OXFORD

human reproduction

Human Reproduction, 2025, **00(0)**, 1–5
https://doi.org/10.1093/humrep/deaf007
Peer Perspectives

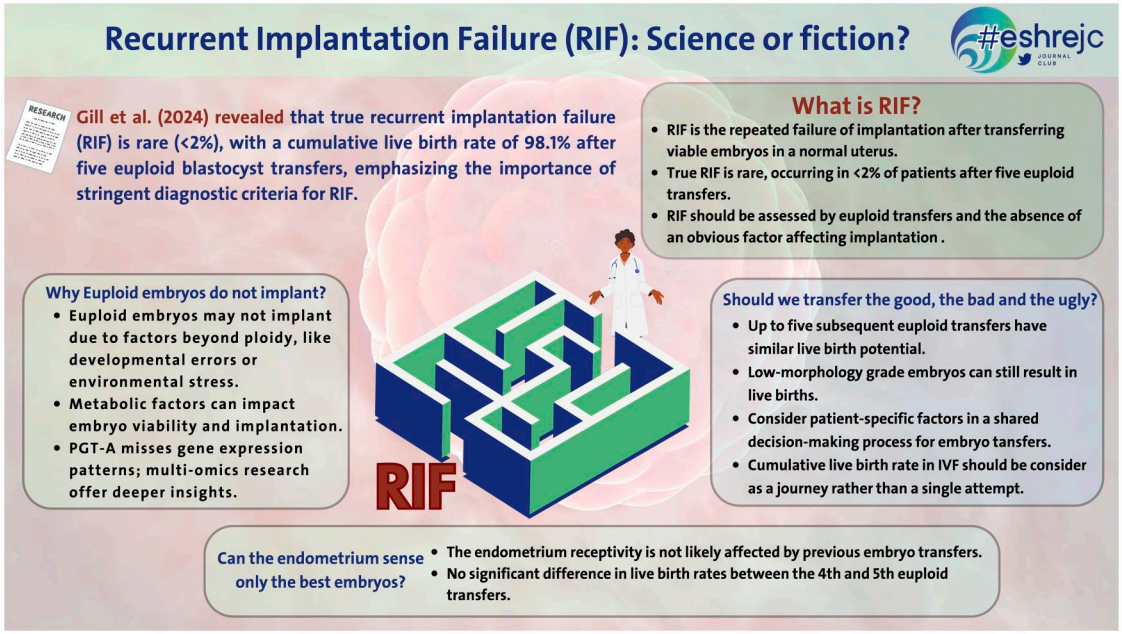
Recurrent implantation failure: science or fiction?

Juan J. Fraire-Zamora [1,*], George Liperis [2,3], Munevver Serdarogullari [4], Omar F. Ammar [5,6], Paul Pirtea [7], Paola Viganò [8], Laurentiu Craciunas [9], Micah J. Hill[10], and Kashish Sharma [11]

[1]EUGIN Group, R&D, Barcelona, Spain
[2]Westmead Fertility Centre, Institute of Reproductive Medicine, University of Sydney, Westmead, NSW, Australia
[3]Embryorigin Fertility Centre, Larnaca, Cyprus
[4]Department of Histology and Embryology, Faculty of Medicine, Cyprus International University, Northern Cyprus, Turkey
[5]Louise, Bordeaux, France
[6]Department of Obstetrics and Gynaecology, College of Medicine, University of Anbar, Ramadi, Iraq
[7]Department of Obstetrics and Gynecology and Reproductive Medicine, Hopital Foch–Faculté de Médecine Paris, Suresnes, France
[8]ART Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[9]Newcastle Fertility Centre, Newcastle upon Tyne, UK
[10]Shady Grove Fertility, Rockville, MD, USA
[11]HealthPlus Fertility Center, HealthPlus Network of Specialty Centers, Abu Dhabi, United Arab Emirates

*Correspondence address. Eugin Group, R&D, C/Balmes 236, 08006, Barcelona, Spain. E-mail: jfraire@eugin.es https://orcid.org/0000-0003-2870-0140

Graphical Abstract



The September 2024 ESHRE Journal Club discussion focused on a study by Gill *et al.* (2024) on the available evidence to define RIF, the embryonic factors that can lead to the implantation failure of euploid blastocyst, the (im)possibility of the endometrium playing a role in RIF, and the implications for patients of transferring all euploid blastocysts from a stimulation cycle. PGT-A, preimplantation genetic testing for aneuploidy; RIF, recurrent implantation failure.

