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Trends in multiple pregnancy rates following medically assisted reproduction: an 18-year UK-based nationwide analysis

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Abstract

Multiple pregnancy (MP) is a long-recognized and mostly preventable iatrogenic complication of medically assisted reproduction (MAR). Given its recent increase in MAR-accessibility, MP rates have risen despite their known association with negative maternal/perinatal outcomes. Hence, primary (*e.g.* policies limiting the number of embryos transferred) and secondary (*e.g.* multifetal pregnancy reduction (MPR)) prevention strategies have emerged to reduce the incidence and burden of these rates worldwide.

This study aimed to assess trends in MP rates following MAR and the clinical impact of MPR in triplet pregnancies on pregnancy and neonatal outcomes. We performed a population-based retrospective analysis of the MP rates incidence included in the anonymized Human Fertilisation and Embryology Authority database between 1999 and 2018. We limited our analysis to pregnancies that occurred following artificial insemination (AI), in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) cycles, including fresh and frozen embryo transfers. The primary outcome was the occurrence of MP. Additionally, we assessed pregnancy outcomes following MPR.

The incidence of MP and triplets seemed to decreased over time following IVF/ICSI cycles, but not following AI. In AI cycles, only ovarian stimulation (OS) was associated with both multiples and triplets (aOR 7.002, 95%CI 5.853-8.378 and aOR 11.791, 95%CI 5.689-24.438, respectively), while in IVF/ICSI pregnancies, transferring 2 or 3 embryos were the strong predictors (aOR 35.688,95%CI 21.641-58.855 and aOR 31.861,95%CI 29.89-33.962, respectively). Frozen embryo transfer significantly reduced the risk of triple pregnancy, when compared with fresh embryos. Regarding MPR, the incidence of preterm birth and low birthweight was significantly lower when compared to expected management.

Our results provide evidence for caution when using OS in AI cycles and for the widespread use of multiple embryo transfers. Furthermore, we provide robust information concerning the outcomes of MPR in triplets after MAR, thus facilitating counselling for patients considering such an approach.

Keywords: Multiple pregnancy, medically assisted reproduction, IVF/ICSI, AI, multifetal pregnancy reduction

Resumo

A gravidez múltipla (GM) é uma complicação iatrogénica maioritariamente evitável da procriação medicamente assistida (PMA). Dada a sua recente acessibilidade, as taxas de GM pós-PMA aumentaram, apesar das repercussões maternas/perinatais associadas. Consequentemente, surgiram estratégias de prevenção primária (como a limitação do número de embriões transferidos) e secundária (nomeadamente a redução da gravidez multifetal (RGM)) para procurar reduzir a incidência e peso destas taxas mundialmente.

Este estudo teve como objetivo avaliar a evolução das taxas de GM após PMA e o impacto clínico da RGM em gravidezes triplas. Realizámos uma análise retrospetiva e populacional da incidência das taxas de GM na base de dados da Autoridade de Fertilização Humana e Embriologia, entre 1999 e 2018. Limitámo-la às gravidezes que ocorreram após ciclos de inseminação artificial (IA), fertilização in vitro (FIV) e injeção intracitoplasmática de espermatozoides (ICSI), incluindo transferências de embriões a fresco e criopreservados. O desfecho primário foi a ocorrência de GM. Adicionalmente, também avaliamos os desfechos obstétricos pós-RGM.

A incidência de GM/trigémeos após FIV/ICSI parece ter diminuído, mas não após IA. Nos ciclos de IA, apenas a estimulação ovárica (EO) esteve associada a GM e trigémeos (aOR 7.002,95%CI 5.853-8.378 e aOR 11.791,95%CI 5.689-24.438, respetivamente), enquanto que nas gravidezes por FIV/ICSI, transferir 2 ou 3 embriões foi dos preditores mais robustos (aOR 35.688,95%CI 21.641-58.855 e aOR 31.861,95%CI 29.89-33.962, respetivamente). A transferência de embriões criopreservados reduziu significativamente o risco de gravidez trigemelar, em relação às transferências a fresco. Relativamente à RGM, a incidência de parto pré-termo e baixo peso ao nascer foi significativamente menor, comparando com uma atitude expectante.

Os resultados obtidos denotam necessidade de cautela na utilização da EO durante IA, bem como no uso generalizado de transferências com múltiplos embriões. Fornecem, igualmente, informação robusta sobre os desfechos pós-RGM em trigémeos, facilitando o aconselhamento de doentes que considerem esta abordagem.

Keywords: Gravidez múltipla, procriação medicamente assistida, FIV/ICSI, IA, redução multifetal

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Introduction

The incidence of twin and higher-order multiple births has increased significantly over the past several decades. This trend is mostly attributable to the availability and high adoption of assisted reproductive technology (ART) techniques in the industrialized world, primarily as a result of motherhood postponement (ACOG, 2021; Sebghati & Khalil, 2021). This staggering increase in the prevalence of multiple pregnancies (MP) has become more significant following particularly in vitro fertilization (IVF) alongside the large-scale use of exogenous ovarian stimulation (Fauser et al., 2005). According to the Centers for Disease Control and Prevention (CDC), in 2018, it is noteworthy that the percentage of multiple births was higher among infants conceived with ART (21.4%) than among all infants born in the total birth population (3.3%). In fact, these data demonstrate the huge contribution that these fertility treatments have on the rate of MP, contributing overall to 12.5% of all multiple births, including 12.5% of all twin births and 13.3% of all triplets and higher-order births (Walensky et al., 2022). These results demonstrate how maximizing the success of these fertility treatments has become frequently the overriding goal, inadvertently leading to a culture of acceptance of MP. However, MP are associated with a wide spectrum of negative consequences for both the mother and the fetuses and, therefore, should not be taken lightly.

The burden of infertility

Infertility has been recognized as a public health issue worldwide by the World Health Organization (WHO). Its prevalence in the population has crucial demographic and health implications, yet it is difficult to evaluate due to different assessment methodologies and the organizational characteristics of existing healthcare systems. Nevertheless, recent demographic surveys have been published with data mostly from the new millennium and they bring a reality that puts the numbers of infertility in the many millions (Inhorn & Patrizio, 2014). Thus, infertility has been reported to range from 3.5% (Webb & Holman, 1992) to 16.7% (Philippov et al., 1998) in more developed countries and from 6.9% (Larsen, 2005) to 9.3% (Che & Cleland, 2002) in less developed ones, adding up to 72.4 million infertile women of whom 40.5 million seek medical care,

at similar rates in both developed and developing countries (Boivin et al., 2007). Other estimates suggest that it affects 8 to 12% of reproductive-aged couples globally, adding up to 48.5 million couples (Mascarenhas et al., 2012; Vander Borght & Wyns, 2018), and that 186 million individuals are living with infertility globally (Rutstein & Iqbal H. Shah, 2004).

Infertility is defined, according to the International Glossary on Infertility and Fertility Care, as a disease of the reproductive system characterized by the failure to achieve a clinical pregnancy after 12 months or more of regular and unprotected sexual intercourse or due to an impairment of a person's capacity to reproduce either as an individual or with his/her partner, which generates disability as an impairment of function. Infertility is further categorized as primary or secondary. Primary female infertility diagnosed when a woman who has never been diagnosed with a clinical pregnancy meets the criteria of infertility, while secondary female infertility is distinguished by the fact that it applies to a woman who had previously had at least one clinical pregnancy. Female infertility encompasses several female factors such as ovulatory disturbances, diminished ovarian reserve, chronic illness, sexual conditions incompatible with coitus, and also anatomical, endocrine, genetic, functional or immunological abnormalities of the reproductive system. The same categorization of infertility might be applicable to the male regarding his ability to initiate a clinical pregnancy and is caused primarily by male factors such as abnormal semen parameters or function, abnormalities of the reproductive system, chronic illness and sexual conditions incompatible with the ability to deposit semen in the vagina (Zegers-Hochschild et al., 2017). In fact, the male factor is thought to contribute to up to 50% of infertility cases overall and may be exclusively responsible for 20 to 30% of all cases (Agarwal et al., 2015). Besides this, it should be noted that 10% to 30% correspond to cases of unexplained infertility (Gunn & Bates, 2016), that is, infertility in couples with apparently no abnormalities of the male or female reproductive system and with adequate coital frequency (Zegers-Hochschild et al., 2017).

The use of MAR to treat infertility and its effect on MP

Evidence of an increase in the reporting of fertility problems to primary care as well as an increase in the scope and accessibility of fertility treatments are expected to have an impact on the number of people seeking ART (Datta et al., 2016). Currently, the terminology used in the field of infertility and fertility care interventions may have different connotations depending on the context, its use in research or clinical interventions, or even among distinct communities. To ensure uniformity in these terms, it is important to distinguish between medically assisted reproduction (MAR) and assisted reproductive technologies (ART). MAR is the broadest of these terms that, according to the International Glossary on Infertility and Fertility Care, includes various interventions, procedures, surgeries and technologies to treat different forms of fertility impairment and infertility, such as ovulation induction, ovarian stimulation (OS), ovulation triggering, all ART procedures, uterine transplantation and intrauterine, intracervical and intravaginal insemination with the semen of partner or donor. Meanwhile, ART involves interventions that only include in vitro handling of both human oocytes and sperm or of embryos for reproductive purposes, such as conventional IVF, intra-cytoplasmatic sperm injection (ICSI), preimplantation genetic testing (PGT), gamete and embryo cryopreservation, semen, oocyte and embryo donation, gestational carrier cycles, among others (Zegers-Hochschild et al., 2017). On the other hand, the Portuguese term used is "Procriação Medicamente Assistida" (PMA), which encompasses artificial insemination (AI), IVF, ICSI, embryo, gamete or zygote transfer, PGT, and other equivalent or additional gametic or embryonic manipulation laboratory techniques, as well as techniques carried out in the context of surrogate pregnancy situations (Decreto-Lei nº 32/2006. Procriação Medicamente Assistida, 2006). In other words, it is equivalent to the Anglo-Saxon term MAR, so it includes not only ART techniques but also AI.

