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Opening the black box: why do euploid blastocysts fail to implant? A systematic review and meta-analysis

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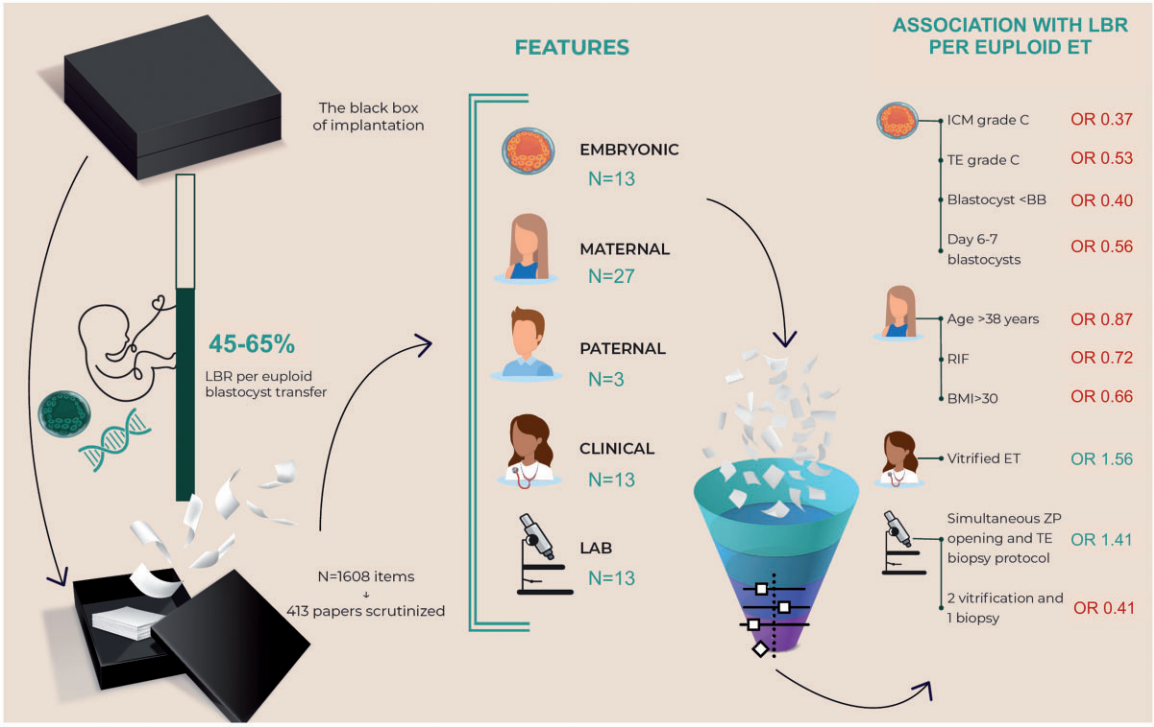
GRAPHICAL ABSTRACT



Opening the black box of implantation: low blastocyst quality and maternal aging, obesity or repeated implantation failures (RIF), as well as poor or excessive embryo manipulations may reduce the live birth rate per euploid blastocyst transfer.

ABSTRACT

BACKGROUND: A normal chromosomal constitution defined through PGT-A assessing all chromosomes on trophectoderm (TE) biopsies represents the strongest predictor of embryo implantation. Yet, its positive predictive value is not higher than 50–60%. This gap of knowledge on the causes of euploid blastocysts' reproductive failure is known as 'the black box of implantation'.

OBJECTIVE AND RATIONALE: Several embryonic, maternal, paternal, clinical, and IVF laboratory features were scrutinized for their putative association with reproductive success or implantation failure of euploid blastocysts.

SEARCH METHODS: A systematic bibliographical search was conducted without temporal limits up to August 2021. The keywords were '(blastocyst OR day5 embryo OR day6 embryo OR day7 embryo) AND (euploid OR chromosomally normal OR preimplantation genetic testing) AND (implantation OR implantation failure OR miscarriage OR abortion OR live birth OR biochemical pregnancy OR recurrent implantation failure)'. Overall, 1608 items were identified and screened. We included all prospective or retrospective clinical studies and randomized-controlled-trials (RCTs) that assessed any feature associated with live-birth rates (LBR) and/or miscarriage rates (MR) among non-mosaic euploid blastocyst transfer after TE biopsy and PGT-A. In total, 41 reviews and 372 papers were selected, clustered according to a common focus, and thoroughly reviewed. The PRISMA guideline was followed, the PICO model was adopted, and ROBINS-I and ROB 2.0 scoring were used to assess putative bias. Bias across studies regarding the LBR was also assessed using visual inspection of funnel plots and the trim and fill method. Categorical data were combined with a pooled-OR. The random-effect model was used to conduct the meta-analysis. Between-study heterogeneity was addressed using I^2. Whenever not suitable for the meta-analysis, the included studies were simply described for their results. The study protocol was registered at http://www.crd.york.ac.uk/PROSPERO/ (registration number CRD42021275329).

OUTCOMES: We included 372 original papers (335 retrospective studies, 30 prospective studies and 7 RCTs) and 41 reviews. However, most of the studies were retrospective, or characterized by small sample sizes, thus prone to bias, which reduces the quality of the evidence to low or very low. Reduced inner cell mass (7 studies, OR: 0.37, 95% CI: 0.27–0.52, $I^2 = 53\%$), or TE quality (9 studies, OR: 0.53, 95% CI: 0.43–0.67, $I^2 = 70\%$), overall blastocyst quality worse than Gardner's BB-grade (8 studies, OR: 0.40, 95% CI: 0.24–0.67, $I^2 = 83\%$), developmental delay (18 studies, OR: 0.56, 95% CI: 0.49–0.63, $I^2 = 47\%$), and (by qualitative analysis) some morphodynamic abnormalities pinpointed through time-lapse microscopy (abnormal cleavage patterns, spontaneous blastocyst collapse, longer time of morula formation I, time of blastulation (tB), and duration of blastulation) were all associated with poorer reproductive outcomes. Slightly lower LBR, even in the context of PGT-A, was reported among women ≥38 years (7 studies, OR: 0.87, 95% CI: 0.75–1.00, $I^2 = 31\%$), while obesity was associated with both lower LBR (2 studies, OR: 0.66, 95% CI: 0.55–0.79, $I^2 = 0\%$) and higher MR (2 studies, OR: 1.8, 95% CI: 1.08–2.99, $I^2 = 52\%$). The experience of previous repeated implantation failures (RIF) was also associated with lower LBR (3 studies, OR: 0.72, 95% CI: 0.55–0.93, $I^2 = 0\%$). By qualitative analysis, among hormonal assessments, only abnormal progesterone levels prior to transfer were associated with LBR and MR after PGT-A. Among the clinical protocols used, vitrified-warmed embryo transfer was

more effective than fresh transfer (2 studies, OR: 1.56, 95% CI: 1.05–2.33, $I^2 = 23\%$) after PGT-A. Lastly, multiple vitrification-warming cycles (2 studies, OR: 0.41, 95% CI: 0.22–0.77, $I^2 = 50\%$) or (by qualitative analysis) a high number of cells biopsied may slightly reduce the LBR, while simultaneous zona-pellucida opening and TE biopsy allowed better results than the Day 3 hatching-based protocol (3 studies, OR: 1.41, 95% CI: 1.18–1.69, $I^2 = 0\%$).

WIDER IMPLICATIONS: Embryo selection aims at shortening the time-to-pregnancy, while minimizing the reproductive risks. Knowing which features are associated with the reproductive competence of euploid blastocysts is therefore critical to define, implement, and validate safer and more efficient clinical workflows. Future research should be directed towards: (i) systematic investigations of the mechanisms involved in reproductive aging beyond *de novo* chromosomal abnormalities, and how lifestyle and nutrition may accelerate or exacerbate their consequences; (ii) improved evaluation of the uterine and blastocyst-endometrial dialogue, both of which represent black boxes themselves; (iii) standardization/automation of embryo assessment and IVF protocols; (iv) additional invasive or preferably non-invasive tools for embryo selection. Only by filling these gaps we may finally crack the riddle behind 'the black box of implantation'.

Keywords: implantation failure / live birth / blastocyst / IVF / miscarriage / PGT-A / trophectoderm biopsy / embryo quality / advanced maternal age / obesity

Introduction

The development of a reliable embryo selection method to improve our prediction of implantation remains a great challenge of modern IVF. Moreover, the establishment of an ongoing pregnancy and the birth of a healthy baby are not solely the result of embryonic characteristics, and a plethora of other features must be carefully considered. Across the years, several non-invasive and invasive methods for embryo selection have been developed, such as static or morphodynamic evaluations, embryo biopsy for preimplantation genetic testing for aneuplodies (PGT-A), and -omic approaches (Bolton *et al.*, 2015; Gardner *et al.*, 2015). In this scenario, static morphological assessment is limited in its prediction of embryo reproductive competence, and even when overcoming a single snapshot-based assessment with a continuous monitoring in time-lapse incubators, only a poor association has been reported between morphokinetics, abnormal cleavage patterns, and embryo chromosomal constitution (Apter *et al.*, 2020). The only accurate approach to uncover embryonic aneuploidies is trophectoderm (TE) biopsy and its analysis through PGT-A assessing all chromosomes (Scott *et al.*, 2012; Tiegs *et al.*, 2020; Capalbo *et al.*, 2022). This technique, by preventing the transfer of aneuploid blastocysts, results in lower miscarriage rates (MRs) per clinical pregnancy and higher live birth rates (LBRs) per embryo transfer (ET) (Chen *et al.*, 2015; Dahdouh *et al.*, 2015b), apparently with no impact on the cumulative live birth rate (CLBR) per treatment (Yan *et al.*, 2021; Hipp *et al.*, 2022). Spent media analyses through metabolomic approaches have been also explored to define a 'fingerprint' of embryo competence; however, their clinical value has been so far insufficient (Lane and Gardner, 2005; Gardner *et al.*, 2011; Siristatidis *et al.*, 2017; Ferrick *et al.*, 2020). Moreover, a healthy pregnancy can only be achieved when a viable, chromosomally normal blastocyst implants in an adequately thick, immunologically tolerant, decidualized, and receptive endometrium within the window of implantation (WOI) (Craciunas *et al.*, 2019). Therefore, this environment cannot be disregarded, especially for its role as 'biosensor' of embryo quality (Macklon and Brosens, 2014; Gurner *et al.*, 2022). A mutual dialogue in fact exists between the embryo and the endometrium, that is mediated by lipid vesicles released in the extracellular environment; in the IVF context, some authors have tried to exploit the mediators of this crosstalk, but the results have been either disappointing or preliminary (Capalbo *et al.*, 2016b; Cimadomo *et al.*, 2019a; Giacomini *et al.*, 2021; Wang *et al.*, 2021b).

