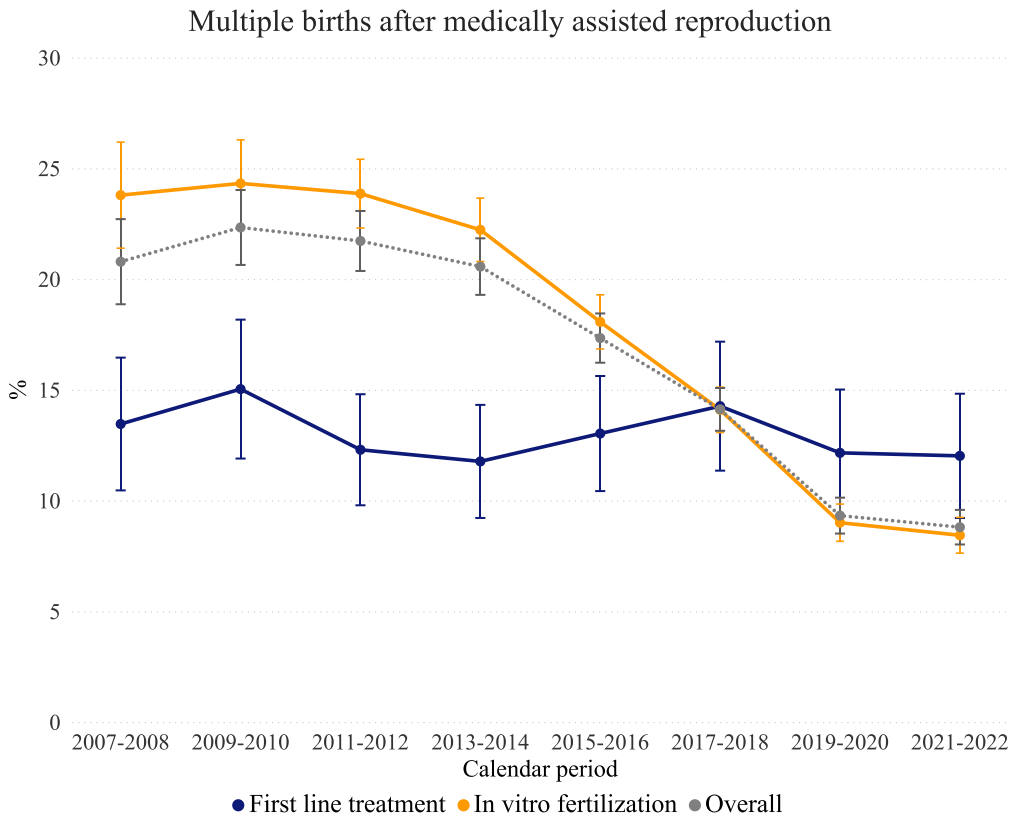

Fig. 1. Proportion of multiple births according to the type of medically assisted reproduction procedures and calendar period. Lombardy (Italy), 2007–2022.

Table 2
Proportion of multiple births according to the type of medically assisted reproduction procedures and calendar period in strata of maternal age. Lombardy (Italy), 2007–2022.

	Multiple births % (95 %CI)			
	First line treatment		In vitro fertilization	
	<35	≥35	<35	≥35
2007–2008	16.9 (12.4–21.5)	9.7 (5.9–13.4)	28.8 (24.8–32.8)	20.3 (17.4–23.3)
2009–2010	18.4 (13.6–23.3)	11.8 (7.8–15.7)	26.6 (23.1–30.0)	23.1 (20.8–25.5)
2011–2012	12.7 (9.1–16.3)	11.9 (8.4–15.4)	24.5 (21.7–27.3)	23.5 (21.7–25.4)
2013–2014	13.1 (9.3–16.8)	10.5 (7.0–13.9)	24.5 (21.9–27.1)	21.1 (19.4–22.8)
2015–2016	14.3 (10.6–18.1)	11.6 (8.0–15.2)	18.6 (16.3–20.8)	17.8 (16.4–19.3)
2017–2018	17.8 (13.5–22.1)	10.0 (6.3–13.6)	15.7 (13.8–17.6)	13.4 (12.2–14.6)
2019–2020	14.4 (10.0–18.8)	9.9 (6.2–13.6)	11.3 (9.7–13.0)	8.0 (7.0–8.9)
2021–2022	13.2 (9.2–17.3)	10.7 (6.8–14.5)	10.2 (8.5–11.8)	7.7 (6.8–8.6)