As previously mentioned, the reason why MP rates are so high in ART cycles is mainly because of the desire to stimulate the most follicles possible and transfer excess embryos with the goal of maximizing pregnancy rates to a number considered to be satisfactory. Regardless of which technique is performed, this veiled pressure for success has consequences for perinatal mortality rates, which are 4-fold higher for twins and 6-

fold higher for triplets than for singletons (The ESHRE Capri Workshop Group, 2000). Moreover, maternal complications also are higher in MP, including an increased risk of miscarriage and other pregnancy complications such as anaemia, pre-eclampsia or gestational diabetes, caesarean section, postpartum haemorrhage, depression, as well as twin-specific complications (such as twin-to-twin transfusion syndrome). Furthermore, other perinatal complications associated with MP comprise a significantly higher risk of prematurity, and low birthweight, as well as long-term complications as intraventricular haemorrhage, cerebral palsy, disability, and learning difficulties (Fauser et al., 2005; Kawwass & Badell, 2018). As a result, MP should be regarded as a serious complication following MAR, and the most important way to decrease these high rates is through prevention.

In fact, a conscious attempt has been made worldwide to reduce the rates of iatrogenic multiple births through legislation and policy changes. The intended goal is to reduce the rate of MP with the least possible impact on treatment outcomes. Primary approaches include being more restrictive in the use of ART techniques, especially in patients who are likely to conceive naturally, less aggressive OS protocols, namely restricting the use of gonadotropins, increasing the use of natural-cycle AI over AI combined with OS, and stricter embryo-transfer policies limiting the number of embryos transferred (Verberg et al., 2007). According to the American Society for Reproductive Medicine, elective single embryo transfer (eSET) is the transfer of a single cleavage- or blastocyst-stage embryo after IVF or ICSI, despite the availability of more than one good-quality embryo (Tobias et al., 2016). Recent evidence suggests eSET along with subsequent frozen and thawed embryo transfer yield similar cumulative live birth rates compared with double or multiple embryo transfers (Thurin et al., 2004), with a more limited significance of these results in women of advanced reproductive age (Fujimoto et al., 2015). However, eSET dramatically lowers the rate of multiple births regardless of female age. With primary prevention in mind, the percentage of SET has increased dramatically in the USA over the past ten years, rising from 20.6% in 2011 to 80.4% in 2020 across all age groups, according to the Society for Assisted Reproductive Technology. In addition to these findings, the percentage of ART-conceived live-birth deliveries that resulted in singletons raised from 72.1% in 2011 to 93.5% in 2020 over the prior decade. Conversely, twin pregnancies declined from 26.8% in 2011 to 6.4% in

2020, while triplets or more decreased from 1.1% to 0.1%, which is most likely influenced by the rising use of SET in recent years (Centers for Disease Control and Prevention, 2022).

Multifetal pregnancy reduction (MPR)

When MP preventive measures are absent or ineffective, secondary prevention usually consists of strategies such as MPR, which entails the elective termination of at least one normal fetus to reduce the chances of miscarriage of the remaining fetus(es) (Boulot et al., 2000). The most common technique uses a transabdominal ultrasound-guided approach with an injection of potassium chloride into the fetal thorax (near or into the heart) to induce asystole (Obič An et al., 2015), while the least frequently involves transvaginal aspiration without an injection (Hessami et al., 2022). It is commonly conducted between 11 and 14 weeks because most spontaneous miscarriages (which could preclude the need for this procedure) as well as eventual chorionic villus sampling and first-trimester ultrasonography (to rule out fetal abnormalities) have already occurred by then (Kim et al., 2019; Sebghati & Khalil, 2021). The fetus or fetuses are chosen primarily for technical reasons, as opposed to selective reduction, in which the termination of one or more specific fetuses occurs due to a known genetic, structural, or other abnormality (Obič An et al., 2015).

Despite the undeniability of improved perinatal and obstetric outcomes following the recourse to MPR with four or more fetuses, the outcomes of reducing triplets to twins compared with expectant management of triplets have remained debatable and are frequently met with controversy due to ethical reasons (Wimalasundera, 2010). Several studies have shown that the probability of preterm birth (PTB) and low birthweight (LBW) is significantly lower in reductions to dichorionic twins without a substantial increase in the risk of miscarriage (Wimalasundera, 2010), as well as in the reduction from a twin to a singleton pregnancy (Gupta et al., 2015; Vieira et al., 2019). Conversely, these approaches do not seem to be correlated with any improvement in more severe adverse outcomes such as very low birthweight, very or extremely preterm birth (Gupta et al., 2015), low 5-minute Apgar score, use of assisted ventilation, intraventricular haemorrhage, and neonatal encephalopathy, convulsions, or sepsis (Razaz et al., 2017).

Thereby, larger studies with more data on this subject are needed to further evaluate outcomes with low incidences and to better measure risk.

Another important topic concerns the decision to undergo fetal reduction to twins versus singletons, which depends on multiple factors and considerations which may be rather challenging for couples considering these options. In fact, despite the insufficient evidence regarding the prognosis of MPR nowadays, researchers have shown that, compared to triplet pregnancies reduced to singletons, MPR to twin pregnancies may be associated with a lower fetal survival rate, lower gestational age at birth, and higher risk of PTB and LBW (Hessami et al., 2022). Additionally, a reduction in the risk of pregnancy complications such as preeclampsia and preterm premature rupture of membranes has also been described in twins-to-singletons MPR (Vieira et al., 2019). Hence, it is essential to have more detailed information so that professional healthcare providers can perform parental counselling in the face of MP regarding these MPR approaches and be aware of the potential risks and benefits of reduction to both twin and singleton pregnancies.

Even though it is widely acknowledged that MAR carries a major risk for MP and subsequent to maternal/perinatal health and that primary prevention strategies (such as the introduction of eSET policies) have generated in some countries an auspicious trend in lowering MP rates, there is still wide variation in attitudes, regulations and practices regarding eSET and other MP prevention strategies. Indeed, whereas triplet deliveries in Europe have fallen significantly over the years and stayed at low rates (Ferraretti et al., 2017), twin births have not followed the same pattern and the consequent low gestational age at birth was still documented in more than half of the deliveries (Wyns et al., 2020). Additionally, the practice of MPR in the prevention of multiple births was reported by almost all countries (Wyns et al., 2020). This illustrates how MPR occurs in a significant portion of fertility care treatments, aiming to address the after-effects of a fervid infertility management. Unfortunately, among these approaches, the one that is having a tangible impact on reducing the rate of MP is not yet established (Wyns et al., 2020). Moreover, while this may be true, with regards to MPR one must take into account not only the general clinical and economic issues but also how this procedure is viewed with all the inherent psychological consequences and ethical concerns. These factors ultimately impact how it is perceived and limit its

applicability in practice as a multiple birth reduction strategy (Bhattacharya & Kamath, 2014). Consequently, unless there are major changes in the way ART is perceived and legislated, the current variations and restrictions in the use of techniques intended to prevent MP are expected to endure.

Thus, this study aimed not only at assessing the trends in MP rates following MAR techniques in the last years using a large national registry but also the clinical impact of MPR in triplet pregnancies on pregnancy and neonatal outcomes.

Material and Methods

Study design and sample

We performed a United Kingdom population-based retrospective analysis of the evolution of the MP rate included in the anonymized database of the Human Fertilisation and Embryology Authority (HFEA) between 1999 and 2018. The HFEA is a regulatory authority of London's Health and Social Services Department which is in charge of regulating the use of reproductive technologies and embryos in research throughout the United Kingdom (UK). Since 1991, this institution has kept records of ART cycles conducted in the UK by a legally mandated reporting policy. This anonymous registry allows researchers to access an extensive collection of data that does not identify any patients or children born as a result of treatment (Human Fertilisation and Embryology Authority, 2022).

Data has been filtered by the HFEA to exclude any patients listed as under 18 and over 50 to reduce small numbers and risk patients of re-identification. Hence, the age range of patients included in the study spanned from 18 to 50 years.

To minimize bias and allow for eventual adjustment for potential confounders, we excluded data from cycles in which the treatment details such as the specific type of treatment, the use or non-use of OS, and the number of transferred embryos were unclear or unknown. Additionally, we restricted our analysis to either AI or IVF/ICSI cycles (including both fresh and frozen embryo transfers), excluding other less frequent or no longer used techniques (*i.e.* gamete or zygote intrafallopian transfer, concomitant transfer of fresh and frozen embryos, cycles with more than three embryos transferred and intravaginal or intracervical insemination). We also excluded cycles in which the indication for treatment was not necessarily infertility, such as IVF for PGT, cycles where female fertility preservation was the goal, as well as patients acting as surrogates. Regarding neonatal outcomes, we excluded ectopic/heterotopic pregnancies, (selective) pregnancy terminations and those lost to follow-up.