In summary, despite the great efforts made to improve it, the LBR per euploid blastocyst ET has been generally reported as between 50% and 60% on aggregated data (Chen *et al.*, 2015; Dahdouh *et al.*, 2015b). There is certainly room to improve our predictive power upon implantation and fill the current gap of knowledge, which currently represents a 'black box'. This systematic review and meta-analysis scrutinized all embryonic, maternal, paternal, clinical, and laboratory features that may directly or indirectly affect the reproductive success or implantation failure of euploid blastocysts.

Methods

Protocol and registration

This study was exempt from institutional review board approval because it did not involve human intervention. We adhered to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA). The study protocol was registered at http://www.crd.york.ac.uk/PROSPERO/ (registration number CRD42021275329) before starting the review process.

Eligibility criteria

We used the Patients, Intervention, Comparison and Outcomes (PICO) model to select our study population. We included only clinical studies (prospective and retrospective studies, and randomized controlled trials) investigating any putative additional feature associated with the LBR per non-mosaic euploid blastocyst transfer in the context of TE biopsy and PGT-A. No time or language restrictions were adopted, and queries were limited to human studies. Case series, case reports, books, congress abstracts, and grey literature were not included in the analysis. Furthermore, we did not include studies where PGT-A was conducted with single cell and/or fluorescent *in situ* hybridization (FISH) analyses, due to their intrinsic technical and clinical limitations (Mastenbroek *et al.*, 2011; Treff *et al.*, 2011; Scott *et al.*, 2012, 2013; Deleye *et al.*, 2017). Similarly, studies where PGT-A was adopted to report chromosome intermediate copy numbers (ICN) as 'mosaic' aneuploidies or where allegedly mosaic embryos were transferred were pre-emptively excluded to minimize the risk of biased analyses. Indeed, the practice of diagnosing mosaicism based on ICN for both whole chromosomes and segmental imbalances (i) is highly prone to false positive/false negative assessments (Capalbo *et al.*, 2017b, 2021; Popovic *et al.*, 2019; Wu *et al.*, 2021; Kim *et al.*, 2022), (ii) significantly reduces the cohort of blastocysts selected for transfer (Kim *et al.*, 2018; Besser *et al.*, 2019), and (iii) is unreliable, since specimens classified in the range 20–50% produced clinical outcomes equivalent to the transfer of euploid blastocysts (<20% ICN) when assessed in a blinded, non-selection, multicenter study (Capalbo *et al.*, 2021).

Search strategy and study selection

We searched PubMed, Web of Science and Scopus without temporal limits up to August 2021 using the keywords '(blastocyst OR day 5 embryo OR day 6 embryo OR day 7 embryo) AND (euploid OR chromosomally normal OR preimplantation genetic testing) AND (implantation OR implantation failure OR miscarriage OR abortion OR live birth OR biochemical pregnancy OR recurrent implantation failure)'. Studies were selected according to the eligibility criteria defined in the previous paragraph. Any discordance was discussed with the senior authors.

Data extraction

Data were extracted independently by the reviewers (DC, ACo, MP, SC, FI, JH, LG, AV) using predefined data fields and study quality indicators. Discrepancies were resolved by discussion with the senior authors (LR, CA, EF, FMU, ACa). In case of partial or missing outcomes, the corresponding authors of the papers selected for the meta-analyses were e-mailed to collect the relevant data.

Risk of bias, summary measures, and synthesis of results

The risk of bias and the quality of the studies included in this meta-analysis were evaluated independently by two authors (DC and ACo). The senior authors resolved conflicts. ROBINS-I and ROB 2.0 scoring were adopted to assess risk of bias in non-randomized and randomized controlled trials, respectively. Bias across studies regarding the primary outcome was assessed using visual inspection of funnel plots, and the trim and fill method (Duval and Tweedie, 2000).

The primary outcome was LBR per ET, namely the number of deliveries that resulted in at least one live birth (>22 gestational weeks) expressed per 100 ETs, and the secondary outcome was MR per clinical pregnancy, namely the number of spontaneous losses (<22 gestational weeks) expressed per 100 clinical pregnancies (i.e. the documented presence of at least one fetus with fetal heartbeat) (Zegers-Hochschild *et al.*, 2017a,b).

Quantitative analysis

Statistical analysis was carried out using Review Manager 5.4 (The Nordic Cochrane Centre, The Cochrane Collaboration). To establish an association between specific embryonic, maternal, paternal, clinical, and IVF laboratory features with the outcomes, categorical data were combined with a pooled odds ratio (OR). The random-effect model was used to conduct the meta-analysis. Between-study heterogeneity was addressed using I^2, which represents the percentage of total variation in the estimated effect across studies. An I^2 value over 50% indicates substantial heterogeneity. *P*-values below 0.05 were considered statistically significant.

Results

The search resulted into 1608 items, which were revised to select a list of eligible manuscripts for inclusion in the review. After evaluation, 372 papers (335 retrospective papers, 30 prospective, and 7 RCTs) and 41 reviews were selected. Among them, 74 papers were quantitatively assessed (Fig. 1). The 41 reviews were included to draft the manuscript and their references were also scrutinized to complete our systematic review. The studies which could be combined in a meta-analysis are summarized in Table 1 and the studies used only in the qualitative analysis are summarized in Table 2.

Embryonic features

The embryonic features potentially associated with euploid blastocysts' reproductive competence were clustered as static and morphodynamic features, and additional molecular analyses.

Static and morphodynamic embryonic features

Although there is an association between blastocyst morphological quality and/or developmental rate to full blastulation (days 5–7) and PGT-A data and/or reproductive competence, the extent of the association is still unclear. The studies are too heterogenous, especially in terms of patient population, clinical and laboratory practice, morphological scoring systems adopted, and PGT-A method, to clearly determine the association.

Inner cell mass, trophectoderm, or whole blastocyst quality

Embryo morphological grading is the most used method for human blastocyst assessment in the daily IVF practice worldwide (Schoolcraft *et al.*, 1999; Gardner and Schoolcraft, 1999b; Gardner et al., 2000). Any scoring system encompasses blastocyst expansion and hatching, inner cell mass (ICM) appearance, TE cohesiveness, and number of cells (Gardner and Schoolcraft, 1999a; Alpha SiRM and ESHRE SIGE, 2011; Hardarson *et al.*, 2012). Of note, a correlation exists between embryo chromosomal status and blastocyst characteristics, with better-quality ICM and TE being associated with higher euploidy rates (Alfarawati *et al.*, 2011; Capalbo *et al.*, 2014; Fragouli *et al.*, 2014; Minasi *et al.*, 2016; Barash *et al.*, 2017b; Guzman *et al.*, 2019; Hernandez-Nieto *et al.*, 2019; Kim *et al.*, 2019; Vinals Gonzalez *et al.*, 2019). Poor-quality ICM and TE often display increased complex aneuploidy rates affecting two or more chromosomes (Alfarawati *et al.*, 2011; Capalbo *et al.*, 2014; Fragouli *et al.*, 2014). Moreover, in the context of ETs involving genetically untested vitrified-warmed embryos, blastocyst expansion, and TE and ICM grades have been all reported to be significantly associated with pregnancy outcomes, with the last two features being the strongest predictor of LB (Ai *et al.*, 2021). Therefore, these features have also been extensively investigated for their putative association with the reproductive competence of euploid blastocysts.

After our systematic search, euploid blastocysts were clustered into two groups according to ICM morphology, namely Gardner's grade C versus A/B, and eight of the studies retrieved reported LBR per SET and/or MR per clinical pregnancy according to this feature (Irani *et al.*, 2017; Zhao *et al.*, 2018; Nazem *et al.*, 2019; Sekhon *et al.*, 2019; Boynukalin *et al.*, 2020, 2021; Murugappan *et al.*, 2020; Peng *et al.*, 2020) (Table 1). One study instead reported only the ongoing pregnancy rate (OPR), and MR based on a 12 gestational weeks threshold and could not be meta-analyzed (Moutos *et al.*, 2021) (Table 2). They were all retrospective single center studies.

In our meta-analysis, grade C ICM (N = 470 overall) was associated with a significantly lower LBR per euploid SET than grade A/B ICM (N = 6403 overall), with an OR 0.37, 95% CI 0.27–0.52, $I^2 = 53\%$, $P < 0.01$ (Fig. 2). The difference in MR per clinical pregnancy (N = 511 from grade C ICM and N = 3108 from grade A/B) was not statistically significant (OR 1.31, 95% CI 0.96–1.80, $I^2 = 0\%$, $P = 0.09$) (Supplementary Fig. S1).

Blastocysts could also be clustered in two groups according to TE morphology grade (i.e. C versus A/B). Ten of the retrieved studies reported LBR per SET and/or MR per clinical pregnancy according to this feature (Irani *et al.*, 2017; Zhao *et al.*, 2018; Nazem *et al.*, 2019; Rienzi *et al.*, 2019; Sekhon *et al.*, 2019; Boynukalin *et al.*, 2020, 2021; Murugappan *et al.*, 2020; Peng *et al.*, 2020; Zhou *et al.*, 2021) (Table 1). One study instead reported only