Keywords: recurrent implantation failure / euploid transfers / embryo implantation potential / uterine factors / cumulative live birth rate

Introduction

Recurrent implantation failure (RIF) is a situation in which *in vitro* fertilized embryos from an IVF patient repeatedly fail to implant after at least three transfer cycles. However, it has been difficult to define RIF within a biological framework since implantation failure has been associated with many different variables such as the morphological grading of the embryo, the total number of embryos transferred, the stage of embryo development, several

Received: December 17, 2024. **Editorial decision:** January 8, 2025.

uterine factors, maternal age, hydrosalpinges, immunological factors and thrombophilic conditions, among others (Coughlan et al., 2014). A large effort has been made to recommend good practices for RIF (ESHRE Working Group on Recurrent Implantation Failure, 2023) and standardize its definition ((The Writing Group) for the Participants to the 2022 Lugano RIF Workshop, 2023) with an emphasis on embryo aneuploidy as the most common reason for implantation failure when other known factors (e.g. hydrosalpinges or uterine factors) are absent. A landmark study for RIF reported that the three consecutive transfers of single euploid blastocysts had a cumulative probability of implantation of 95.2%, leaving the incidence of RIF as <5% in IVF patients (Pirtea et al., 2021). In a recent study by Gill et al. (2024), published in *Human Reproduction*, the authors investigated the clinical pregnancy and live birth rates after the fourth and fifth euploid blastocyst transfers in women who have suffered the failure of three previous euploid transfers in the absence of a known factor that affects implantation. Gill et al. analysed a cohort of 123 987 IVF patients with an exhaustive list of exclusion criteria. From this cohort, only 105 patients met the inclusion criteria and had at least one more euploid blastocyst transferred after three failed euploid blastocyst transfers. These data point to an extremely small patient population that can be defined as true RIF (0.085%; 105 out of 123 987 patients) and showed that both clinical pregnancies and live births in the fourth euploid blastocyst transfers did not differ significantly from the outcomes in the fifth euploid blastocyst transfers, supporting the idea that the prevalence of RIF could be <5% as indicated by Pirtea et al. (2021). Moreover, Gill et al. (2024) showed that the overall cumulative live birth rate (CLBR) with five euploid blastocysts transferred was 98.1% (95% CI: 96.5–99.6%) in the absence of an obvious factor affecting implantation, placing the prevalence of true unexplained RIF as low as <2%. The study by Gill et al. (2024) has strong implications in the actual diagnosis of RIF and highlights that the embryo is the main determinant for implantation success and live birth. For these reasons, the September 2024 ESHRE Journal Club discussion focused on the available evidence to define RIF, the embryonic factors that can lead to the implantation failure of euploid blastocysts, the (im)possibility of the endometrium playing a role in RIF, and the implications for patients of transferring all euploid blastocysts from a stimulation cycle.

What is RIF anyway?

Given the historical difficulties of defining RIF in our field, the first task in analysing its true incidence is to ask what the clinical profile of RIF patients would look like. Recently, several experts have proposed clinical approaches to diagnose the actual RIF aetiology and developed statistical models to try to understand its occurrence (Smith et al., 2015; Ata et al., 2021; Rozen et al., 2021; ESHRE Working Group on Recurrent Implantation Failure, 2023; (The Writing Group) for the Participants to the 2022 Lugano RIF Workshop, 2023). This discussion has led to define RIF as the lack of implantation after the transfer of embryos considered to be viable, or of 'good' morphology grade, in a uterus that is morphologically normal and implying that a standardized fertility workup has already been completed for the female patient. Importantly, patients with obvious non-treatable uterine pathologies should be considered separately from possible RIF patients seeking treatment. Thus, when performing clinical ART treatments, the remaining patient population would consist of two groups: (i) those patients with a realistic probability to conceive and deliver when a viable embryo is transferred and (ii) those with unidentifiable factors that contribute to a biological resistance to embryo implantation and, therefore, unlikely to achieve a successful pregnancy. Estimating the true size of RIF prevalence could then be assessed by a decline in the rate of implantation over successive embryo transfers. This is because, conceptually, patients with true RIF would be expected to ever achieve implantation and will remain in the RIF group after multiple transfers. Under these premises, it was already shown that, when excluding uterine pathologies and transferring up to three consecutive euploid embryos, RIF is a rare condition likely occurring in <5% of infertile couples (Pirtea et al., 2021). Furthermore, Gill et al. (2024) extended the assessment to five euploid embryo transfers searching for a possible decline in the rate of implantation. Nevertheless, the authors confirmed that RIF is a rare condition, reducing its occurrence to <2% of patients without uterine pathologies and undergoing up to five euploid embryo transfers. These findings not only suggest that RIF is likely an epiphenomenon of IVF rather than an actual aetiology of infertility, but they also emphasize that clinical investigation and guidance by professional criteria are necessary to avoid overdiagnosis, unnecessary testing, or the use of therapies without evidence of benefit.