AI and IVF

AI cycles included in this study were defined as the assisted conception technique that involved the deposition of a processed semen sample in the upper uterine cavity to attempt a pregnancy (Zegers-Hochschild et al., 2017), overcoming the natural barriers of sperm ascent in the female reproductive tract. AI in the UK is frequently the first-line treatment for anovulatory infertility and mild male factor infertility. OS can be performed in this technique. However, a strict controlled ovarian hyperstimulation policy should be employed to achieve a suitable number of follicles while avoiding the known unwanted effects, namely ovarian hyperstimulation syndrome (OHSS) and MP (Allahbadia, 2017). This methodology is critical because, unlike ART, which can be regulated downstream by limiting the number of embryos transferred, strategies to control MP rates are less extensive and effective during AI and rely heavily on cycle cancellation or even conversion to IVF (Evans et al., 2020).

Conversely, extracorporeal gamete fertilization, followed by embryo transfer via the cervix into the uterus, constitutes the definition of ART used in this study. It entailed both conventional IVF and ICSI, a subtype of IVF that involves injecting a single spermatozoon into the cytoplasm of an oocyte (Zegers-Hochschild et al., 2017). Direct referral to IVF/ICSI for patients with advanced maternal age, severe male factor infertility, tubal factor or severe endometriosis (Allahbadia, 2017) is common, including in the setting of this study. When viable embryos are available, a fresh embryo transfer may be followed by one or more frozen embryo transfers in subsequent cycles in conventional IVF/ICSI treatment. As an alternative, it is also feasible to freeze all suitable embryos and only transfer frozen embryos during the following cycles are performed in such cases. This method is frequently referred to as the "freeze all" strategy (Zaat et al., 2021).

The major safety issues of conventional IVF are MP and OHSS, an exaggerated systemic response to OS, which can include ascites, electrolyte imbalance and hypercoagulability (Carson & Kallen, 2021). Most ART cycles begin with the administration of exogenous OS, which is a pharmacological treatment used to induce multi-follicular development while suppressing endogenous ovulation caused by the hypothalamus-pituitary axis (Carson & Kallen, 2021). OS is frequently utilized to

encourage the emergence of numerous dominant follicles, the maturation of a large number of oocytes and the formation of multiple embryos in an effort to increase the likelihood of pregnancy (Macklon et al., 2006). Since the beginning of modern-day ART, this OS pattern has modelled the fundamentals of clinical practice. Conversely, natural cycle ART and mild OS are regimens in which the ovaries are unstimulated or minimally stimulated with the goal of minimizing the number of oocytes produced during IVF stimulation (Zegers-Hochschild et al., 2017). These approaches are linked to improved stimulation tolerance, less treatment-related stress, reduced cost and a greater safety profile, in terms of the incidence of MP and OHSS (Allersma et al., 2013; Kumar Datta et al., 2020), with several studies finding no significant difference in pregnancy outcomes (Nargund et al., 2017).

Exposure/Independent variables assessed

Oocyte source age at treatment, number of previous AI or IVF/ICSI cycles, parity, specific treatment type, whether OS was performed, embryo development stage, number of transferred embryos, transfer of a fresh or frozen embryo and year of treatment were the independent variables considered. These variables were available at the beginning of the treatment in all included cases, and they may affect the results of MAR cycles. Specifically, according to the American College of Obstetricians and Gynecologists, women over the age of 35 are more likely than younger women to release two or more eggs during a single menstrual cycle, which increases their likelihood of having multiples. This, coupled with the fact that there is greater use of ovulation-inducing drugs, as a consequence of the increase in maternal age at conception, may end up having a compounding impact on the increase in the incidence of MP. Moreover, the number of transferred embryos may affect the outcomes of treatment and increase the risk of MP and, consequently, pregnancy complications (Fujimoto et al., 2015; Pandian et al., 2013; Thurin et al., 2004). Meanwhile, the stage of development of the transferred embryos can also affect MP, since blastocyst stage transfer is associated with an increase in pregnancy rates (Dirican et al., 2022; Martins et al., 2017; Papanikolaou et al., 2008). Regarding the status of the embryo (fresh or frozen-thawed), although it is still unclear whether the acting protocol of frozen

transfers reduces MP rates (Wong et al., 2017), it has been found to lower risk of adverse obstetric and perinatal outcomes (i.e. PTB, LBW and perinatal death) compared with fresh embryo transfers (Z. Li et al., 2019; Wong et al., 2017), which can affect our results if unaccounted for.

Main Outcome Measures

The endpoints were defined according to definitions of International Committee Monitoring Assisted Reproductive Technologies (ICMART) in The International Glossary on Infertility and Fertility Care (Zegers-Hochschild et al., 2017).

The primary outcome assessed was the occurrence of multiple pregnancy, defined as a pregnancy with two or more embryos/fetuses. The secondary outcomes evaluated included the occurrence of triplet pregnancies, and also the neonatal outcomes in which MPR was performed.

Regarding the offspring, the neonatal endpoints contemplated were livebirth as well as the birthweight and the gestational age at birth. Concerning birth weight, the following indicators were also evaluated: low birth weight was considered if a newborn weighed less than 2500g, very low birth weight if a newborn weighed less than 1500g and extremely low birth weight if a newborn weighed less than 1000g. A preterm birth (PTB) was defined as a birth that occurs before 37 completed weeks of gestational age, while very preterm birth was defined before 32 weeks of gestational age. An additional interval of preterm birth taking place before 30 completed weeks of gestational age was also assessed.

Statistical analysis

Crude categorical baseline characteristics and outcome parameters were compared between groups using the χ^2 test. The incidence of MP and triplet pregnancy were also assessed accounting for the before-mentioned potential confounding using multivariable logistic regression.

The statistical significance of the trends in MP rates and relevant potential confounders observed over time were determined using Poisson regression. All

graphical depictions of trends were smoothed using moving averages, adjusting for the mean MP rates of the year before and after, in order to minimize spurious variations which could limit the visual interpretation of the temporal trend.

A p-value was considered significant when below 0.05, followed by Bonferroni-adjusted pairwise comparisons whenever warranted. The statistical analysis was performed using the Stata software version 13.1 (StataCorp, College Station, TX, USA).

Results

Baseline characteristics of the population

In this UK-based nationwide retrospective analysis we included 244.678 intrauterine pregnancies following MAR cycles performed between 1999 and 2018 following the above-mentioned inclusion and exclusion criteria. The baseline patient characteristics are presented in Tables 1A and 1B. Among these, 94% (n=230.010) pregnancies occurred following IVF/ICSI cycles and 6% (n=14.668) following AI cycles. Among the included cycles, 80.3% (n=196.363) were singleton, 17.3% (n=48.315) twin and 0.6% (n=1.571) triplet pregnancies. The main analysis (Table 1A) was performed by subgrouping pregnancies according to whether they were singleton or multiple (2 or 3 intrauterine fetal heart pulsations detected). However, a separate analysis was also performed subgrouping the pregnancies according to whether they were or not triplet pregnancies (Table 1B).

Most of the women achieving pregnancy with these MAR techniques (both AI and IVF) were aged between 18-34 years (51.2% in singleton pregnancies and 55.5% in multiple pregnancies vs 52.1% in non-triplet pregnancies and 55.0% in triplet pregnancies). Regarding AI, pregnancies obtained following this MAR technique used OS in 47.1% of the included cycles (n=6.915), culminating with a greater preponderance in multiple (84.4% vs 44.5% in singleton pregnancies) and triplet pregnancies (91.3% vs 46.9% in non-triplet pregnancies). In total, in 30.25% of the IVF/ICSI cycles (n=69.576) only one embryo was transferred, while in 64.01% of cycles (n=147.232) two embryos were transferred, and in 5.74% of cycles (n=13.202) three embryos were transferred. Double embryo transfer (DET) was the procedure used in 89.8% of the multiple pregnancies and 60.8% of the triplet pregnancies and triple embryo transfer (TET) was used in 7.5% of the multiple pregnancies and 37.8% of the triplet pregnancies.