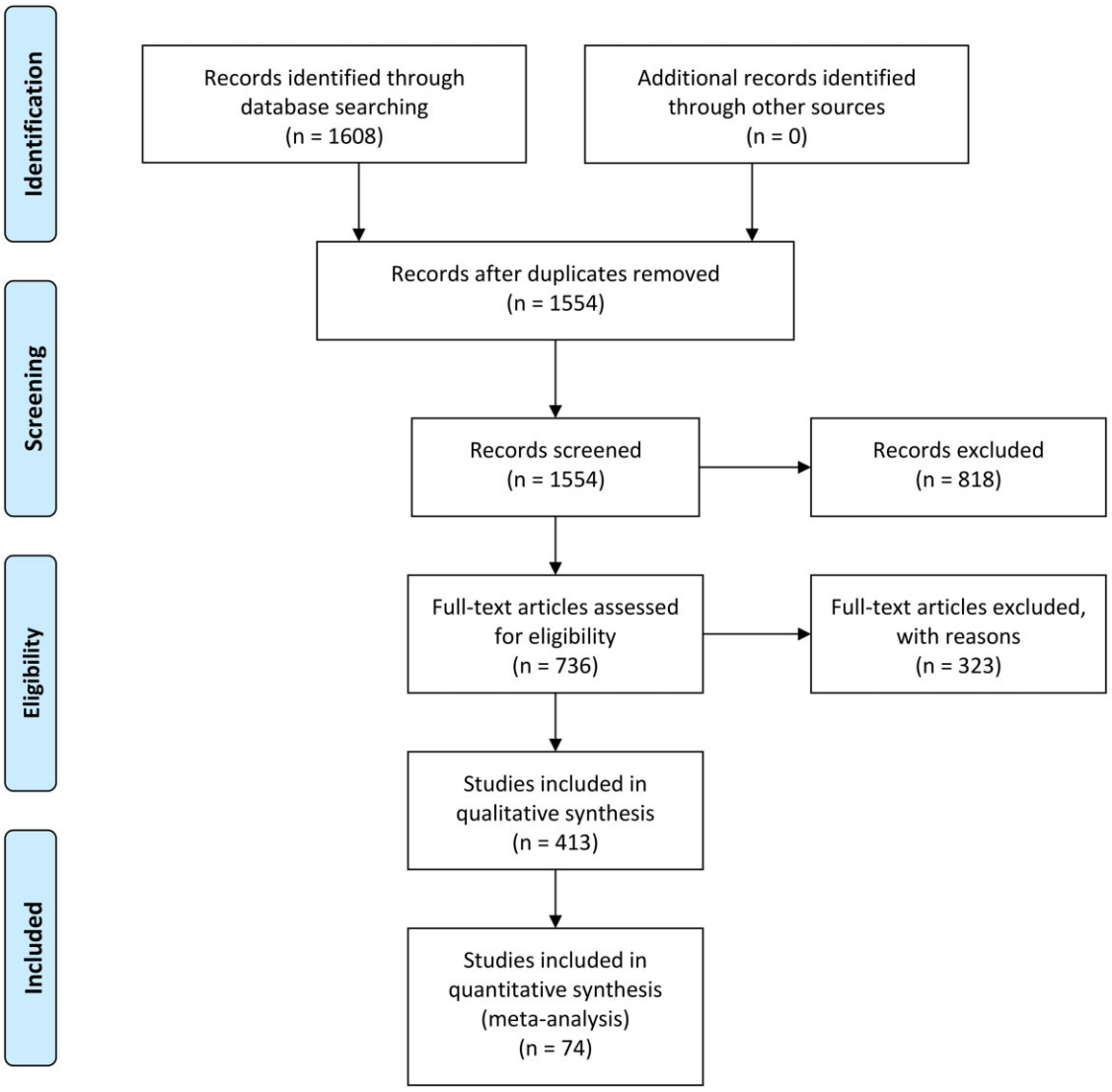


Figure 1. **Flowchart.**

the OPR and MR based on a 12 gestational weeks threshold and could not be meta-analyzed (Moutos *et al.*, 2021) (Table 2). They were all retrospective single center studies, except for a multicenter one (Rienzi *et al.*, 2019).

In our meta-analysis, grade C TE (N = 1909 overall) was associated with a significantly lower LBR per euploid SET than grade A/B TE (N = 6110 overall), with an OR 0.53, 95% CI 0.43–0.67, $I^2 = 70\%$, $P < 0.01$ (Fig. 3). The MR per clinical pregnancy (N = 527 from grade C TE and N = 3230 from grade A/B) was also significantly higher for the former group (OR 1.44, 95% CI 1.09–1.90, $I^2 = 10\%$, $P = 0.01$) (Supplementary Fig. S2).

In six single center (Irani *et al.*, 2018b; Cimadomo *et al.*, 2019b; Vinals Gonzalez *et al.*, 2019; Ji *et al.*, 2021; Wang *et al.*, 2021a; Chen *et al.*, 2022) and two multicenter retrospective studies (Capalbo *et al.*, 2014; Cimadomo *et al.*, 2018a), specific ICM and TE quality were not reported, but overall blastocyst quality was categorized as good (Gardner's score >BB) or poor (≤BB), and LBR per SET and/or MR per clinical pregnancy were retrievable from the manuscripts (Table 1). One study instead reported only OPR and MR based on a 12 gestational weeks threshold and could not be meta-analyzed (Moutos *et al.*, 2021) (Table 2).

In our meta-analysis, poor-quality blastocysts (N = 722 overall) resulted in a significantly lower LBR per euploid SET than high-quality ones (N = 4384 overall) with an OR 0.40, 95% CI 0.24–0.67, $I^2 = 83\%$, $P < 0.01$ (Fig. 4). The difference in MR per clinical pregnancy (N = 230 from poor-quality blastocysts and N = 1907 from high-quality ones) was not statistically significant (OR 1.42, 95% CI 0.63–3.22, $I^2 = 68\%$, $P = 0.40$) (Supplementary Fig. S3).

Day of biopsy

According to ESHRE and Alpha recommendations, full blastocyst expansion should be assessed at 116 ± 2 h post-insemination (hpi) (Alpha SiRM and ESHRE SIGoE, 2011), and day5 blastocyst development rate should be adopted as a critical Key Performance Indicator (KPI) in IVF (ESHRE SIGoE and Alpha SiRM, 2017). Nevertheless, a consistent cohort of blastocysts develops beyond day5, and up to day7. Recently, extended culture has been proposed as an effective strategy, especially when no suitable embryo can be obtained earlier (Hammond *et al.*, 2018), and several studies have outlined the reproductive competence of slower-growing embryos.

Eighteen of the retrieved studies assessed LBR per SET and MR per clinical pregnancy after euploid SETs in two groups: day6–7 versus day5 (Capalbo *et al.*, 2014; Taylor *et al.*, 2014c; Minasi *et al.*, 2016; Piccolomini *et al.*, 2016; Barash *et al.*, 2017b; Cimadomo *et al.*, 2018a; Irani *et al.*, 2018b; Hernandez-Nieto *et al.*, 2019;

Table 1. List of articles available for the meta-analyses.

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
EMBRYONIC FEATURES								
Inner cell mass morphology								
Irani *et al.*, 2017	Retrospective single center	aCGH	January 2013–December 2015	USA	417 euploid SETs	Grade C	Grade A/B	LBR: 5/37, 13.5% (study) versus 222/380, 58.4% (control), $P < 0.01$ MR: 2/27, 7.4% (study) versus 20/242, 8.3% (control), $P < 0.01$
Zhao *et al.*, 2018	Retrospective single center	aCGH and SNP-array	June 2011–May 2016	China	914 euploid SETs	Grade C	Grade A/B	LBR: 2/16, 12.5% (study) versus 387/898, 43.1% (control), $P = 0.02$ MR: 2/4, 50.0% (study) versus 80/467, 17.1% (control), $P = 0.14$
Nazem *et al.*, 2019	Retrospective single center	qPCR and NGS	January 2012–December 2017	USA	2236 euploid SETs	Grade C	Grade A/B	LBR: 41/127, 32.3% (study) versus 1102/2109, 52.3% (control), $P < 0.01$ MR: 3/44, 6.8% (study) versus 112/1214, 9.2% (control), $P = 0.79$
Sekhon *et al.*, 2019	Retrospective single center	qPCR, aCGH, and NGS	January 2012–June 2017	USA	1107 euploid SETs	Grade C	Grade A/B	LBR: 11/50, 22% (study) versus 541/1057, 51.2% (control), $P < 0.01$ MR: not reported
Boynukalin *et al.*, 2020	Retrospective single center	NGS	October 2015–January 2018	Turkey	690 euploid SETs	Grade C	Grade A/B	LBR: 25/70, 35.7% (study) versus 369/620, 59.5% (control), $P < 0.01$ MR: not reported
Murugappan *et al.*, 2020	Retrospective single center	qPCR, aCGH, and NGS	January 2012–December 2018	USA	660 euploid SETs	Grade C	Grade A/B	LBR: 19/38, 50% (study) versus 389/622, 62.5% (control), $P = 0.13$ MR: 5/24, 20.8% (study) versus 68/457, 14.9% (control), $P = 0.39$
Peng *et al.*, 2020	Retrospective single center	Not Reported	January 2014–January 2018	China	849 euploid SETs	Grade C	Grade A/B	LBR: 42/132, 31.8% (study) versus 334/717, 46.6% (control), $P < 0.01$ MR: 13/55, 23.6% (study) versus 62/396, 15.7% (control), $P = 0.17$
Boynukalin *et al.*, 2021	Retrospective single center	NGS	January 2016–July 2019	Turkey	1051 euploid SETs	Grade C	Grade A/B	LBR: not reported MR: 56/357, 15.7% (study) versus 44/332, 13.3% (control), $P = 0.37$
Trophectoderm morphology								
Irani *et al.*, 2017	Retrospective single center	aCGH	January 2013–December 2015	USA	417 euploid SETs	Grade C	Grade A/B	LBR: 16/58, 27.6% (study) versus 211/359, 58.8% (control), $P < 0.01$ MR: 9/25, 36.0% (study) versus 40/251, 15.9% (control), $P = 0.02$
Zhao *et al.*, 2018	Retrospective single center	aCGH and SNP-array	June 2011–May 2016	China	914 euploid SETs	Grade C	Grade A/B	LBR: 23/84, 27.4% (study) versus 366/830, 44.1% (control), $P < 0.01$ MR: 7/30, 23.3% (study) versus 75/441, 17.0% (control), $P = 0.45$
Nazem *et al.*, 2019	Retrospective single center	qPCR and NGS	January 2012–December 2017	USA	2236 euploid SETs	Grade C	Grade A/B	LBR: 185/463, 40.0% (study) versus 958/1773, 54.0% (control), $P < 0.01$ MR: 23/208, 11.0% (study) versus 92/1050, 8.8% (control), $P = 0.29$
Rienzi *et al.*, 2019	Retrospective multicenter	qPCR, aCGH, and NGS	January 2016–June 2018	Italy, Spain	830 euploid SETs	Grade C	Grade A/B	LBR: 56/237, 23.6% (study) versus 288/593, 48.6% (control), $P < 0.01$ MR: not reported

(continued)