Gill et al. defined their unit of analysis as the euploid blastocyst transferred and assessed the occurrence of RIF through the CLBR in the RIF cohort after five euploid embryo transfers. This is an important point to note, since not all cycles will result in more than five blastocysts available for transfer and implies that not all the embryos transferred necessarily originate from a single ovarian stimulation cohort, especially in women of advanced maternal age (>35 years old) and diminished ovarian reserve. Interestingly, it has been suggested that the chances of obtaining euploid blastocysts are not affected by a prior ART cycle with only aneuploid embryos (Herlihy et al., 2022). Thus, the definition of RIF should not be considered based on a single cohort of embryos but rather on the number of euploid blastocysts transferred or the number of untested embryo transfers adjusted to patient's age.

Why do not all euploid embryos implant?

The data from both Pirtea et al. (2021) and Gill et al. (2024) showed that CLBR with up to five euploid blastocyst transfers can reach 98.1%. Interestingly, these studies also showed a CLBR of ~65% after one euploid transfer, indicating that not all euploid embryos will result in a live birth and that embryo quality goes beyond ploidy status. Embryonic factors influencing reduced implantation and subsequent live birth outcomes are diverse, encompassing both intrinsic and extrinsic elements. Abnormal cleavage patterns, delayed morula formation, prolonged blastulation times, poor morphological grades, spontaneous blastocyst collapse, and developmental delays are all associated with lower implantation rates and reduced pregnancy success (Cimadomo et al., 2023a). Besides a genetic origin, these embryonic phenotypes could arise from environmental or metabolic disturbances. For instance, preimplantation embryos are particularly sensitive to various environmental stressors, including temperature fluctuations, CO_2 levels, exposure to light, and aerosols, all of which can disrupt critical developmental processes and impact implantation success (Ramos-Ibeas et al., 2019). These environmental stressors could trigger the unfolded protein response and endoplasmic reticulum stress pathways, resulting in cellular damage during *in vitro* development of preimplantation embryos (Basar et al., 2014; Sahin et al., 2023). Additionally, metabolic factors, such as amino acid flux patterns, have been shown to predict

blastocyst viability, the likelihood of successful implantation, and chances of live birth (Lee and Rinaudo, 2024).

Furthermore, many embryonic abnormalities that affect implantation and developmental potential cannot be detected through preimplantation genetic testing for aneuploidy (PGT-A), as this approach only assesses chromosomal abnormalities. There are over 20 000 protein-coding genes in the human genome, and mutations in these genes, which are often undetected by PGT-A, can influence embryo quality and implantation potential. For instance, specific mutations in genes encoding the subcortical maternal complex, which is essential for embryo development, have been associated with human infertility and impaired offspring health (Bebbere *et al.*, 2021; Tannorella *et al.*, 2022; Sahin *et al.*, 2023). In general, preimplantation embryos undergo dynamic changes in gene expression, with each developmental stage being defined by a unique pattern of genes expressed and for which improper expression can lead to the disruption of critical developmental processes affecting embryo viability (Paonessa *et al.*, 2021).

The advent of single-cell omics and multi-omics technologies has allowed for a more comprehensive understanding of early embryo development, providing access to genetic, epigenetic, and transcriptomic profiles. These techniques, including single-cell RNA sequencing, as well as innovations in RNA modifications, translation, and proteomics, promise to revolutionize the study of blastocyst cell fate decisions, offering new avenues for improving implantation success and pregnancy outcomes (Paonessa *et al.*, 2021; Ju *et al.*, 2023). Although these techniques warrant further investigation for their utility in embryo selection, they can provide a more nuanced understanding of the intricate factors governing embryo development and implantation potential and a promise to refine ART beyond the ploidy status of the embryo.

Can the endometrium sense only the best embryos?