	Single (n=196363)	Multiple (n=48315)	p-value
Oocyte source (age)			<0.001
Donor (<36)	8835 (4.5%)	2473 (5.1%)	
Autologous (18-34)	100624 (51.2%)	26811 (55.5%)	
Autologous (35-37)	46880 (23.9%)	11429 (23.7%)	
Autologous (38-39)	23580 (12.0%)	4975 (10.3%)	
Autologous (40-42)	14533 (7.4%)	2453 (5.1%)	
Autologous (>42)	1911 (1.0%)	174 (0.4%)	
Number of previous AI cycles			<0.001
None	181362 (92.4%)	46161 (95.5%)	
1	3601 (1.8%)	414 (0.9%)	
2	3001 (1.5%)	386 (0.8%)	
≥3	8399 (4.3%)	1354 (2.8%)	
Number of previous IVF cycles			<0.001
None	110952 (56.5%)	24124 (49.9%)	
1	38674 (19.7%)	10387 (21.5%)	
2	21923 (11.2%)	6341 (13.1%)	
≥3	24814 (12.6%)	7463 (15.4%)	
Number of previous livebirth deliveries after AI/IVF			<0.001
Nulliparous	159703 (88.0%)	41763 (88.7%)	
Parous	21809 (12.0%)	5304 (11.3%)	
Treatment type			<0.001
AI	13683 (7.0%)	985 (2.0%)	
With ovarian stimulation	6084 (44.5%)	831 (84.4%)	
IVF	182680 (93.0%)	47330 (98.0%)	
Embryos transferred			<0.001
1	68283 (37.4%)	1293 (2.7%)	
2	104740 (57.3%)	42492 (89.8%)	
3	9657 (5.3%)	3545 (7.5%)	
Embryo stage			<0.001
Cleavage stage	110475 (60.5%)	32960 (69.6%)	
Blastocyst stage	72205 (39.5%)	14370 (30.4%)	
Type of transfer			<0.001
Fresh ET	171254 (93.7%)	44730 (94.5%)	
Frozen ET	11426 (6.3%)	2600 (5.5%)	
Treatment year			<0.001
1999-2003	30068 (15.3%)	10072 (20.8%)	
2004-2007	31513 (16.0%)	10222 (21.2%)	
2008-2011	43589 (22.2%)	12807 (26.5%)	
2012-2015	52064 (26.5%)	10302 (21.3%)	
2016-2018	39129 (19.9%)	4912 (10.2%)	

Table 1A - Distribution of baseline characteristics of the study population according to single or multifetal pregnancies. AI, artificial insemination; IVF, in vitro fertilization; ET, embryo transfer.

	Singleton/Twin (n=243107)	Triplet (n=1571)	p-value
Oocyte source (age)			0.002
Donor (<36)	11229 (4.6%)	79 (5.0%)	
Autologous (18-34)	126571 (52.1%)	864 (55.0%)	
Autologous (35-37)	57948 (23.8%)	361 (23.0%)	
Autologous (38-39)	28417 (11.7%)	138 (8.8%)	
Autologous (40-42)	16864 (6.9%)	122 (7.8%)	
Autologous (>42)	2078 (0.9%)	7 (0.4%)	
Number of previous AI cycles			0.49
None	226069 (93.0%)	1454 (92.6%)	
1	3989 (1.6%)	26 (1.7%)	
2	3369 (1.4%)	18 (1.1%)	
≥3	9680 (4.0%)	73 (4.6%)	
Number of previous IVF cycles			<0.001
None	134383 (55.3%)	693 (44.1%)	
1	48720 (20.0%)	341 (21.7%)	
2	28031 (11.5%)	233 (14.8%)	
≥3	31973 (13.2%)	304 (19.4%)	
Number of previous livebirth deliveries after AI/IVF			0.34
Nulliparous	200119 (88.1%)	1347 (87.4%)	
Parous	26918 (11.9%)	195 (12.6%)	
Treatment type			0.82
AI	14576 (6.0%)	92 (5.9%)	
With ovarian stimulation	6831 (46.9%)	84 (91.3%)	<0.001
IVF	228531 (94.0%)	1479 (94.1%)	
Embryos transferred			<0.001
1	69555 (30.4%)	21 (1.4%)	
2	146333 (64.0%)	899 (60.8%)	
3	12643 (5.5%)	559 (37.8%)	
Embryo stage			<0.001
Cleavage stage	142414 (62.3%)	1021 (69.0%)	
Blastocyst stage	86117 (37.7%)	458 (31.0%)	
Type of transfer			0.37
Fresh ET	214587 (93.9%)	1397 (94.5%)	
Frozen ET	13944 (6.1%)	82 (5.5%)	
Treatment year			<0.001
1999-2003	39507 (16.3%)	633 (40.3%)	
2004-2007	41518 (17.1%)	217 (13.8%)	
2008-2011	56077 (23.1%)	319 (20.3%)	
2012-2015	62079 (25.5%)	287 (18.3%)	
2016-2018	43926 (18.1%)	115 (7.3%)	

Table 1B - Distribution of baseline characteristics of the study population according to non-triplet or triplet pregnancies. AI, artificial insemination; IVF, in vitro fertilization; ET, embryo transfer.

Multiple and triplet pregnancy rates

The trend in MP following ART has been evaluated according to ART treatment (AI versus IVF/ICSI, Fig.1). By using the Poisson regression analysis, we can observe that the incidence of multiple and triplet pregnancy has decreased following IVF/ICSI (incidence rate ratio (IRR) 0.976, 95%CI 0.962-0.989 and IRR 0.998, 95%CI 0.996-1.001, respectively), but not following AI (IRR 0.995, 95%CI 0.984-1.007 and IRR 0.988, 95%CI 0.954-1.024, respectively).

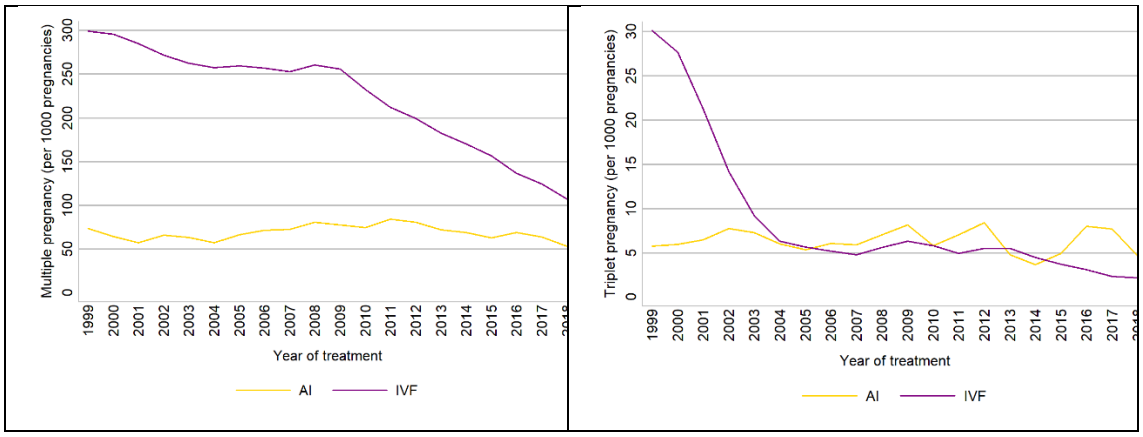

Figure 1 - Multiple and triplet pregnancy rates per 1000 pregnancies by AI or IVF treatment in the UK between 1999 and 2018.

Predictors of multiple and triplet pregnancies

In order to determine which are the main predictors of multiple and triplet pregnancy, we performed multivariable regression analysis for each infertility treatment (Tables 2A and 2B).

In AI cycles, multivariable logistic regression (adjusting for female age, parity following MAR, OS, year of treatment and number of previous AI cycles), revealed that having performed OS was associated with an increased risk of both multiples and triplets (aOR 7.002, 95%CI 5.853-8.378 and aOR 11.791, 95%CI 5.689-24.438, respectively). In IVF/ICSI cycles (adjusting for female age, parity following MAR, number of previous IVF cycles, year of treatment, number of embryos transferred, stage and type of embryo transfer), female age and transferring 2 (aOR 35.688, 95%CI 21.641-58.855, and aOR 31.861, 95%CI 29.89-33.962, respectively) or 3 embryos (aOR 405.346, 95%CI 239.452-686.171,

and aOR 44.234, 95%CI 40.826-47.925, respectively) were among the predictors of multiple and triplet pregnancies.

	Adjusted odds ratios (95% CI)	
	Multiple	**Triplet**
Oocyte source (age)		
Autologous (18-34)	Ref.	Ref.
Autologous (35-37)	1.149 (0.98-1.348)	1.209 (0.75-1.948)
Autologous (38-39)	**0.696 (0.543-0.892)**	0.579 (0.248-1.351)
Autologous (40-42)	**0.702 (0.511-0.964)**	0.328 (0.08-1.352)
Autologous (>42)	0.595 (0.214-1.65)	NE
Ovarian stimulation during AI		
No	Ref.	Ref.
Yes	**7.002 (5.853-8.378)**	**11.791 (5.689-24.438)**
Treatment year		
Per year	0.995 (0.984-1.007)	0.988 (0.954-1.024)
Number of previous AI cycles		
None	Ref.	Ref.
1	0.914 (0.75-1.113)	1.026 (0.555-1.896)
2	0.857 (0.689-1.066)	0.734 (0.351-1.534)
≥3	0.841 (0.697-1.014)	0.933 (0.52-1.672)
Number of previous livebirth deliveries after AI		
Nulliparous	Ref.	Ref.
Parous	1.006 (0.844-1.201)	1.088 (0.634-1.865)

Table 2A - Predictors of multiple and triplet pregnancy by AI. aORs were calculated with multivariable logistic regression. Statistically significant predictors are presented in bold. AI, artificial insemination; CI, confidence interval; NE, not estimable; Ref, reference.