Table 1. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
Sekhon *et al.*, 2019	Retrospective single center	qPCR and aCGH	January 2012–June 2017	USA	1107 euploid SETs	Grade C	Grade A/B	LBR: 87/220, 39.5% (study) versus 465/887, 52.5% (control), $P < 0.01$ MR: not reported
Boynukalin *et al.*, 2020	Retrospective single center	NGS	October 2015–January 2018	Turkey, Cyprus, Spain	690 euploid SETs	Grade C	Grade A/B	LBR: 222/407, 54.5% (study) versus 172/283, 60.8% (control), $P = 0.12$ MR: not reported
Murugappan *et al.*, 2020	Retrospective single center	qPCR, aCGH, and NGS	January 2012–December 2018	USA	660 euploid SETs	Grade C	Grade A/B	LBR: 33/71, 46.5% (study) versus 375/589, 63.7% (control), $P < 0.01$ MR: 11/44, 25.0% (study) versus 62/437, 14.2% (control), $P = 0.07$
Peng *et al.*, 2020	Retrospective single center	Not Reported	January 2014–January 2018	China	849 euploid SETs	Grade C	Grade A/B	LBR: 111/270, 41.1% (study) versus 265/579, 45.8% (control), $P = 0.21$ MR: 22/133, 16.5% (Study) versus 53/318, 16.7% (control), $P = 0.59$
Boynukalin *et al.*, 2021	Retrospective single center	NGS	January 2016–July 2019	Turkey	1051 euploid SETs	Grade C	Grade A/B	LBR: not reported MR: 8/53, 15.1% (study) versus 92/636, 14.5% (control), $P = 0.90$
Zhou *et al.*, 2021	Retrospective single center	NGS	2016–2020	China	316 euploid SETs	Grade C	Grade A/B	LBR: 24/99, 24.2% (study) versus 81/217, 37.3% (control), $P = 0.03$ MR: 10/34, 29.4% (study) versus 16/97, 13.1% (control), $P = 0.14$
Overall blastocyst morphological quality from Excellent to Poor								
Capalbo *et al.*, 2014	Retrospective multicenter	aCGH	January 2009–August 2013	Italy, USA	215 euploid SETs	<BB	≥BB	LBR: 7/13, 53.8% (study) versus 99/202, 49.0% (control), $P = 0.78$ MR: not reported
Cimadomo *et al.*, 2018a	Retrospective multicenter	qPCR	June 2016–August 2017	Italy	962 euploid SETs	<BB	≥BB	LBR: 5/68, 7.4% (study) versus 385/894, 43.1% (control), $P < 0.01$ MR: not reported
Irani *et al.*, 2018b	Retrospective single center	aCGH	January 2013–December 2016	USA	701 euploid SETs	<BB	≥BB	LBR: 33/112, 29.5% (study) versus 336/589, 57.0% (control), $P < 0.01$ MR: 9/42, 21.4% (study) versus 32/368, 8.7% (control), $P = 0.02$
Cimadomo *et al.*, 2019b	Retrospective single center	qPCR and NGS	April 2013–May 2018	Italy	1883 euploid SETs	<BB	≥BB	LBR: 21/193, 10.9% (study) versus 757/1690, 44.8% (control), $P < 0.01$ MR: 12/33, 36.4% (study) versus 122/879, 13.9% (control), $P < 0.01$
Vinals Gonzalez *et al.*, 2019	Retrospective single center	NGS	December 2015–February 2018	UK	179 euploid SETs	<BB	≥BB	LBR: 6/10, 60% (study) versus 115/169, 68.0% (control), $P = 0.73$ MR: 1/8, 12.5% (study) versus 10/140, 7.1% (control), $P = 0.47$
Ji *et al.*, 2021	Retrospective single center	NGS	January 2017–May 2019	China	360 euploid SETs	<BB	≥BB	LBR: 58/145, 40.0% (study) versus 111/215, 51.6% (control), $P = 0.03$ MR: 9/69, 13.0% (study) versus 11/126, 8.7% (control), $P = 0.34$
Chen *et al.*, 2022	Retrospective single center	NGS	January 2017–December 2019	China	469 euploid SETs	<BB	≥BB	LBR: 44/112, 39.3% (study) versus 193/357, 54.1% (control), $P < 0.01$ MR: 3/47, 6.4% (study) versus 29/222, 13.1% (control), $P = 0.32$

(continued)

Table 1. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
Wang *et al.*, 2021a	Retrospective single center	NGS	April 2017–December 2019	China	337 euploid SETs	<BB	≥BB	LBR: 30/69, 43.5% (study) versus 146/268, 54.5% (control), $P = 0.11$ MR: 1/31, 3.2% (study) versus 26/172, 15.1% (control), $P = 0.09$
					Day of biopsy			
Capalbo *et al.*, 2014	Retrospective multicenter	qPCR	January 2009–August 2013	Italy, USA	215 euploid SETs	Day 6/7	Day 5	LBR: 24/47, 51.1% (study) versus 82/168, 48.8% (control), $P = 0.87$ MR: not reported
Taylor *et al.*, 2014c	Retrospective single center	aCGH	January 2011–April 2013	USA	89 euploid SETs	Day 6	Day 5	LBR: 23/39, 58.9% (study) versus 26/50, 52.0% (control), $P = 0.51$ MR: not reported
Minasi *et al.*, 2016	Retrospective single center	aCGH	September 2012–April 2014	Italy	229 euploid SETs	Day 6/7	Day 5	LBR: 40/116, 34.5% (study) versus 52/113, 46.0% (control), $P = 0.08$ MR: 11/51, 21.6% (study) versus 7/59, 11.9% (control), $P = 0.17$
Piccolomini *et al.*, 2016	Retrospective single center	aCGH	February 2014–May 2015	Brazil	191 euploid SETs	Day 6	Day 5	LBR: 22/60, 36.7% (study) versus 45/131, 34.4% (control), $P = 0.76$ MR: 5/27, 18.5% (study) versus 12/57, 21% (control), $P = 0.79$
Barash *et al.*, 2017b	Retrospective single center	SNP-array	January 2013–January 2016	USA	503 euploid SETs	Day 6	Day 5	LBR: 109/233, 46.8% (study) versus 166/270, 61.5% (control), $P < 0.01$ MR: 16/125, 12.8% (study) versus 13/179, 7.3% (control), $P = 0.1$
Cimadomo *et al.*, 2018a	Retrospective multicenter	qPCR	June 2016–August 2017	Italy	962 euploid SETs	Day 6/7	Day 5	LBR: 176/532, 33.1% (study) versus 214/430, 49.8% (control), $P < 0.01$ MR: not reported
Irani *et al.*, 2018b	Retrospective single center	aCGH	January 2013–December 2016	USA	701 euploid SETs	Day 6	Day 5	LBR: 150/335, 44.8% (study) versus 221/366, 60.4% (control), $P < 0.01$ MR: 16/166, 9.6% (study) versus 23/244, 9.4% (control), $P = 0.9$
Hernandez-Nieto *et al.*, 2019	Retrospective single center	qPCR and NGS	January 2012–March 2018	USA	3818 euploid SETs	Day 6/7	Day 5	LBR: 568/1497, 37.9% (study) versus 1311/2321, 56.5% (control), $P < 0.01$ MR: 154/812, 19.0% (study) versus 209/1520, 13.8% (control), $P < 0.01$
Kimelman *et al.*, 2019	Retrospective single center	SNP-array and NGS	2015–2016	USA	112 euploid SETs	Day6	Day 5	LBR: 11/19, 57.9% (study) versus 60/93, 64.5% (control), $P = 0.6$ MR: 3/14, 21.4% (study) versus 4/64, 6.3% (control), $P = 0.15$
Sekhon *et al.*, 2019	Retrospective single center	qPCR, aCGH, and NGS	January 2012–June 2017	USA	1107 euploid SETs	Day 6/7	Day 5	LBR: 167/396, 42.2% (study) versus 394/739, 53.3% (control), $P < 0.01$ MR: not reported
Whitney *et al.*, 2019	Retrospective single center	NGS	January 2015–March 2016	USA	253 euploid SETs	Day 6/7	Day 5	LBR: 69/108, 63.9% (study) versus 112/145, 77.2% (control), $P = 0.02$ MR: 3/72, 4.2% (study) versus 3/115, 2.6% (control), $P = 0.56$
Boynukalin *et al.*, 2020	Retrospective single center	NGS	October 2015–January 2018	Turkey, Cyprus, Spain	707 euploid SETs	Day 6	Day 5	LBR: 69/166, 41.6% (study) versus 334/541, 61.7% (control), $P < 0.01$ MR: not reported

(continued)

Table 1. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
Ji et al., 2021	Retrospective single center	NGS	January 2017–May 2019	China	360 euploid SETs	Day 6	Day 5	LBR: 79/176, 44.9% (study) versus 90/184, 48.9% (control), $P = 0.44$ MR: 11/90, 12.2% (study) versus 15/105, 14.3% (control), $P = 0.67$
Peng et al., 2020	Retrospective single center	Not reported	January 2014–January 2018	China	849 euploid SETs	Day 6	Day 5	LBR: 79/233, 33.9% (study) versus 297/616, 48.2% (control), $P < 0.01$ MR: 25/104, 24.0% (study) versus 50/347, 14.4% (control), $P = 0.02$
Sardana et al., 2020	Retrospective single center	NGS	January 2016–December 2017	India	97 euploid SETs	Day 6	Day 5	LBR: 10/25, 40.0% (study) versus 38/72, 52.8% (control), $P = 0.27$ MR: 1/11, 9.1% (study) versus 12/50, 24.0% (control), $P = 0.27$
Chen et al., 2022	Retrospective single center	NGS	January 2017–December 2019	China	469 euploid SETs	Day 6	Day 5	LBR: 91/232, 39.2% (study) versus 146/237, 61.6% (control), $P < 0.01$ MR: 17/108, 15.7% (study) versus 15/161, 9.3% (control), $P = 0.11$
Wang et al., 2021a	Retrospective single center	NGS	April 2017–December 2019	China	337 euploid SETs	Day 6/7	Day 5	LBR: 68/168, 40.5% (study) versus 108/169, 63.9% (control), $P < 0.01$ MR: 12/80, 15.0% (study) versus 15/123, 12.2% (control), $P = 0.67$
Zhou et al., 2021	Retrospective single center	NGS	2016–2020	China	316 euploid SETs	Day6	Day 5	LBR: 70/245, 28.6% (study) versus 35/71, 49.3% (control), $P < 0.01$ MR: 23/93, 24.7% (study) versus 3/38, 7.9% (control), $P = 0.03$
Combined trophectoderm biopsy and spent media chromosomal analysis								
Rubio et al., 2019	Prospective single center pilot blinded study	NGS	November 2017–March 2018	Italy	29 euploid SETs	TE biopsy euploid—spent media aneuploid	TE biopsy euploid—spent media euploid	LBR: 2/12, 16.7% (study) versus 9/17, 52.9% (control), $P = 0.06$ MR: 2/4, 50.0% (study) versus 0/9, 0% (control), $P = 0.08$
Yeung et al., 2019	Prospective single center observational	aCGH and NGS	March 2017–June 2018	China	14 euploid SETs	TE biopsy euploid—spent media aneuploid	TE biopsy euploid—spent media euploid	LBR: 3/7, 42.9% (study) versus 3/7, 42.9% (control), $P = 0.99$ MR: 3/6, 50.0% (study) versus 2/5, 40.0% (control), $P = 0.99$
MATERNAL FEATURES								
Age at oocyte retrieval								
Harton et al., 2013	Retrospective multicenter	aCGH	–	USA	343 euploid SETs	Women ≥38 years	Women <38 years	LBR: 67/133, 50.4% (study) versus 131/210, 62.4% (control), $P = 0.03$ MR: 5/72, 6.9% (study) versus 12/143, 8.4% (control), $P = 0.80$
Barash et al., 2017a	Retrospective single center	SNP-array	January 2013–January 2015	USA	368 euploid SETs	Women ≥38 years	Women <38 years	LBR: 105/189, 55.5% (study) versus 98/179, 54.7% (control), $P = 0.92$ MR: not reported
Irani et al., 2019	Retrospective single center	aCGH	2013–2016	USA	785 euploid ETs (700 SETs and 85 DETs)	Women ≥38 years	Women <38 years	LBR: 179/330, 54.2% (study) versus 242/455, 53.2% (control), $P = 0.77$ MR: not reported