The endometrium has been proposed as a biosensor for embryo quality during its receptive window (Macklon and Brosens, 2014). In this model, the decidualized endometrium is thought to allow for the implantation of embryos that produce a robust amount of beta hCG to ensure continuous ovarian progesterone production during early pregnancy and, consequently, select against embryos with an insufficient hCG production as they are perceived to lack fitness for a sustained pregnancy. If this is the case, and hCG might play a role in endometrial receptivity, each embryo transfer might influence endometrial receptivity beyond the length of the menstrual cycle through hCG administration, affecting also endometrial receptivity in subsequent transfers. Interestingly, a systematic review concluded that there is no substantive difference in clinical pregnancy in women undergoing blastocyst transfers with or without intracavity administration of hCG, and no evidence was found that miscarriage is reduced following the same treatment (Craciunas *et al.*, 2018). Therefore, it is not likely that prior embryo transfers could disturb the intrinsic receptivity of the endometrium in the long term. Although Gill *et al.* (2024) reported a live birth rate of 53% in the fifth euploid transfer compared to 40% in the fourth transfer after three euploid failures, this increase was not statistically significant. It is noteworthy that there were only 105 such euploid transfers in the dataset (out of 123 987 patients), suggesting that the precision of this estimated success might be low. However, given the data by Gill *et al.* (2024), and considering there is no apparent increase in the probability of live birth with consecutive euploid transfers (presumptively of diverse morphology grades), we can only conclude that each euploid transfer has a similar chance of live birth when obvious uterine anomalies have been excluded.

Should we transfer the good, the bad, and the ugly?

Blastocyst morphology has been previously associated with pregnancy outcomes in genetically untested embryo transfers (Ahlström *et al.*, 2011; Hill *et al.*, 2013). Other observational studies have demonstrated an association with pregnancy outcomes and morphology grade in euploid blastocysts, ranging from 13 to 35% of ongoing pregnancies for poor-morphology grade embryos to 52–76% for top-morphology grade ones (Irani *et al.*, 2017; Nazem *et al.*, 2019). Gill *et al.* (2024) demonstrated that, after three failed euploid transfers, the subsequent fourth and fifth euploid transfers had live birth rates of 40% and 53%, respectively. Most clinics choose the euploid embryo transfer order based on the morphology grade of the embryos and the day of blastulation. If this is the case, most of the fourth and fifth euploid transfers from a single stimulation embryo cohort would represent lower-grade blastocysts. In this regard, an earlier study found that the implantation potential of euploid blastocysts was similar despite different morphology grades and developmental rates in 956 blastocysts (Capalbo *et al.*, 2014), while a more recent study showed that, in 1966 blastocysts, slow-developing or low morphology grade embryos can still result in live births (Cimadomo *et al.*, 2022). This would suggest that, after three failed euploid transfers, subsequent euploid transfers do have a good chance of a successful live birth outcome.

It is important to note that clinical IVF practice varies globally, with some clinics performing trophectoderm biopsy and vitrification only on low-grade blastocysts versus other clinics only performing these procedures on blastocysts with a good morphology grade, more likely resulting in better per-transfer outcomes. Therefore, the term low-grade euploid blastocyst may not apply to all clinical scenarios and could have varying meanings across clinics and countries.

RIF can be an ambiguous term affecting patient's well-being. Nevertheless, the results by Pirtea *et al.* (2021), Gill *et al.* (2024), and the evidence discussed above indicate that we can assure patients that after failed attempts of euploid transfers, each subsequent euploid transfer still has a good chance of implantation. In this sense, it is a coherent clinical approach to transfer all embryos from a patient's cohort before embarking on another cycle of ovarian stimulation or using untested IVF add-ons with their associated risks. However, it is important to also engage patients and clinicians in a shared decision-making process to subsequently transfer all remaining embryos, including lower-grade euploid blastocysts (Cimadomo *et al.*, 2023b). Factors to consider in an informed decision include the patient's age, desired family size, grade of the remaining embryos, insurance or state mandates, and the cost of each option. As in all clinical decision making, one size does not fit all scenarios, and reproductive medicine professionals must keep in mind that the CLBR should be used to define IVF as a journey rather than a single attempt (Rienzi *et al.*, 2021).

Conclusion

For many years, RIF has been a puzzling condition in our field resulting in the development of add-on treatments offered to

patients without sufficient evidence of their efficacy. The article by Gill et al. (2024) steps up on previous work by Pirtea et al. (2021) to strongly indicate that RIF might mostly be a statistical misinterpretation and that, after excluding any obvious uterine anomalies and performing only euploid transfers, the prevalence of true unexplained RIF is <2%. This is a good example of how knowledge builds on previous work and evidence-based definitions to improve clinical practice and avoid the use of untested IVF add-ons. Moreover, the discussion of this article reopens the debate on how to manage ART treatments based on patients' characteristics and that one size does not fit all. Undoubtedly, new research and technologies will help to select euploid embryos with the highest implantation and live birth potential, making the IVF journey more efficient for patients.