Discussion

Among births after MAR, the incidence of multiple births showed a continuous decreasing trend, halving from more than 20 % in 2007 to less than 10 % in 2022. However, different trends emerged according to the type of MAR procedure used. The proportion of multiple births resulting from first line treatments was significantly lower than that resulting from IVF techniques in the early years of observation. However, this percentage remained relatively stable at around 15 % from 2007 to 2022, whereas, concomitantly, there was a continuous and sharp decrease in the rate of multiple pregnancies with conventional IVF or ICSI. Since 2017–2018, the tendency has reversed and the rate of multiple pregnancies with first line treatments overcame the rate observed in IVF pregnancies. This marked a departure from the pattern observed in the first study period, and highlighted a notable change in the landscape of multiple births in the context of MAR.

The increase in the incidence of multiple births associated with fertility treatment can be attributed to several factors. A heightened sense of urgency often leads many couples facing infertility to opt for more aggressive treatments, such as the use of exogenous gonadotropins in the first line approaches or to accept the transfer of a larger number of embryos during IVF. Interestingly, multiple birth rates tend to be lower in countries where MAR is covered by healthcare insurance. It is unclear whether this is due to more conservative embryo transfer practices or to the demographic characteristics of the patient population with greater access to such treatments. In cases where there is inadequate or no insurance coverage for MAR procedures, women may opt for ovulation induction as a more economical alternative or choose to transfer a larger number of embryos within the limited resources available for one or a few other cycles [15–17]. For instance, access to fertility treatment in the USA is not uniform, as health insurance coverage varies from state to state. As of September 2023, 21 states have passed fertility insurance coverage laws, 15 of which include IVF coverage and 17 cover fertility preservation for medically induced infertility [18]. A study of births between 2007 and 2009 compared Massachusetts, which has a comprehensive infertility insurance mandate, with Florida and Michigan, which do not. In Massachusetts, births after assisted reproductive technologies were significantly higher than in the other two states, and the use of eSET was three times more common, resulting in a lower