(continued)

Table 1. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
Lee *et al.*, 2019a	Retrospective single center	aCGH	November 2012–January 2015	Taiwan	235 euploid ETs (both SETs and DETs)	Women ≥38 years	Women <38 years	LBR: 33/61, 54.1% (study) versus 95/174, 54.6% (control), $P = 0.99$ MR: 7/40, 17.5% (study) versus 11/110, 10% (controls), $P = 0.26$
Boynukalin *et al.*, 2020	Retrospective single center	NGS	October 2015–January 2018	Turkey	707 euploid SETs	Women ≥38 years	Women <38 years	LBR: 144/253, 56.9% (study) versus 259/454, 57.0% (control), $P = 0.99$ MR: 33/177, 18.6% (study) versus 39/298, 13.1% control), $P = 0.11$
Reig *et al.*, 2020	Retrospective single center	qPCR and NGS	2011–2018	USA	8175 euploid SETs	Women ≥38 years	Women <38 years	LBR: 1159/2186, 53.0% (study) versus 3550/5989, 59.3% (control), $P < 0.01$ MR: 174/1333, 13.1% (study) versus 473/4023, 11.8% (control), $P = 0.21$
Tong *et al.*, 2021	Retrospective single center	NGS	August 2018–September 2019	China	125 euploid ETs (both SETs and DETs) in RIF women	Women ≥38 years	Women <38 years	LBR: 8/23, 34.8% (study) versus 41/102, 40.2% (control), $P = 0.8$ MR: 1/9, 11.1% (study) versus 8/49, 16.3% (control), $P = 0.99$
Unexplained infertility								
Taylor *et al.*, 2014a	Retrospective single center	aCGH	January 2010–January 2014	USA	114 euploid ETs (both SETs and DETs)	Infertile patients	Unexplained infertility	LBR: 42/81, 54.3% (study) versus 25/33, 75.8% (control), $P = 0.02$ MR: 2/44, 4.5% (study) versus 3/28, 10.7% (control), $P = 0.37$
Boynukalin *et al.*, 2020	Retrospective single center	NGS	October 2015–January 2018	Turkey	707 euploid SETs	Infertile patients	Unexplained infertility	LBR: 334/608, 54.9% (study) versus 69/99, 69.7% (control), $P < 0.01$ MR: not reported
Boynukalin *et al.*, 2021	Retrospective single center	NGS	January 2016–July 2019	Turkey	1051 euploid SETs	Infertile patients	Unexplained infertility	LBR: not reported MR: 69/488, 14.1% (study) versus 31/201, 15.4% (control), $P = 0.72$
Meng *et al.*, 2021	Retrospective nationally reported 2014 IVF data to SART CORS	aCGH and NGS	2014	USA	4148 euploid ETs (both SETs and DETs)	Infertile patients	Unexplained infertility	LBR: 1000/1901, 52.6% (study) versus 267/495, 53.9% (control), $P = 0.61$ MR: 166/1169, 14.2% (study) versus 45/312, 14.4% (control), $P = 0.93$
Polycystic ovarian syndrome								
Luo *et al.*, 2017	Retrospective single center 1:3 matched-pair study	SNP-array	January 2010–September 2015	China	268 euploid SETs	Lean PCOS	Lean non-PCOS (matched for age, BMI, and embryo quality)	LBR: 25/67, 37.3% (study) versus 97/201, 48.3% (control), $P < 0.01$ MR: 9/34, 26.5% (study) versus 14/111, 12.6% (control), $P = 0.06$
Boynukalin *et al.*, 2020	Retrospective single center	NGS	October 2015–January 2018	Turkey	617 euploid SETs	PCOS	No PCOS	LBR: 48/90, 53.3% (study) versus 320/550, 58.2% (control), $P = 0.42$ MR: not reported
Boynukalin *et al.*, 2021	Retrospective single center	NGS	January 2016–July 2019	Turkey	994 euploid SETs	PCOS	No PCOS	LBR: not reported MR: 13/57, 22.8% (study) versus 74/513, 14.4% (control), $P = 0.12$

(continued)

Table 1. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
Meng *et al.*, 2021	Retrospective nationally reported 2014 IVF data to SART CORS	aCGH and NGS	2014	USA	4148 euploid ETs (both SETs and DETs)	PCOS	No PCOS	LBR: 117/226, 51.8% (study) versus 1150/2170, 53.0% (control), $P = 0.72$ MR: 19/137, 13.9% (study) versus 192/1344, 14.4% (control), $P = 0.99$
					Diminished ovarian reserve			
Boynukalin *et al.*, 2020	Retrospective single center	NGS	October 2015–January 2018	Turkey	617 euploid SETs	DOR	No DOR	LBR: 65/123, 52.8% (study) versus 290/494, 58.7% (control), $P = 0.26$ MR: not reported
Boynukalin *et al.*, 2021	Retrospective single center	NGS	January 2016–July 2019	Turkey	994 euploid SETs	DOR	No DOR	LBR: not reported MR: 13/93, 14.0% (study) versus 74/477, 15.5% (control), $P = 0.87$
Meng *et al.*, 2021	Retrospective nationally reported 2014 IVF data to SART CORS	aCGH and NGS	2014	USA	4148 euploid ETs (both SETs and DETs)	DOR	No DOR	LBR: 201/390, 51.5% (study) versus 1066/2006, 53.1% (control), $P = 0.99$ MR: 33/235, 14.0% (study) versus 178/1246, 14.3% (control), $P = 0.99$
					Endometriosis			
Bishop *et al.*, 2021	Retrospective multicenter	aCGH and NGS	January 2016–March 2018	USA	459 euploid ETs (both SETs and DETs)	Endometriosis	No Endometriosis	LBR: 33/54, 61.1% (study) versus 202/405, 49.9% (control), $P = 0.15$ MR: 6/39, 15.4% (study) versus 60/262, 22.9% (control), $P = 0.41$
Boynukalin *et al.*, 2020	Retrospective single center	NGS	October 2015–January 2018	Turkey	617 euploid SETs	Endometriosis	No Endometriosis	LBR: 44/74, 59.4% (study) versus 311/543, 57.3% (control), $P = 0.8$ MR: not reported
Boynukalin *et al.*, 2021	Retrospective single center	NGS	January 2016–July 2019	Turkey	994 euploid SETs	Endometriosis	No Endometriosis	LBR: not reported MR: 6/43, 14.0% (study) versus 81/527, 15.4% (control), $P = 0.99$
Meng *et al.*, 2021	Retrospective nationally reported 2014 IVF data to SART CORS	aCGH and NGS	2014	USA	4148 euploid ETs (both SETs and DETs)	Endometriosis	No Endometriosis	LBR: 32/64, 50.0% (study) versus 1235/2332, 53.0% (control), $P = 0.70$ MR: 4/36, 11.1% (study) versus 207/1445, 14.3% (control), $P = 0.81$
Vaiarelli *et al.*, 2021	Retrospective case–control multicenter	qPCR	April 2014–March 2018	Italy	485 euploid SETs	Endometriosis	No Endometriosis	LBR: 67/158, 42.4% (study) versus 132/327, 40.4% (control), $P = 0.69$ MR: 11/78, 14.1% (study) versus 24/156, 15.4% (control), $P = 0.84$
					Tubal factor			
Boynukalin *et al.*, 2020	Retrospective single center	NGS	October 2015–January 2018	Turkey	617 euploid SETs	Tubal factor	No Tubal factor	LBR: 40/71, 56.3% (study) versus 315/546, 57.7% (control), $P = 0.90$ MR: not reported
Boynukalin *et al.*, 2021	Retrospective single center	NGS	January 2016–July 2019	Turkey	994 euploid SETs	Tubal factor	No Tubal factor	LBR: not reported MR: 6/25, 24.0% (study) versus 81/545, 14.9% (control), $P = 0.24$