Data availability

No datasets were generated or analysed in the current manuscript.

Acknowledgements

The authors thank the Editor-in-Chief of Human Reproduction, Prof. Cornelis (Nils) Lambalk, and all the participants of the ESHRE journal club.

Authors' roles

J.J.F-Z., G.L., M.S., O.F.A., and K.S., conceptualized and moderated the discussion; J.J.F-Z. organized and led the discussion; P.P., P.V, L.C., and M.J.H contributed to the discussion as experts; O.F.A. prepared the graphical abstract; all authors provided outlines for the manuscript; and J.J.F-Z drafted the manuscript with the help of K.S. All authors provided critical revision to the manuscript/graphical abstract/and approved the final version.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors have no conflicts of interest to declare.

References

Ahlström A, Westin C, Reismer E, Wikland M, Hardarson T. Trophectoderm morphology: an important parameter for predicting live birth after single blastocyst transfer. *Hum Reprod* 2011;**26**:3289–3296.

Ata B, Kalafat E, Somigliana E. A new definition of recurrent implantation failure on the basis of anticipated blastocyst aneuploidy rates across female age. *Fertil Steril* 2021;**116**:1320–1327.

Basar M, Bozkurt I, Guzeloglu-Kayisli O, Sozen B, Tekmen I, Schatz F, Arici A, Lockwood CJ, Kayisli UA. Unfolded protein response prevents blastocyst formation during preimplantation embryo development in vitro. *Fertil Steril* 2014;**102**:1777–1784.

Bebbere D, Albertini DF, Coticchio G, Borini A, Ledda S. The subcortical maternal complex: emerging roles and novel perspectives. *Mol Hum Reprod* 2021;**27**:gaab043.

Capalbo A, Rienzi L, Cimadomo D, Maggiulli R, Elliott T, Wright G, Nagy ZP, Ubaldi FM. Correlation between standard blastocyst morphology, euploidy and implantation: an observational study in two centers involving 956 screened blastocysts. *Hum Reprod* 2014;**29**:1173–1181.

Cimadomo D, Forman EJ, Morbeck DE, Liperis G, Miller K, Zaninovic N, Sturmey R, Rienzi L. Day7 and low-quality blastocysts: opt in or opt out? A dilemma with important clinical implications. *Fertil Steril* 2023b;**120**:1151–1159.

Cimadomo D, Rienzi L, Conforti A, Forman E, Canosa S, Innocenti F, Poli M, Hynes J, Gemmell L, Vaiarelli A *et al.* Opening the black box: why do euploid blastocysts fail to implant? A systematic review and meta-analysis. *Hum Reprod Update* 2023a;**29**:570–633.

Cimadomo D, Soscia D, Casciani V, Innocenti F, Trio S, Chiappetta V, Albricci L, Maggiulli R, Erlich I, Ben-Meir A *et al.* How slow is too slow? A comprehensive portrait of Day 7 blastocysts and their clinical value standardized through artificial intelligence. *Hum Reprod* 2022;**37**:1134–1147.

Coughlan C, Ledger W, Wang Q, Liu F, Demirol A, Gurgan T, Cutting R, Ong K, Sallam H, Li TC. Recurrent implantation failure: definition and management. *Reprod Biomed Online* 2014;**28**:14–38.

Craciunas L, Tsampras N, Raine-Fenning N, Coomarasamy A. Intrauterine administration of human chorionic gonadotropin (hCG) for subfertile women undergoing assisted reproduction. *Cochrane Database Syst Rev* 2018;**10**:CD011537.

ESHRE Working Group on Recurrent Implantation Failure; Cimadomo D, de Los Santos MJ, Griesinger G, Lainas G, Le Clef N, McLernon DJ, Montjean D, Toth B, Vermeulen N, Macklon N. ESHRE good practice recommendations on recurrent implantation failure. *Hum Reprod Open* 2023;**2023**:hoad023.

Gill P, Ata B, Arnanz A, Cimadomo D, Vaiarelli A, Fatemi HM, Ubaldi FM, Garcia-Velasco JA, Seli E. Does recurrent implantation failure exist? Prevalence and outcomes of five consecutive euploid blastocyst transfers in 123 987 patients. *Hum Reprod* 2024;**39**:974–980.