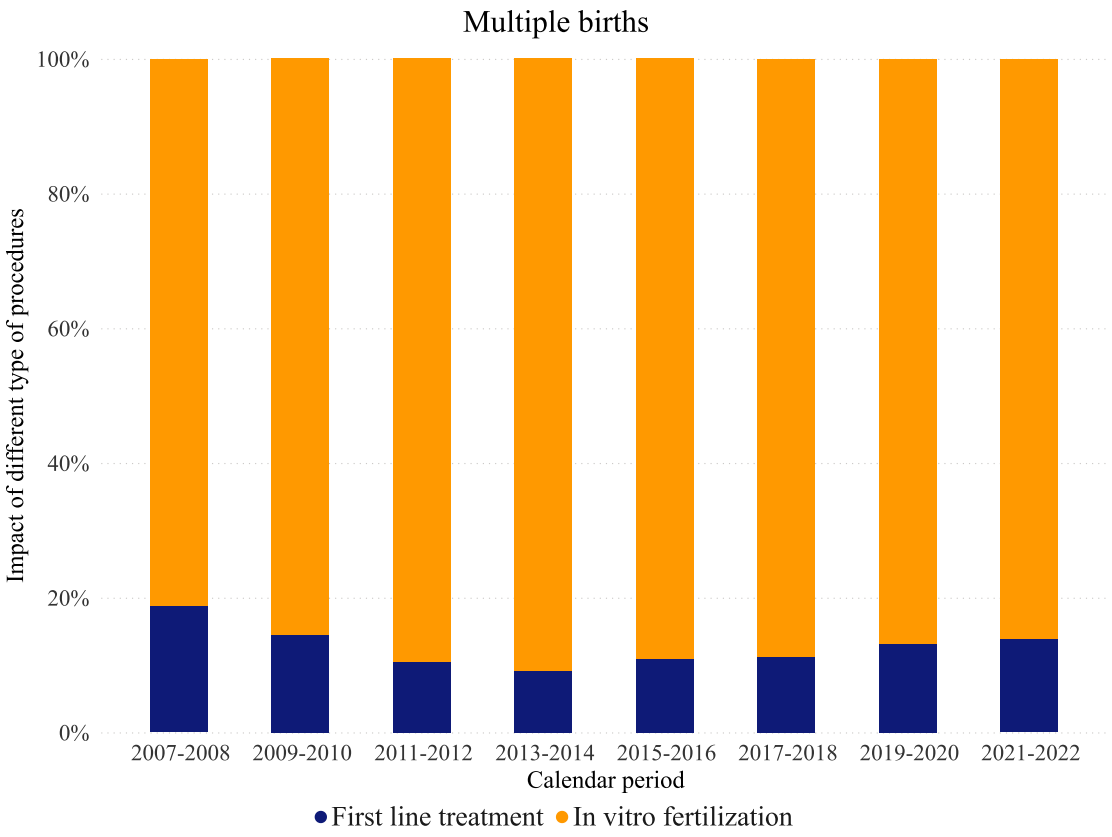

Fig. 2. Impact (%) of medically assisted reproduction procedures on the rate of multiple birth according to calendar period. Lombardy (Italy), 2007–2022.

incidence of twins and higher order births. The relative risk of higher order births was about 2.5 times higher [19]. Similarly, in Italy, a country where assisted reproduction is covered by NHS, the national registry, which reports annually on assisted reproduction activity, shows a small difference in the percentage of twin/multiple births recorded between women assisted in public centres where the healthcare system covers the costs (6.8 %) and strictly private centres (7.9 %) [20].

In the past, several embryos were routinely transferred to increase the chances of pregnancy; the transfer of two or more embryos was common practice, resulting in a dramatic increase in the rate of multiple pregnancies associated with MAR [21]. Nowadays, eSET emerges as the approach of choice for MAR to result in a full-term healthy newborn, reducing multiple pregnancies and, at the same time, ensuring satisfactory success rates [22]. Until 2009 in Italy, 50 % of transfers were carried out with at least three embryos [20]. This was partly due to an Italian law (Law 90/2004), adopted from 2004 to 2009, which restricted embryo freezing. This encouraged the transfer of multiple embryos in a single IVF cycle. The transfer of multiple embryos, and thus multiple births, has gradually decreased since the law was repealed. There has been a notable increase in eSET with the expanded use of extended embryo culture and blastocyst vitrification. In 2021, transfers with one embryo exceeded 50 % of transfers in fresh cycles for the first time, with a variation of + 3 % compared to 2020 and + 32 % compared to 2005. Transfers with three embryos showed a rapid and continuous downward trend, reaching about 5 % in 2021. The percentage of transfers with two embryos has also continued to decrease in recent years, falling below eSET in 2020 and 2021 [20]. The use of cumulative live birth rate per oocyte retrieval as the primary outcome of conventional IVF-ICSI rather than the live birth rate per transfer also contributed to this change in practice. According to the European Society of Human Reproduction and Embryology (ESHRE) registries [23], eSET was the most commonly chosen option in 2018–2019 (more than 50 %), but differences emerged when looking at individual countries. In 2019, some countries reported less than 30 % eSET, i.e., Armenia, Greece, Montenegro and Serbia. The lowest percentage was observed in Albania, where 1.5 % was eSET and the rest were all double embryo transfers. None of the countries performed more than 50 % of their transfers with three embryos. Among countries reporting transfers with four or more embryos, the highest proportions were found in Serbia and Greece (3–6 %).

Ovulation stimulation results in more twinning than conventional IVF or ICSI [10]. In contrast, the ESHRE registries did not show an overall higher twin rate in those who underwent IUI than in those who underwent IVF/ICSI, possibly due to the different approach in some countries that have not yet adopted eSET as the primary approach [23].

By the late 1990s, it was accepted in the USA that most of the increase in multiple births, especially higher order births, was due to IVF. A study of the relative contributions of assisted reproductive technologies and ovarian induction to multiple births reported that the latter was responsible for a significant proportion. During the period 1997–2003, the contribution of IVF to the twin birth rate continued to increase, while it contributed less to higher order multiple births. Conversely, the proportion of triplets attributed to ovarian induction increased from 31 % to 36 % and quadruplets from 39 % to 62 % [24].