	Adjusted odds ratios (95% CI)	
	Multiple	**Triplet**
Oocyte source (age)		
Donor (<36)	Ref.	Ref.
Autologous (18-34)	1.242 (0.977-1.579)	1.029 (0.977-1.083)
Autologous (35-37)	0.897 (0.696-1.155)	**0.772 (0.731-0.814)**
Autologous (38-39)	**0.575 (0.431-0.768)**	**0.547 (0.516-0.581)**
Autologous (40-42)	**0.317 (0.233-0.432)**	**0.359 (0.335-0.384)**
Autologous (>42)	**0.124 (0.057-0.273)**	**0.182 (0.154-0.216)**
Number of previous IVF cycles		
None	Ref.	Ref.
1	0.998 (0.867-1.149)	**0.906 (0.881-0.933)**
2	1.035 (0.881-1.216)	**0.897 (0.866-0.929)**
≥3	0.972 (0.83-1.139)	**0.908 (0.876-0.941)**
Number of previous livebirth deliveries after IVF		
Nulliparous	Ref.	Ref.
Parous	1.105 (0.929-1.314)	**1.127 (1.085-1.171)**
Treatment year		
Per year	**0.976 (0.962-0.989)**	0.998 (0.996-1.001)
Embryos transferred		
1	Ref.	Ref.
2	**35.688 (21.641-58.855)**	**31.861 (29.89-33.962)**
3	**405.346 (239.452-686.171)**	**44.234 (40.826-47.925)**
Embryo stage		
Cleavage stage	Ref.	Ref.
Blastocyst stage	**2.915 (2.53-3.359)**	**1.825 (1.773-1.879)**
Type of transfer		
Fresh ET	Ref.	Ref.
Frozen ET	0.876 (0.693-1.107)	**0.871 (0.831-0.914)**

Table 2B - Predictors of multiple and triplet pregnancy by IVF. aORs were calculated with multivariable logistic regression. Statistically significant predictors are presented in bold. IVF, in vitro fertilization; ET, embryo transfer; CI, confidence interval; NE, not estimable; Ref, reference.

Ovarian stimulation and the number of embryos transferred

As shown in Figure 2, while visually the proportion of patients submitted to AI with OS has remained nearly constant over time, the number of embryos transferred in IVF/ICSI cycles has significantly decreased over time in all age groups.

In terms of AI using OS, the rates per 1000 pregnancies seem to have had little variation over the study period, with a greater one present in autologous cycles over 42 years of age, who continue to have higher rates than the other age groups. Regarding

IVF/ICSI, the transfer of at least two embryos has been decreasing, with the greatest decrease occurring in the youngest age groups (namely autologous 18-34, donor <36 and autologous 35-37) and has been happening primarily since 2008. The decline in TET began earlier, right at the beginning of the millennium, reaching residual rates in the younger age groups from 2005 onwards. Patients aged >42 and between 38-39, however, seem to show a much slower rate of decrease.

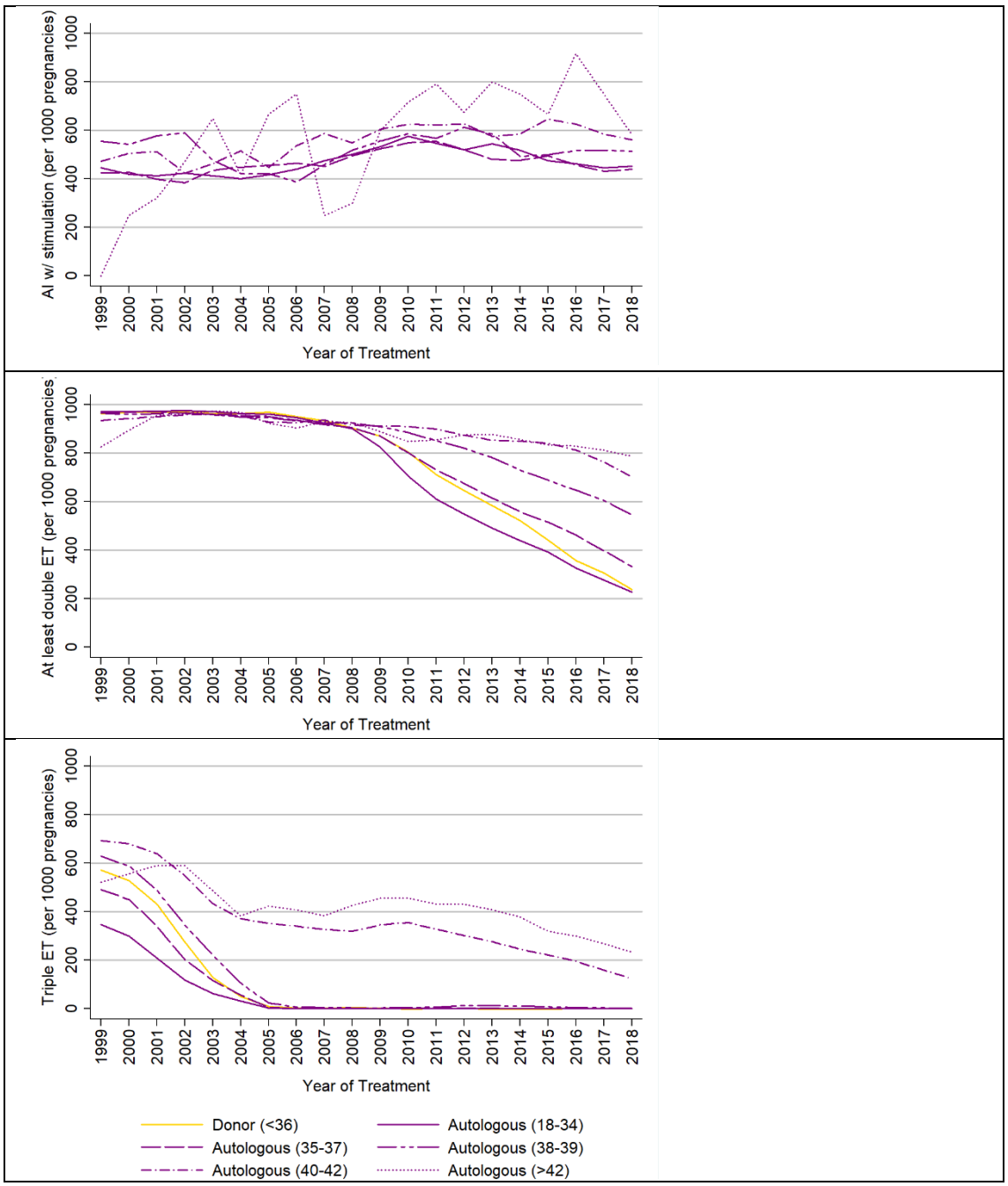

Figure 2 - AI with OS and embryo transfer rates per 1000 pregnancies by the age of oocyte source. AI, artificial insemination; ET, embryo transfer

Absolute frequency of multiple and triplet pregnancies

The influence of female age on the absolute frequency of MP over time is shown in Figure 3 and of triplets in Figure 4. The absolute frequency of MP and multiple births after ART seems to have decreased over the past few decades but has remained highest in the 18- to 34-year-old age range. For AI, the rates remained roughly constant, with only a few variations over the years, with women aged 18-34 and 35-37 having the highest rate of multiples. Among IVF/ICSI pregnancies that resulted in multiple births, there seems to be a preponderance of DET. TET resulting in MP/triplet pregnancy was more prevalent at the beginning of the 2000s, and its use has seemed to decrease substantially since then.

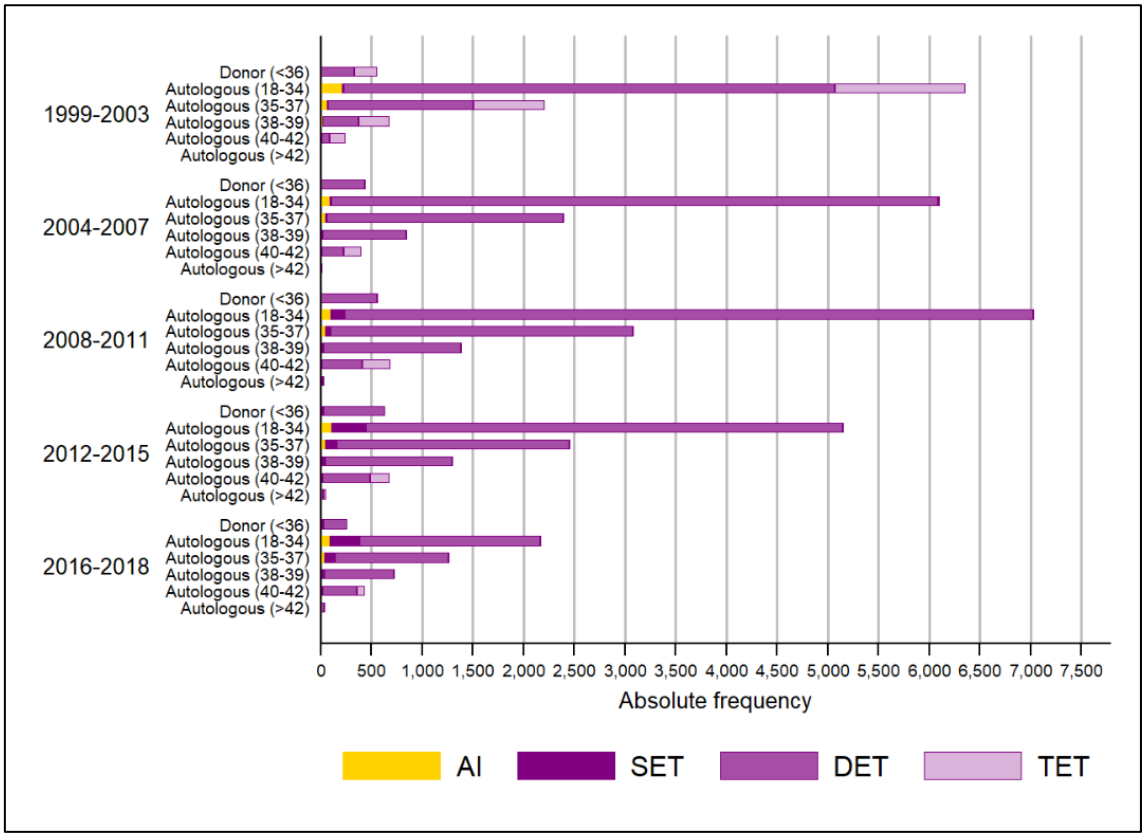

Figure 3 - Absolute frequency of MP over time by age of oocyte source. AI, artificial insemination; SET, single embryo transfer; DET, double embryo transfer; TET, triple embryo transfer.