Herlihy NS, Klimczak AM, Cheung JKW, Seli E, Scott RT Jr. The chances of obtaining a euploid embryo and subsequent live birth remain consistent with national age-based rates after an in vitro fertilization cycle that produced only aneuploid embryos. *Fertil Steril* 2022;**118**:484–491.

Hill MJ, Richter KS, Heitmann RJ, Graham JR, Tucker MJ, DeCherney AH, Browne PE, Levens ED. Trophectoderm grade predicts outcomes of single-blastocyst transfers. *Fertil Steril* 2013;**99**:1283–1289.e1.

Irani M, Reichman D, Robles A, Melnick A, Davis O, Zaninovic N, Xu K, Rosenwaks Z. Morphologic grading of euploid blastocysts influences implantation and ongoing pregnancy rates. *Fertil Steril* 2017;**107**:664–670.

Ju LF, Xu HJ, Yang YG, Yang Y. Omics views of mechanisms for cell fate determination in early mammalian development. *Genomics Proteomics Bioinformatics* 2023;**21**:950–961.

Lee SH, Rinaudo PF. Metabolic regulation of preimplantation embryo development in vivo and in vitro: molecular mechanisms and insights. *Biochem Biophys Res Commun* 2024;**726**:150256.

Macklon NS, Brosens JJ. The human endometrium as a sensor of embryo quality. *Biol Reprod* 2014;**91**:98.

Nazem TG, Sekhon L, Lee JA, Overbey J, Pan S, Duke M, Briton-Jones C, Whitehouse M, Copperman AB, Stein DE. The correlation between morphology and implantation of euploid human blastocysts. *Reprod Biomed Online* 2019;**38**:169–176.

Paonessa M, Borini A, Coticchio G. Genetic causes of preimplantation embryo developmental failure. *Mol Reprod Dev* 2021;**88**:338–348.

Pirtea P, De Ziegler D, Tao X, Sun L, Zhan Y, Ayoubi JM, Seli E, Franasiak JM, Scott RT Jr. Rate of true recurrent implantation failure is low: results of three successive frozen euploid single embryo transfers. *Fertil Steril* 2021;**115**:45–53.

Ramos-Ibeas P, Heras S, Gómez-Redondo I, Planells B, Fernández-González R, Pericuesta E, Laguna-Barraza R, Pérez-Cerezales S, Gutiérrez-Adán A. Embryo responses to stress induced by assisted reproductive technologies. *Mol Reprod Dev* 2019;**86**:1292–1306.

Rienzi L, Cimadomo D, Vaiarelli A, Gennarelli G, Holte J, Livi C, Aura Masip M, Uher P, Fabozzi G, Ubaldi FM. Measuring success in IVF is a complex multidisciplinary task: time for a consensus? *Reprod Biomed Online* 2021;**43**:775–778.

Rozen G, Rogers P, Teh WT, Stern CJ, Polyakov A. An algorithm to personalise the diagnosis of recurrent implantation failure based on theoretical cumulative implantation rate. *Hum Reprod* 2021; **36**:1463–1468.

Sahin GN, Yildirim RM, Seli E. Embryonic arrest: causes and implications. *Curr Opin Obstet Gynecol* 2023;**35**:184–192.

Smith AD, Tilling K, Lawlor DA, Nelson SM. External validation and calibration of IVFpredict: a national prospective cohort study of 130,960 in vitro fertilisation cycles. *PLoS One* 2015;**10**:e0121357.

Tannorella P, Calzari L, Daolio C, Mainini E, Vimercati A, Gentilini D, Soli F, Pedrolli A, Bonati MT, Larizza L *et al.* Germline variants in genes of the subcortical maternal complex and Multilocus Imprinting Disturbance are associated with miscarriage/infertility or Beckwith-Wiedemann progeny. *Clin Epigenetics* 2022;**14**:43.

(The Writing Group) for the Participants to the 2022 Lugano RIF Workshop; Pirtea P, Cedars MI, Devine K, Ata B, Franasiak J, Racowsky C, Toner J, Scott RT, de Ziegler D, Barnhart KT. Recurrent implantation failure: reality or a statistical mirage?: consensus statement from the July 1, 2022 Lugano Workshop on recurrent implantation failure. *Fertil Steril* 2023;**120**:45–59.

Human Reproduction, 2025, 00, 1–5
https://doi.org/10.1093/humrep/deaf007
Peer Perspectives