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Table 1. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
Meng *et al.*, 2021	Retrospective nationally reported 2014 IVF data to SART CORS	aCGH and NGS	2014	USA	4148 euploid ETs (both SETs and DETs)	Tubal factor	No Tubal factor	LBR: 49/101, 48.5% (study) versus 1218/2295, 53.1% (control), $P = 0.42$ MR: 11/60, 18.3% (study) versus 200/1421, 14.1% (control), $P = 0.35$
Repeated implantation failure								
Greco *et al.*, 2014	Prospective single center pilot	aCGH	March 2012–March 2013	Italy	85 euploid SETs	RIF	Non-RIF	LBR: 28/41, 68.3% (study) versus 31/44, 70.5% (control), $P = 0.99$ MR: 0/28, 0% (study) versus 0/31, 0% (control), $P = 0.99$
Cimadomo *et al.*, 2021a	Retrospective single center	qPCR and NGS	April 2013–December 2019	Italy	1580 euploid SETs	RIF	Non-RIF	LBR: 93/255, 36.5% (study) versus 599/1326, 45.2% (control), $P = 0.01$ MR: 16/109, 14.7% (study) versus 94/693, 13.6% (control), $P = 0.76$
Zhou *et al.*, 2021	Retrospective single center	NGS	2016–2020	China	316 euploid SETs	RIF	Non-RIF	LB: 4/14, 28.6% (study) versus 101/302, 33.4% (control), $P = 0.99$ MR: 2/6, 33.3% (study) versus 24/125, 19.2% (control), $P = 0.6$
Recurrent pregnancy loss								
Boynukalin *et al.*, 2020	Retrospective single center	NGS	October 2015–January 2018	Turkey	707 euploid SETs	RPL	Non-RPL	LBR: 83/168, 49.4% (study) versus 320/539, 59.4% (control), $P = 0.03$ MR: not reported
Liu *et al.*, 2020	Retrospective single center	SNP-array and NGS	January 2015–December 2018	China	290 euploid ETs (287 SETs + 3 DETs)	RPL	Non-RPL	LBR: 34/89, 38.2% (study) versus 119/201, 59.2% (control), $P < 0.01$ MR: 11/45, 24.4% (study) versus 9/128, 7.0% (control), $P < 0.01$
Cimadomo *et al.*, 2021a	Retrospective single center	qPCR and NGS	April 2013–December 2019	Italy	1580 euploid SETs	RPL	Non-RPL	LBR: 61/136, 44.9% (study) versus 631/1444, 43.7% (control), $P = 0.86$ MR: 11/72, 15.3% (study) versus 99/730, 13.6% (control), $P = 0.72$
Zhou *et al.*, 2021	Retrospective single center	NGS	2016–2020	China	316 euploid SETs	RPL	Non-RPL	LB: 15/43, 34.9% (study) versus 90/273, 33.0% (control), $P = 0.86$ MR: 6/21, 28.6% (study) versus 20/110, 18.2% (control), $P = 0.36$
BMI and body fat								
Cozzolino *et al.*, 2020b	Retrospective multicenter	aCGH and NGS	January 2016–July 2019	Spain	3480 euploid ETs (both SETs and DETs)	BMI: <25 25–29.9 ≥30		LBR: 1209/2704, 44.7% (<25), 265/591, 44.8% (25–30), 63/185, 34.3% (≥30), $P = 0.02$ MR: 96/1305, 7.4% (<25), 26/291, 8.9% (25–30), 13/76, 17.1% (≥30), $P = 0.01$
Meng *et al.*, 2021	Retrospective nationally reported 2014 IVF data to SART CORS	aCGH and NGS	2014	USA	4148 euploid ETs (both SETs and DETs)	BMI: <25 25–29.9 ≥30		LBR: 1125/1987, 56.6% (<25), 336/666, 50.5% (25–29.9), 167/369, 45.3% (≥30), $P < 0.01$ MR: 179/1304, 13.7% (<25), 60/396, 15.2% (25–29.9), 40/207, 19.3% (≥30), $P = 0.11$

(continued)

Table 1. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
Endometrial receptivity array (ERA) test: performed versus not performed								
Neves *et al.*, 2019	Retrospective single center	aCGH	October 2012–December 2018	–	143 euploid ETs (both SETs and DETs) in patients with ≥1 previous implantation failure	ERA performed	ERA not performed	LBR: 11/24, 45.8% (study) versus 64/119, 53.8% (control), $P=0.51$ MR: 3/14, 21.4% (study) versus 20/84, 23.8% (control), $P=0.99$
Cozzolino *et al.*, 2020a	Retrospective multicenter	aCGH and NGS	2013–2018	Spain	216 euploid ETs (both SETs and DETs) in moderate (≥3 previous failures) or severe (≥5 previous failures) RIF patients	ERA performed	ERA not performed	LBR: 9/19, 47.4% (study) versus 110/197, 55.8% (control), $P=0.48$ MR: not reported
Riestenberg *et al.*, 2021a	Prospective single center observational	NGS	January 2018–April 2019	USA	228 euploid SETs	ERA performed	ERA not performed	LBR: 83/147, 56.5% (study) versus 45/81, 55.6% (control), $P=0.89$ MR: 15/99, 15.2% (study) versus 7/53, 13.2% (control), $P=0.75$
Endometrial receptivity array (ERA) test: receptive versus not receptive (personalized ET)								
Tan *et al.*, 2018	Retrospective single center	aCGH and NGS	October 2014–July 2017	Canada	36 euploid ETs (both SETs and DETs) in patients with ≥1 previous implantation failure	ERA non-receptive (personalized-ET)	ERA receptive	LBR: 5/16, 31.3% (study) versus 8/20, 40.0% (control), $P=0.59$ MR: not reported
Neves *et al.*, 2019	Retrospective single center	aCGH	October 2012–December 2018	–	24 euploid ETs (both SETs and DETs) in patients with ≥1 previous implantation failure	ERA non-receptive (personalized-ET)	ERA receptive	LBR: 1/8, 12.5% (study) versus 10/16, 62.5% (control), $P=0.03$ MR: 3/4, 75.0% (study) versus 0/10, 10.0% (control), $P=0.051$
Barrenetxea *et al.*, 2021	Retrospective single center	Not Reported	September 2018–June 2019	Spain	85 euploid SETs	ERA non-receptive (personalized-ET)	ERA receptive	LBR: 28/40, 70.0% (study) versus 25/45, 55.6% (control), $P=0.19$ MR: 4/32, 12.5% (study) versus 2/27, 7.4% (control), $P=0.68$
Riestenberg *et al.*, 2021a	Prospective single center observational	NGS	January 2018–April 2019	USA	147 euploid SETs	ERA non-receptive (personalized-ET)	ERA receptive	LBR: 53/87, 60.9% (study) versus 30/60, 50.0% (control), $P=0.19$ MR: 6/60, 10.0% (study) versus 9/39, 23.1% (control), $P=0.08$
PATERNAL FEATURES								
Age								
Tiegs *et al.*, 2017	Retrospective single center	aCGH	January 2011–November 2014	USA	473 SETs	Men ≥40 years	Men <40 years	LBR: 123/234, 52.6% (study) versus 182/339, 53.7% (control), $P=0.80$ MR: 12/135, 8.9% (study) versus 20/202, 9.9% (control), $P=0.85$

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Table 1. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
Hanson *et al.*, 2020	Retrospective single center	qPCR and NGS	January 2012–December 2018	USA	3769 euploid SETs with LB outcomes + 2959 clinical pregnancies from euploid SETs with miscarriage data	Men ≥40 years	Men <40 years	LBR: 577/965, 59.7% (study) versus 1713/2804, 61.1% (control), $P = 0.42$ MR: 86/770, 11.3% (study) versus 208/2189, 9.5% (control), $P = 0.13$
					Male factor			
Mazzilli *et al.*, 2017	Retrospective single center	qPCR	April 2013–December 2015	Italy	901 euploid ETs (888 SETs and 13 DETs)	Severe male factor (OAT (sperm concentration <15 mil/ml, motility <40%, morphology <4%), cryptozoospermia, surgical sperm retrieval)	No severe male factor	LBR: 82/201, 40.8% (study) versus 294/700, 42.0% (control), $P = 0.81$ MR: 10/92, 10.9% (study) versus 40/334, 12.0% (control), $P = 0.86$
Denomme *et al.*, 2018	Prospective single center matched case–control	qPCR	2010–2014	USA	241 euploid ETs (both SETs and DETs)	Male factor (motility <40%, morphology <3%, sperm count <20 ml/ml, and total motile count <13 mil/ml)	No male factor	LBR: 87/128, 68.0% (study) versus 87/113, 77.0% (control), $P = 0.12$ MR: 15/102, 14.7% (study) versus 2/89, 2.2% (control), $P < 0.01$
Tarozzi *et al.*, 2019	Retrospective single center	aCGH	May 2013–December 2017	Italy	186 euploid ETs (both SETs and DETs)	Severe male factor (sperm concentration <0.1 mil/ml)	No severe male factor	LBR: 7/24, 29.2% (study) versus 39/164, 23.8% (control), $P = 0.61$ MR: 1/8, 12.5% (study) versus 11/50, 22.0% (control), $P = 0.99$
Boynukalin *et al.*, 2020	Retrospective single center	NGS	October 2015–January 2018	Turkey	617 euploid SETs	Male factor (undefined)	No male factor	LBR: 102/183, 55.7% (study) versus 253/434, 58.3% (control), $P = 0.65$ MR: not reported
Boynukalin *et al.*, 2021	Retrospective single center	NGS	January 2016–July 2019	Turkey	994 euploid SETs	Male factor (undefined)	No male factor	LBR: not reported MR: 18/151, 11.9% (study) versus 69/419, 16.5% (control), $P = 0.23$
Meng *et al.*, 2021	Retrospective nationally reported 2014 IVF data to SART CORS	aCGH and NGS	2014	USA	4148 euploid ETs (both SETs and DETs)	Male factor (undefined)	No male factor	LBR: 202/384, 52.6% (study) versus 1065/2012, 52.9% (control), $P = 0.91$ MR: 28/230, 12.2% (study) versus 183/1251, 14.6% (control), $P = 0.36$
Zhou *et al.*, 2021	Retrospective single center	NGS	2016–2020	China	316 euploid SETs	Male factor (undefined)	No male factor	LB: 17/42, 40.5% (study) versus 88/274, 32.1% (control), $P = 0.30$ MR: 2/19, 10.5% (study) versus 24/112, 21.4% (control), $P = 0.36$

(continued)