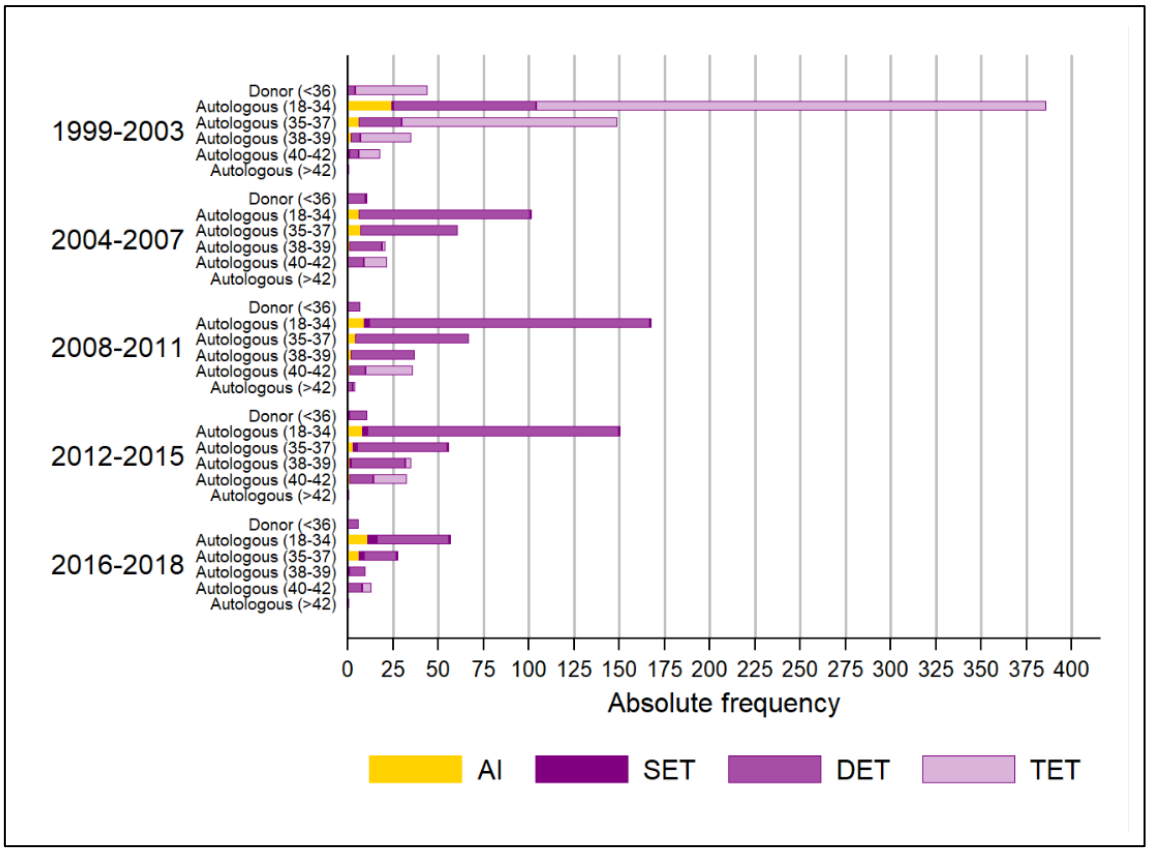

Figure 4 - Absolute frequency of triplets over time by age of oocyte source. AI, artificial insemination; SET, single embryo transfer; DET, double embryo transfer; TET, triple embryo transfer.

Sub-analysis of triplet pregnancies

The sub-analysis of triplets compared clinical outcomes in patients undergoing expectant management (EM), both twin (n=46.744) and triplet (n=1.382), MPR 3-to-2 (n=129) and MPR 3-to-1 (n=60). Baseline patient demographics are presented in Table 3, while further information regarding neonatal outcomes is detailed in Table 4.

The highest percentage of MPR occurred in the 18-34 age group. This approach was used more following IVF cycles than AI cycles (3-to-2 IVF 87.6% vs AI 12.4% and 3-to-1 IVF 95% vs AI 5.0%).

	Expectant management		Triple MPR		p-value
	Twin (n=46.744)	Triplet (n=1.382)	3-to-2 (n=129)	3-to-1 (n=60)	
Oocyte source (age)					
Donor (<36)	2394 (5.1%)	64 (4.6%)	9 (7.0%)	6 (10.0%)	<0.001
Autologous (18-34)	25947 (55.5%)	774 (56.0%)	63 (48.8%)	27 (45.0%)	
Autologous (35-37)	11068 (23.7%)	315 (22.8%)	31 (24.0%)	15 (25.0%)	
Autologous (38-39)	4837 (10.3%)	119 (8.6%)	13 (10.1%)	6 (10.0%)	
Autologous (40-42)	2331 (5.0%)	103 (7.5%)	13 (10.1%)	6 (10.0%)	
Autologous (>42)	167 (0.4%)	7 (0.5%)	0 (0.0%)	0 (0.0%)	
Number of previous AI cycles					
0	44707 (95.6%)	1289 (93.3%)	115 (89.1%)	50 (83.3%)	<0.001
1	388 (0.8%)	20 (1.4%)	5 (3.9%)	1 (1.7%)	
2	368 (0.8%)	15 (1.1%)	1 (0.8%)	2 (3.3%)	
≥3	1281 (2.7%)	58 (4.2%)	8 (6.2%)	7 (11.7%)	
Number of previous IVF cycles					
0	23431 (50.1%)	605 (43.8%)	58 (45.0%)	30 (50.0%)	<0.001
1	10046 (21.5%)	305 (22.1%)	19 (14.7%)	17 (28.3%)	
2	6108 (13.1%)	203 (14.7%)	26 (20.2%)	4 (6.7%)	
≥3	7159 (15.3%)	269 (19.5%)	26 (20.2%)	9 (15.0%)	
Number of previous livebirth deliveries after AI/IVF					
Nulliparous	40416 (88.8%)	1196 (88.2%)	107 (83.6%)	44 (75.9%)	0.004
Parous	5109 (11.2%)	160 (11.8%)	21 (16.4%)	14 (24.1%)	
Type of treatment					
AI	893 (1.9%)	73 (5.3%)	16 (12.4%)	3 (5.0%)	<0.001
With ovarian stimulation	747 (83.7%)	66 (90.4%)	16 (100.0%)	2 (66.7%)	
IVF	45851 (98.1%)	1309 (94.7%)	113 (87.6%)	57 (95.0%)	
SET	1272 (2.8%)	20 (1.5%)	1 (0.9%)	0 (0.0%)	<0.001
DET	41593 (90.7%)	803 (61.3%)	42 (37.2%)	54 (94.7%)	
TET	2986 (6.5%)	486 (37.1%)	70 (61.9%)	3 (5.3%)	
Treatment year					
1999-2003	9439 (20.2%)	553 (40.0%)	75 (58.1%)	5 (8.3%)	<0.001
2004-2007	10005 (21.4%)	181 (13.1%)	19 (14.7%)	17 (28.3%)	
2008-2011	12488 (26.7%)	278 (20.1%)	15 (11.6%)	26 (43.3%)	
2012-2015	10015 (21.4%)	263 (19.0%)	16 (12.4%)	8 (13.3%)	
2016-2018	4797 (10.3%)	107 (7.7%)	4 (3.1%)	4 (6.7%)	

Table 3 - Baseline characteristics of expectant management versus multifetal pregnancy reduction. AI, artificial insemination; IVF, in vitro fertilization; SET, single embryo transfer; DET, double embryo transfer; TET, triple embryo transfer. MPR, multifetal pregnancy reduction.

In terms of how MPR rates seem to have changed over time, peaking in the early 2000s and dropping ever since, as shown in Figure 5. In addition, over time, MPR 3-to-2 seemed to outperform 3-to-1 in terms of relative frequency, with the exception of the years 2008 to 2011 when 3-to-1 reached its peak incidence.