It is challenging to control the number of ovulated oocytes when using fertility drugs that stimulate the ovaries, and there are no consistent guidelines for reducing twins and high-order multiple pregnancies due to ovarian induction [12–14]. Initially, reducing the dose of ovarian induction drugs was criticised because it was thought that the multiple birth rate could not be reduced without lowering the fertility rate [12]. However, some attempts have been made to minimise the rate of multiple births, especially the high-order ones, by modulating the dose of gonadotropins while ensuring a good pregnancy success rate

[4,25–28]. Therefore, in cases requiring the use of gonadotropins, it is recommended to use the lowest dose possible and to strictly monitor the ovaries with ultrasound [10]. Also, a policy of strict cancellation of ovarian induction cycles based on ultrasound criteria was considered a precautionary measure by the American College of Obstetrics and Gynaecology (ACOG) and the Royal College of Obstetricians and Gynaecologists (RCOG) [29,30].

It is recommended to consider the cycle cancellation if > 2 follicles $\geq$ 16 mm or > 3 follicles ≥ 14 mm develop in women aged less than 40 years undergoing ovarian induction or stimulation that will not be followed by an IVF procedure [10]. However, despite these efforts, our real-life data showed that no significant improvement has been observed over the 15 years study period. In this perspective, transvaginal ultrasound, a safe and reliable procedure, plays a central role in monitoring and optimising follicular development. Regular ultrasound monitoring ensures that the ovarian response is adequate but not excessive, minimising the risk of multiple pregnanices and ovarian hyperstimulation syndrome [31–33].

From a public health perspective, the impact of first line treatments on the overall number of multiple pregnancies is modest in our context because IVF pregnancies represented the vast majority of MAR pregnancies. Over the period studied, the use of IVF procedures has increased, while the use of first line treatments has decreased from around 30 % at the beginning to less than 10 % at the end. NHS coverage of MAR may explain this finding. Physicians and patients may more easily opt for IVF procedures in the absence of economic barriers. In the studied context where IVF procedures have overcome ovarian stimulation with or without IUI in the rate of singleton births, the possible overuse of more complex MAR procedures may have a beneficial drawback for the NHS because the higher costs of conventional IVF or ICSI could be balanced with the significant costs of preventing multiple pregnancies.

The population-based design, with a large cohort of births available over a period of more than 10 years, is the major strength of the study. However, some weaknesses of this study need to be considered. First, the data recorded on the MAR procedures were reported by the woman and hence some reporting bias is possible. Furthermore, detailed information on the zygosity, the indication for MAR (e.g., female or male infertility), and on laboratory procedures (e.g., number and stage of embryo, characteristic of cycles, oocytes donation) was not reported in the administrative databases. Additionally, laboratory data on hormone levels such as basal follicle-stimulating or anti-Mullerian hormone levels were not available. Such clinical information would have been useful for further considerations of which practices or patients' characteristics are risk factors for multiple births and influence their trends. For example, according to recent evidence, fresh embryo transfer and blastocyst transfer have been associated with monozygotic twinning after assisted reproductive technology [34,35].

In conclusion, changes in the Italian legislation in 2009 and improvements in IVF procedures have substantially influenced the reduction of multiple pregnancies. In contrast, the percentage of multiple pregnancies from first line treatments has remained constant over time. The rate of multiple births is still an order of magnitude higher than that of spontaneous pregnancies. Further efforts are needed to reduce multiple pregnancies in infertility treatment, especially when first line treatments are used.

CRediT authorship contribution statement

Giovanna Esposito: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Fabio Parazzini:** Writing – review & editing, Supervision, Funding acquisition. **Paola Viganò:** Writing – review & editing. **Anna Cantarutti:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Matteo Franchi:** Writing – review & editing, Formal analysis, Data curation. **Giovanni Corrao:** Supervision. **Carlo La Vecchia:** Writing – review & editing, Supervision. **Edgardo Somigliana:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Edgardo Somigliana reports grants from Ferring, grants and personal fees from Merck-Serono, grants and personal fees from Theramex, personal fees from Gedeon-Richter, outside the submitted work. Giovanni Corrao received research support from the European Community (EC), the Italian Agency of Drugs (AIFA), and the Italian Ministry for University and Research (MIUR). He took part in a variety of projects that were funded by pharmaceutical companies (i.e. Novartis, GSK, Roche, AMGEN and BMS). He also received honoraria as a member of the advisory board to Roche. The other authors declare that they have no conflicts of interest to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejogrb.2024.07.004.

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