Table 1. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
CLINICAL or IVF LABORATORY FEATURES								
Gonadotrophins dosage								
Barash *et al.*, 2017a	Retrospective single center	SNP-array	January 2013–January 2015	USA	368 euploid SETs	Gn dosage >3000 IU	Gn dosage <3000 IU	LBR: 130/233, 55.8% (study) versus 73/135, 54.1% (control), $P = 0.83$ MR: not reported
Wu *et al.*, 2018	Retrospective single center	aCGH	January 2013–June 2017	China	683 euploid SETs	Gn dosage >3000 IU	Gn dosage <3000 IU	LBR: 41/78, 52.6% (study) versus 319/605, 52.7% (control), $P = 0.99$ MR: not reported
Double stimulation in a single ovarian cycle (DuoStim)								
Ubaldi *et al.*, 2016	Prospective single center paired non-inferiority	qPCR	January–September 2015	Italy	15 euploid SETs	Second stimulation in the same ovarian cycles	Conventional OS	LBR: 5/8, 62.5% (study) versus 5/7, 71.4% (control), $P = 0.99$ MR: 1/6, 16.7% (study) versus 1/6, 16.7% (control), $P = 0.99$
Vaiarelli *et al.*, 2020	Prospective multicenter observational	qPCR and NGS	October 2015–March 2019	Italy	389 euploid SETs *(in 126 cases, the euploid blastocyst transferred was randomly chosen from either the I or II stimulation in the same ovarian cycle)*	Second stimulation in the same ovarian cycles	Conventional OS	LBR: 102/207, 49.3% (study) versus 80/182, 44.0% (control), $P = 0.3$ MR: 16/118, 13.6% (study) versus 14/94, 14.9% (control), $P = 0.8$
Trigger for final oocyte maturation								
Makhijani *et al.*, 2020	Retrospective single center	aCGH and NGS	January 2013–April 2019	USA	263 euploid SETs	hCG trigger	GnRH-agonist trigger	LBR: 77/118, 65.3% (study) versus 93/145, 64.1% (control), $P = 0.90$ MR: 8/85, 9.4% (study) versus 7/100, 7.0% (control), $P = 0.38$
Tan *et al.*, 2020	Retrospective single center	aCGH and NGS	January 2014–January 2017	Canada	233 euploid SETs in hyper-responder patients (>15 oocytes collected)	hCG trigger	GnRH-agonist trigger	LBR: 26/77, 33.8% (study) versus 80/156, 51.3% (control), $P = 0.02$ MR: 15/38, 39.5% (study) versus 30/97, 30.9% (control), $P = 0.99$
Cimadomo *et al.*, 2021c	Retrospective single center	qPCR and NGS	April 2013–July 2018	Italy	1523 euploid SETs	hCG trigger	GnRH-agonist trigger	LBR: 280/608, 46.0% (study) versus 403/915, 44.0% (control), $P = 0.46$ MR: not reported
Oocyte vitrification								
Forman *et al.*, 2012	RCT single center on sibling oocytes	SNP-array	September 2010–August 2011	USA	26 paired euploid ETs (DET with 1 blastocyst from the control and 1 from the study group) + 23 euploid SETs	Vitrified-warmed oocytes	Fresh oocytes	LBR: 16/29, 55.2% (study) versus 24/46, 52.2% (control), $P = 0.82$ MR: not reported

(continued)

Table 1. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
Goldman *et al.*, 2015	Retrospective single center matched case–control study	aCGH	December 2011–July 2014	USA	64 euploid ETs (52 SETs and 4 DETs)	Vitrified-warmed oocytes	Fresh oocytes	LBR: 10/16, 62.5% (study) versus 22/40, 55.0% (control), $P = 0.8$ MR: 0/10, 0% (study) versus 1/23, 4.3% (control), $P = 0.99$
Culture media								
Cimadomo *et al.*, 2018c	Prospective single center quasi-RCT	qPCR	September 2013–September 2015	Italy	619 euploid ETs (607 SETs and 12 DETs)	Continuous media (Continuous single culture medium, CSCM, Irvine Scientific)	Sequential media (Quinn's advantage cleavage + blastocyst, Sage)	LBR: 168/428, 39.3% (study) versus 81/203, 39.9% (control), $P = 0.93$ MR: 28/195, 14.4% (study) versus 9/89, 10.1% (control), $P = 0.34$
Deng *et al.*, 2020b	Retrospective single center	NGS	July 2013–December 2017	USA	375 euploid SETs	Continuous media (One-step, Sage)	Sequential media (Quinn's advantage cleavage + blastocyst, Sage)	LBR: 105/204, 51.5% (study) versus 94/171, 55.0% (control), $P = 0.53$ MR: 20/125, 16.0% (study) versus 9/103, 8.7% (control), $P = 0.11$
Trophectoderm biopsy protocol								
Zhao *et al.*, 2019	RCT single center	NGS	November 2015–July 2016	China	163 euploid SETs	Simultaneous zona opening and trophectoderm biopsy method	Day3 hatching-based method	LBR: 48/81, 59.3% (study) versus 41/82, 50.0% (control), $P = 0.24$ MR: 4/52, 7.7% (study) versus 6/47, 12.8% (control), $P = 0.40$
Rubino *et al.*, 2020	Retrospective single center matched case–control study	NGS	October 2016–September 2017	USA	1668 euploid SETs	Simultaneous zona opening and trophectoderm biopsy method	Day3 hatching-based method	LBR: 491/834, 58.9% (study) versus 416/834, 46.2% (control), $P < 0.01$ MR: 54/545, 11.7% (study) versus 44/460, 9.6% (control), $P = 0.91$
Xiong *et al.*, 2021b	Retrospective single center	NGS	January–October 2018 (control), November 2018–May 202 (study)	China	69 euploid SETs	Simultaneous zona opening and trophectoderm biopsy method	Day3 hatching-based method	LBR: 20/35, 57.1% (study) versus 21/34, 61.7% (control), $P = 0.81$ MR: 2/23, 8.7% (study) versus 1/22, 4.5% (control), $P = 0.61$
Blastocyst re-biopsy								
Bradley *et al.*, 2017a	Retrospective single center	aCGH and NGS	January 2013–September 2016	Australia	1490 euploid SETs	Two biopsy and vitrification-warming cycles	One biopsy and vitrification-warming cycle	LBR: 6/22, 27.3% (study) versus 734/1468, 50.0% (control), $P = 0.051$ MR: 0/6, 0% (study) versus 52/786, 6.6% (control), $P = 0.99$
Cimadomo *et al.*, 2018b	Retrospective multicenter	qPCR	April 2013–September 2017	Italy	2874 euploid SETs	Two biopsy and vitrification-warming cycles	One biopsy and vitrification-warming cycle	LBR: 19/49, 38.8% (study) versus 1211/2825, 42.9% (control), $P = 0.66$ MR: 2/21, 9.5% (study) versus 168/1379, 12.2% (control), $P = 0.99$
Aluko *et al.*, 2021	Retrospective single center	Not Reported	July 2013–July 2017	USA	2618 euploid SETs	Two biopsy and vitrification-warming cycles	One biopsy and vitrification-warming cycle	LBR: 7/15, 46.7% (study) versus 1434/2603, 55.1% (control), $P = 0.6$ MR: 0/7, 0% (study) versus 171/1624, 10.5% (control), $P = 0.99$

(continued)

Table 1. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
Biopsy and second vitrification-warming of previously vitrified untested blastocysts								
Bradley *et al.*, 2017a	Retrospective single center	aCGH and NGS	January 2013–September 2016	Australia	1494 euploid SETs	One biopsy and two vitrification-warming cycles	One biopsy and vitrification-warming cycle	LBR: 10/26, 38.5% (study) versus 734/1468, 50.0% (control), $P = 0.32$ MR: 0/10, 0% (study) versus 52/786, 6.6% (control), $P = 0.99$
Aluko *et al.*, 2021	Retrospective single center	Not Reported	July 201–July 2017	USA	2698 euploid SETs	One biopsy and two vitrification-warming cycles	One biopsy and vitrification-warming cycle	LBR: 27/95, 28.4% (study) versus 1434/2603, 55.1% (control), $P < 0.01$ MR: 8/37, 21.6% (study) versus 171/1624, 10.5% (control), $P = 0.053$
Fresh or vitrified-warmed transfer								
Rodriguez-Purata *et al.*, 2016	Retrospective single center	qPCR and aCGH	January 2011–December 2015	USA	744 euploid ETs (both SETs and DETs)	Vitrified-warmed ET (freeze-all or after a first fresh ET)	Fresh ET	LBR: 236/428, 55.1% (study) versus 147/316, 46.5% (control), $P = 0.02$ MR: not reported
Coates *et al.*, 2017	RCT single center	NGS	December 2013–August 2015	USA	107 euploid ETs (both SETs and DETs)	Vitrified-warmed ET	Fresh ET	LBR: 47/61, 77.0% (study) versus 27/46, 58.7% (control), $P = 0.04$ MR: not reported
Endometrial preparation protocol for vitrified-warmed transfer								
Greco *et al.*, 2016	RCT single center	aCGH	2015	Italy	222 euploid SETs	Hormone replacement	Modified natural cycle	LBR: 47/113, 41.5% (study) versus 50/109, 45.8% (control), $P = 0.61$ MR: 8/57, 14.0% (study) versus 6/59, 10.2% (control), $P = 0.57$
Melnick *et al.*, 2017	Retrospective single center	aCGH and SNP-array	October 2011–December 2014	USA	113 euploid SETs in anovulatory women	Hormone replacement	Modified natural cycle	LBR: 18/48, 37.5% (study) versus 41/65, 63.1% (control), $P < 0.01$ MR: 3/21, 14.3% (study) versus 2/43, 4.7% (control), $P = 0.32$
Zhou *et al.*, 2021	Retrospective single center	NGS	2016–2020	China	316 euploid SETs	Hormone replacement	Modified natural cycle	LBR: 70/207, 33.8% (study) versus 35/109, 32.1% (control), $P = 0.8$ MR: 19/89, 21.3% (study) versus 7/42, 16.7% (control), $P = 0.64$

Grade A, B, or C is defined according to Gardner and Schoolcraft's criteria.
CCT, comprehensive chromosome testing; aCGH, array comparative genomic hybridization; qPCR, quantitative polymerase chain reaction; SNP-array, single nucleotide polymorphisms array; NGS, next generation sequencing; SET, single embryo transfer; DET; double embryo transfer; LBR, live birth rate; MR, miscarriage rate; TE, trophectoderm; PCOS, polycystic ovarian syndrome; DOR, diminished ovarian reserve; RIF, repeated implantation failure; RPL, recurrent pregnancy loss; BMI, body mass index; ERA, endometrial receptivity array; OAT, oligoasthenoteratozoospermia; Gn, gonadotrophins; OS, ovarian stimulation; hCG, human chorionic gonadotrophin; GnRH, gonadotrophin releasing hormone.

Table 2. Articles included in the review but not meta-analyzed because (i) the primary and/or secondary outcomes of this meta-analysis were not retrievable, (ii) only one or two articles were available for the meta-analysis, and/or (iii) the main variables under investigation were continuous and could not be categorized into similar groups used in other studies.