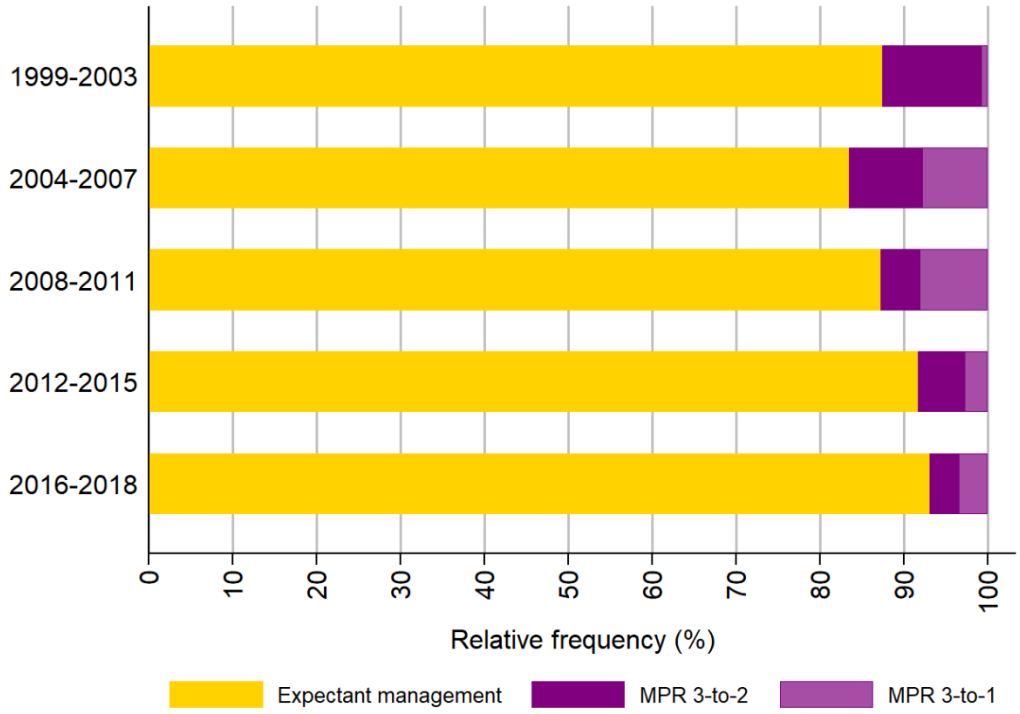

Figure 5 - Relative frequency of triple MPR and expectant management over time. MPR, multifetal pregnancy reduction.

Regarding neonatal outcomes, the categories of PTB <37 weeks and LBW <2500g differ between all approaches significantly, with higher numbers present in triplet EM (70.9% and 70.8%, respectively). Preterm delivery <30 weeks which had a triplet EM varied significantly from the other approaches, accounting for 13% of these births. The same seemed true for birth weight, where a triplet EM showed a higher rate of extremely LBW (11.6%), when compared to the other approaches. With regard to survival rates, twins and MPR 3-to-2 have a survival rate of all fetuses higher than the triplet EM (82.7% and 79.8% vs 63.2%, respectively). The comparisons of delivering at least two liveborns were not statistically significant. Moreover, a lower mortality rate of all remaining fetuses was observed in twins than in EM triplets (4.9% vs 7.9%, respectively). Conversely, patients undergoing 3-to-2 MPR had higher fetal non-survival rates when compared to twin pregnancy (13.2% vs 4.9%, respectively).

	Expectant management		Triplet MPR		p-value
	Twin (n=46744)	**Triplet (n=1382)**	**3-to-2 (n=129)**	**3-to-1 (n=60)**	
Preterm birth					
<37 weeks	20561 (44.0%)*	980 (70.9%)*	44 (34.1%)*	4 (6.7%)*	<0.001
<32 weeks	3750 (8.0%)*	322 (23.3%)*†	13 (10.1%)†‡	1 (1.7%)*‡	<0.001
<30 weeks	2158 (4.6%)*	180 (13.0%)*†‡	8 (6.2%)†	1 (1.7%)‡	<0.001
Birthweight					
<2500 g	26993 (57.7%)*	978 (70.8%)*	61 (47.3%)*	5 (8.3%)*	<0.001
<1500 g	4546 (9.7%)*	458 (33.1%)*†	17 (13.2%)†‡	1 (1.7%)*‡	<0.001
<1000 g	1557 (3.3%)*	161 (11.6%)*†‡	5 (3.9%)†	1 (1.7%)‡	<0.001
Survival					
All	38645 (82.7%)*	874 (63.2%)*†	103 (79.8%)†‡	56 (93.3%)*‡	<0.001
None	2308 (4.9%)*†	109 (7.9%)*	17 (13.2%)†	4 (6.7%)	<0.001
At least 2	38645 (82.7%)	1122 (81.2%)	103 (79.8%)	N.A.	0.252

Table 4 - Neonatal outcomes by expectant management versus multifetal pregnancy reduction. *†‡Pairwise comparisons P-value<0.05 for all figures with the same superscript.

Discussion

Our study sought to assess how MP rates, including twin and triplet pregnancies, have changed in the latest decades following MAR. The findings showed that, despite a decline in their incidence, multiple births are still a reality today and a, despite undesired, common side effect of fertility treatments. The use of OS in AI, and a high number of embryos transferred in IVF/ICSI appeared to be the main causes of these sustained rates, being especially relevant when the oocytes derive from younger women.

As previously stated, MP account for an increasing share of total pregnancies in the industrialized world as MAR procedures have evolved. However, after more than four decades of increases (Kulkarni et al., 2013; ACOG, 2021), our study provides evidence demonstrating that multiple and triplet pregnancies have started to see their numbers decline, especially the latter, accompanied by a concomitant rise in single pregnancies. This is consistent with earlier research that demonstrates that triplet and higher-order multiple births rates reached their peak at the turn of the millennium and have since been sharply declining in both the US and the majority of European countries (Ferraretti et al., 2017; Kulkarni et al., 2013). However, twin pregnancy rates (Wyns et al., 2020) have not had the same noticeable, albeit present, decrease. It is reasonable to assume that these variations are due to changes in the policies and practices of MAR techniques over the last few decades. In fact, according to the data we gathered, the decrease in the number of triple and higher-order pregnancies in IVF/ICSI cycles coincides with a decrease in the number of embryos transferred in these cycles across all age groups, owing to a greater dependence on SET and DET strategies. Transfers of at least two embryos have been declining, notably since 2008 and among younger age groups. In TET, the decline commenced years earlier, approaching nearly imperceptible rates in the lower age categories, while still displaying substantial levels in the oldest groups. This is consistent with expert advice recommendations that those receiving multiple embryos during a single transfer are more likely to have a poorer prognosis (HFEA, 2022). Furthermore, corroborating these data, DET was dominant in pregnancies resulting in multiple births, while SET played a minimal role in multiple births and even less so in triplets. TET, on the other hand, occurred in the vast majority of triplet pregnancies.

Nonetheless, the substantial increase in the usage of SET in recent years, particularly among younger age groups, is noteworthy. In fact, the HFEA intern report shows that in 2019, compared to just 13% in 1991, one embryo was transferred in 75% of IVF cycles. Further, policies limiting TET implemented in 2003, followed by encouragement to transfer one embryo and cryopreservation of any surplus embryos, resulted in fewer DET and TET and, as a result, fewer multiple births (HFEA, 2021). Supporting the results mentioned above, the literature describes that, although DET remains the most commonly used approach (Ferraretti et al., 2017; Wyns et al., 2020), there has been a marked increase in the use of eSET, especially in the last ten years, being even the preponderant choice in some countries (Wyns et al., 2020). The practice of transferring three embryos has been supplanted by SET (Ferraretti et al., 2017), and it is now become uncommon and still practiced in only a few countries (Wyns et al., 2020). As a result of this increased awareness and change in practices, the percentage of MP conceived through ART has decreased (Centers for Disease Control and Prevention, 2022; Kulkarni et al., 2013), although room for improvement still exists. As a matter of fact, considering the absolute frequency of MP over time (as shown in Figure 3), eliminating DET in women below the age of 38 would potentially have a profound impact on MP and triplet cases.

Since the effectiveness of these techniques is crucial, a potential deterrent to SET is the worry that it will decrease the likelihood of pregnancy, which has a clinical impact on couples' decisions. Regarding the efficacy of SET, a 2013 meta-analysis found that while SET is linked with a lower live birth rate than DET, the cumulative live birth rate after one cycle of DET did not vary significantly from the rate after two cycles of SET. However, these findings are more common in younger individuals with a good prognosis, whereas they are more contentious and have less significance in older reproductive ages (Fujimoto et al., 2015; Pandian et al., 2013), so further clarification on this topic is important. In light of the institutions' policies encouraging multiple birth reduction techniques, these SET recommendations reflect the significant increase in younger women that we have observed.

As a result of this study, we may conclude that transferring two or more embryos seems to be a major predisposing factor for MP. Moreover, we also found other significant predictors in IVF, such as blastocyst and frozen embryos transfers. Embryo

transfers after ART are commonly performed at either the cleavage (which occurs on days 2-3 after fertilization) or at the blastocyst stage (days 5-6). Transfers at the blastocyst stage have become more common in the UK, to the detriment of cleavage transfers (HFEA, 2022; Papanikolaou et al., 2008), resulting in a higher implantation potential (Dirican et al., 2022; Martins et al., 2017; Papanikolaou et al., 2008). That being said, blastocyst stage transfer has drawbacks that make its superiority debatable, namely since many embryos do not reach this stage in vitro, resulting in more cancelled cycles (American Society for Reproductive Medicine, 2018) and fewer embryos cryopreserved (Martins et al., 2017; Papanikolaou et al., 2008). Nonetheless, if combined with SET, blastocyst transfer may be used as a preventive measure for MP because it allows for the transfer of fewer, but potentially more competent, embryos. In fact, the Society for Assisted Reproductive Technology advises that eSET be used consistently to reduce multiple gestation due to blastocyst's high implantation rate (American Society for Reproductive Medicine, 2018).

On the other hand, frozen embryo transfer revealed lower triplet pregnancy rates when compared to fresh embryos in our study, but no significant difference in non-triplet pregnancies. The HFEA report indicate that frozen embryo transfers are rising and that their success, which historically were lower than fresh embryo transfers, have improved significantly due to the advances in embryo cryopreservation technologies, namely with the introduction of vitrification. However, one must account for the fact that these embryos are normally cryopreserved as a "second choice", since the best quality embryos are typically selected for replacement in a preceding fresh transfer. In this way, this may act as a bias, giving the misleading impression that triple pregnancies are less likely. The literature on this topic is very sparse and there are few data on the risk of MP in frozen embryo transfers. Those that do exist reveal uncertainty about whether the freezing strategy reduces these MP rates (Wong et al., 2017), including the risk of monozygotic twinning (Busnelli et al., 2019).

As far as the number of previous IVF cycles is concerned, the rates of MP after them did not vary significantly from those without any prior cycles, but the rates of triplets did. Embryo quality is usually the limiting factor for patients with reiterated unsuccessful IVF cycles. In fact, the number of previous IVF cycles performed has a significant impact on the implantation rate, regardless of the woman's age or the use of fresh or frozen

embryos (Y. Wang et al., 2021). Thus, the poorer prognosis of these patients, as well as the likelihood of recurrent failure in subsequent IVF cycles, may explain the apparent lower risk in triple pregnancies.

Despite the decrease in the incidence of MP and triplets after IVF/ICSI, this did not occur after AI. In fact, triplet pregnancies following AI did not seem to vary over the years, and the women undergoing this procedure who showed a higher rate of multiples belonged to the lowest age groups. Such a result may lead one to postulate that, since the only modifiable predictor associated with multiple and triplet pregnancies in AI is OS, its use continues to play a role in maintaining these rates, especially in younger women. OS was used in nearly half of all AI cycles included in this study, with a greater contribution in MP and even more in triplets. In fact, there is evidence that the use of OS has a considerable impact on more than half of multiple births and even on higher-order ones (Kulkarni et al., 2013). However, unlike IVF strategies, it is more complex to restrict multiple births resulting from OS, not only because of the dynamics and follicular growth itself (Chaabane et al., 2015; Kulkarni et al., 2013), the heterogeneity of the population undergoing these MAR procedures, which acts as a confounding factor, but also because of the lack of reliable predictors or threshold parameters (Chaabane et al., 2015; Practice Committee of American Society for Reproductive Medicine, 2012). Thus, the tactics employed in OS to decrease higher-order MP involve trying to use the lowest feasible doses of OS with the goal of inducing the growth of only one follicle (Practice Committee of American Society for Reproductive Medicine, 2012). When several mature follicles develop, the alternative options to cancel the treatment cycle are to convert to IVF, aspirate supernumerary follicles or perform MPR as a secondary preventive measure (Dickey, 2009; Evans et al., 2020). So, given the need to reduce the incidence of MP rates resulting not only from IVF but also from AI, it is critical to raise extra awareness and conduct additional research on this topic to reach a consensus.

In terms of MPR, it is a key secondary prevention technique in reducing the adverse events associated with higher-order pregnancies. It is, after all, the most successful and effective intervention in this area (Stone & Kohari, 2015). It is worth noting that our sample size for this MPR procedure is considerably larger or at least comparable to previous studies that have addressed this topic (Boulot et al., 2000; Razaz et al., 2017; Zemet et al., 2020). Previous literature regarding this topic, however, has been rather

controversial. When compared to expected management, the incidence of PTB and LBW was significantly lower in both the MPR groups, which is in line with previous data (Gupta et al., 2015; Wimalasundera, 2010). The reduction in preterm birth was the most significant advantage in the outcomes investigated compared to EM. When looking at the two most severe cut-offs of both of these outcome categories (PTB and LBW), it is observed that there is a higher percentage of births in the presence of an EM. This demonstrates that the pattern appears to be similar in the worst neonatal scenarios, in which MPR may have a protective role. However, there are other critical aspects to consider. When there were significant differences between the two approaches of MPR (namely the first two cut-offs of PTB and LBW), MPR 3-to-1 seems to include a smaller proportion of newborns in these categories, as well as improved fetal survival, although the sample size was smaller. When compared to reductions to singletons, previous studies report that 3-to-2 reductions have a lower fetal survival rate, lower gestational age at birth, higher risk of preterm birth, and lower birth weight (Hessami et al., 2022; Zemet et al., 2020), so they postulated that 3-to-1 reductions should be considered in situations where the risk of prematurity is quite high. However, we must not forget that ethical issues aside, this may come at the cost of fewer healthy newborns per couple. Moreover, MPR 3-to-2 reductions occurred more commonly than 3-to-1 reductions. One causal factor may be that many infertile couples continue to prefer multiple gestation after several years of fertility treatments as their outcome (Barishansky et al., 2022), despite the known comparative risks of multiple vs singleton pregnancies, as well as the inherent ethical issues and disagreement in outcomes (Wimalasundera, 2010). In terms of fetal survival of all fetuses, triplet EM had the lowest percentage compared to twin EM and both MPR groups. However, when we look at the mortality figures of all fetuses, MPR 3-to-2 was associated with more women losing their entire pregnancy when compared to twins without reduction. Thus, it is crucial to note that having performed a reduction did not reduce the risk that had already been observed in the triplets. Interestingly, this evidence is not in line with that of previous reports that revealed no substantial increase in miscarriage risk and perinatal mortality (Jin et al., 2020; Razaz et al., 2017; Wimalasundera, 2010) following MPR. Therefore, with MPR 3-to-2 being the most common technique, the data obtained may reinforce the importance of primary prevention, rather than simply attempting to prevent the adverse outcomes of triplet

pregnancies downstream. In addition to these facts, we must also consider the ethical ramifications of ending the lives of otherwise healthy fetuses in order to enhance the potential of the remaining. The emotional impact of this approach on women and/or couples should not be underestimated, especially at a time when they are in a volatile psychological state. In fact, after dealing with infertility for several years, the emphasis is often on getting pregnant rather than avoiding a MP (Collopy, 2004). Those who actually choose MPR may experience guilt and sadness after the procedure without regretting their decision (Collopy, 2004), as opposed to accepting a reduction and then suffering a total pregnancy loss (Obič An et al., 2015). So, although health professionals should rely on statistics regarding maternal and neonatal outcomes, considering the mothers' future physical and mental health is also important.

Strengths and limitations

The major strength of the current study is that it provides insight into the trends of MP rates following MAR techniques over a period of 18 years in the UK and investigates how MP predictors have evolved over time and their impact on the overall incidence of MAR-related MP. It also explored the trends and clinical impact of MPR in triplet pregnancies, as well as the neonatal outcomes of these pregnancies. In addition, we relied on one of the world's largest fertility data registries (the Human Fertilisation and Embryology Authority registry). To overcome potential limitations, we also performed a robust analysis that adjusted for potential confounders. With over 240.000 cycles analysed, we believe this study has significant robustness to provide valuable data on MP rates and their evolution over the last years, as well as to have an impact on clinical counselling for MPR.

However, we must also acknowledge the limitations of our research. First, this study has the limitation of being retrospective, which resulted in a limited control for confounding variables and data may be missing, erroneous or miscategorized. In this regard, it is worth noting that prior to 2006 the data were covered by the Historic Audit Project, which followed up on outcomes of these cycles, and that double-entry and multiple rule validation has been applied to any data related to dates after March 2002, ensuring the highest level of data integrity and accuracy. Second, since new regulations went into effect in October 2009, patients have had the option to refuse the disclosure

of their personal information or/and that of prospective children born as a result of reproductive treatments shared for research purposes. This implies that the data no longer represents the entire UK population undergoing these MAR techniques. Whilst important to mention, this limitation is unlikely to have a non-at-random impact on the results obtained. Moreover, because the data is anonymous, the same patient may have underwent multiple transfer cycles, leaving us unable to link the treatment cycle to the individual patient and, consequently, to adjust for this clustering or to calculate the cumulative outcomes per patient. For the same reason, data regarding clinic-specific protocols were unavailable.

Conclusions

There is no doubt that MP related to MAR techniques entails significant negative consequences in both maternal and neonatal outcomes and, therefore, preventive efforts have been made to decrease these iatrogenic rates. Despite the reduction achieved in MP incidence, they still exist, owing primarily to the widespread use of OS and multiple embryo transfers. Thus, future efforts remain essential and should emphasize the prudent use of OS in AI cycles and the widespread adoption of an eSET policy.

These findings may also improve couples' counselling regarding the management of triplet pregnancies, which are still an, albeit decreasing, reality in our daily practice. Thus, when MPR is recommended in triplets, counselling should be provided regarding the lower risk of PTB and LBW. However, one must not lose sight of the importance of acting upstream in the primary prevention of MP, so this approach should not be viewed as a fertility treatment safety net.

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