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
EMBRYONIC FEATURES								
Inner cell mass morphology								
Moutos *et al.*, 2021	Retrospective single center	NGS	June 2007–December 2018	USA	539 euploid SETs	Grade C	Grade A/B	LBR (>12 gestational weeks): 12/31, 38.7% (study) versus 290/508, 57.1% (control), $P = 0.06$ MR (<12 gestational weeks): 2/14, 14.3% (study) versus 49/339, 14.5% (control), $P = 0.99$
Trophectoderm morphology								
Moutos *et al.*, 2021	Retrospective single center	NGS	June 2007–December 2018	USA	539 euploid SETs	Grade C	Grade A/B	OPR (>12 gestational weeks): 294/511, 57.5% (control) versus 8/28, 28.6% (study), $P < 0.01$ MR (<12 gestational weeks): 49/343, 14.2% (control) versus 2/10, 20% (study), $P = 0.34$
Overall blastocyst morphological quality from Excellent to Poor								
Moutos *et al.*, 2021	Retrospective single center	NGS	June 2007–December 2018	USA	539 euploid SETs	<BB	≥BB	OPR (>12 gestational weeks): 16/40, 40.0% (study) versus 286/499, 57.3% (control), $P = 0.05$ MR (>12 gestational weeks): 1/17, 5.9% (study) versus 50/336, 14.9% (control), $P = 0.49$
Day of biopsy								
Moutos *et al.*, 2021	Retrospective single center	NGS	June 2007–December 2018	USA	539 euploid SETs	Day 6/7	Day 5	OPR (>12 gestational weeks): 75/156, 48.1% (study) versus 227/383, 59.3% (control), $P = 0.02$ MR (<12 gestational weeks): 13/88, 14.8% (study) versus 38/256, 14.8% (control), $P = 0.99$
Mono-pronuclear zygotes								
Bradley *et al.*, 2017b	Retrospective single center	aCGH and NGS	June 2013–August 2016	Australia	1098 euploid SETs	1PN-derived blastocysts	2PN-derived blastocysts	CPR (>4 gestational weeks): 9/26, 34.6% (study) versus 573/1072, 53.5% (control), $P = 0.07$ MR: not reported
Multinucleation in day2								
Balakier *et al.*, 2016	Retrospective single center	aCGH	–	Canada	74 euploid SETs	MN at the 2-cell stage	No MN at the 2-cell stage	OPR (>12 gestational weeks): 12/36, 33.3% (study) versus 29/38, 76% (control), $P < 0.01$ MR: not reported

(continued)

Table 2. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
Number of blastomeres in day3 of preimplantation development								
Pons *et al.*, 2019	Retrospective single center	aCGH	July 2014–June 2017	Spain	297 euploid SETs	Number of blastomeres in day3: >11 9–11 8 <8		LBR: 27/50, 54.0% (>11 cells), versus 45/79, 57.0% (9–11 cells), 69/133, 51.9% (8 cells), 10/35, 28.6% (<8 cells), $P = 0.04$ MR: 7/34, 20.6% (>11 cells),7/52, 13.5% (9–11 cells), 9/78, 11.5% (8 cells), 4/14, 28.6% (<8 cells), $P = 0.3$
Abnormal cleavage patterns								
Ozbek *et al.*, 2021	Retrospective single center	aCGH and NGS	April 2015–October 2017	Turkey	291 euploid SETs	Reverse or direct cleavage	No abnormal cleavage	LBR: 14/53, 25.4% (study) versus 133/238, 55.9% (control), $P < 0.01$ MR: 5/20, 25% (study) versus 31/166, 18.7% (control), $P < 0.01$
Morula compaction								
Lagalla *et al.*, 2020	Retrospective single center	aCGH	May 2013–July 2017	Italy	1271 embryos from PGT-A cycles	Partial morula compaction	Complete morula compaction	OPR (undefined): 31/137, 22.6% (study) versus 28/89, 33.8% (control), $P = 0.16$ MR: not reported
Blastocyst expansion dynamics								
Gazzo *et al.*, 2020b	Retrospective single center	NGS	–	Peru	114 euploid SETs	Blastocysts undergoing spontaneous collapse(s)	Blastocysts that did not collapse	OPR (undefined): 14/30, 46.7% (study) versus 53/84, 63.1% (control), $P = 0.012$ MR: not reported
Huang *et al.*, 2021	Retrospective single center	NGS	January 2018–December 2019	USA	66 euploid SETs	Blastocyst expansion dynamics: Group 1 (Blastocyst area >20 000 μ^2 and tSB < 110 hpi) Group 2 (Blastocyst area >20 000 μ^2 and tSB > 110 hpi) Group 3 (Blastocyst area <20 000 μ^2 and tSB < 110 hpi) Group 4 (Blastocyst area <20 000 μ^2 and tSB > 110 hpi)		LBR: 85.0% (group 1), 68.7% (group 2), 63.6% (group 3), 58.3% (group 4), P-value < 0.05 MR: not reported
Timings of preimplantation development								
Yang *et al.*, 2014	Prospective multicenter on sibling oocytes	aCGH	February–December 2012	USA	45 euploid ETs (19 SETs and 26 DETs)	tSB ≥96.1 h	tSB <96.1 h	OPR: 11/18, 61.1% (study) versus 20/27, 74.1% (control), $P = 0.51$ MR: 0/11, 0% (study) versus 1/21, 4.8% (control), $P = 0.99$
Mumusoglu *et al.*, 2017	Retrospective single center	aCGH	April 2015–October 2016	Turkey	129 euploid SETs	tB-tSB: continuous variable		tB-tSB: 9.5 ± 3.4 h (no-OP) versus 8.1 ± 3.2 h (OP, >12 gestational weeks), $P = 0.014$, OR 0.81, 95% CI 0.70–0.93
Hung *et al.*, 2018	Retrospective single center	aCGH and NGS	March 2013–March 2017	Taiwan	34 euploid SETs	Early blastulation in day4	No early blastulation in day4	OPR (>12th gestational weeks): 10/14, 71.4% (study) versus 10/20, 50% (control), $P = 0.29$ MR: not reported
Rienzi *et al.*, 2019	Retrospective multicenter	qPCR, aCGH, and NGS	January 2016–June 2018	Italy, Spain	830 euploid SETs	tM ≥80 h	tM <80 h	LBR: 252/662, 38.1% (study) versus 92/168, 54.7% (control), $P < 0.01$

(continued)

Table 2. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
McQueen *et al.*, 2021	Retrospective single center	SNP-array and NFS	October 2015–January 2018	USA	192 euploid SETs	tPNf, t2, t3, t4, t8, tM, and tB: continuous variables		MR: not reported LB: no difference Miscarriage: no difference
Mitochondrial DNA score from a trophectoderm biopsy								
Diez-Juan *et al.*, 2015	Retrospective single center	aCGH	–	Spain	65 euploid SETs	Mitoscore: A (<18.19) B (18.19–24.15) C (24.15–50.58) D (>50.58)		OPR (undefined): 13/16, 81.3% (A), versus 8/16, 50.0% (B), 10/16, 62.5% (C), 3/17, 17.6% (D), $P < 0.01$ MR: not reported
Fragouli *et al.*, 2015	Prospective non-selection multicenter	aCGH	–	–	42 euploid ETs	qPCR- or NGS-based mtDNA relative quantification >0.003	qPCR- or NGS-based mtDNA relative quantification <0.003	OPR (undefined): 0/15, 0% (study) versus 16/27, 59.3% (control), $P < 0.01$ MR: not reported
Fragouli *et al.*, 2017	Prospective non-selection single center	NGS	–	USA	199 euploid SETs	Elevated mtDNA content (i.e. relative mtDNA >0.0004 (mitochondrial 16 s rRNA assay) or >0.000335 (MajArc assay))	Normal or low mtDNA content (i.e. relative mtDNA <0.0004 (mitochondrial 16 s rRNA assay) or <0.000335 (MajArc assay))	OPR (undefined): 0/9, 0% (study) versus 121/190, 63.7% (control), $P < 0.01$ MR (undefined): 0/0, – (study) versus 10/131, 7.6% (control)
Ravichandran *et al.*, 2017	Non-selection multicenter center	aCGH and NGS	–	USA	282 euploid SETs	qPCR-based mtDNA quantification >0.0004	qPCR-based mtDNA quantification <0.0004	OPR (undefined): 0/33, 0% (study) versus 185/249, 74.3% (control), $P < 0.01$ MR: not reported
Treff *et al.*, 2017	Non-selection single center	qPCR	January 2010–July 2016	USA	187 euploid DETs of different sex embryos (in 69 cases a singleton was obtained)	qPCR-based relative mtDNA quantification		Mean 0.16 (no LB) versus 0.19 (LB), $P = 0.6$ (sub-analysis within the 69 pairs where one implanted and one did not: $P = 0.81$) MR: not reported
Victor *et al.*, 2017	Non-selection single center	NGS	.	USA	241 euploid SETs (in 24 cases paired from the same patient, one implanted and one not implanted)	qPCR- or NGS-based relative mtDNA quantification		No association between mtDNA score and OP (>5 gestational weeks) ($P = 0.231$). MR: not reported
Lledo *et al.*, 2018	Prospective non-selection single center	NGS	January 2017–December 2017	Spain	159 euploid SETs	NGS-based mtDNA relative quantification >0.003	NGS-based mtDNA relative quantification <0.003	OPR (undefined): 3/17, 17.7% (study) versus 61/142, 43.0% (control), $P = 0.05$ MR (undefined): 2/5, 40.0% (study) versus 4/65, 6.2% (control), $P = 0.01$
Lee *et al.*, 2019b	Prospective non-selection single center	NGS	January 2016–September 2018	Taiwan	267 euploid SETs	NGS-based adjusted mtDNA relative quantification: continuous variable		CPR (>4 gestational weeks): median 0.00088 (not implanted) versus 0.00097 (implanted), $P = 0.21$ MR: not reported
Boynukalin *et al.*, 2020	Retrospective single center	NGS	October 2015–January 2018	Turkey	707 euploid SETs	Mitoscore: continuous variable		median 20.6, quartile 1 16.4—quartile 3 25.2 (no LB) versus median 18.7, quartile 1 15.5—quartile 3 23.7 (LB), $P < 0.01$ MR: not reported

(continued)

Table 2. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
Scott et al., 2020	Non-selection single center	NGS	July 2016–June 2017	USA	615 euploid SETs plus 78 euploid SETs from 39 patients (one implanted and one not implanted)	qPCR-based relative mtDNA quantification		No difference between embryo resulting in OP (>9 gestational weeks) versus no OP ($P = 0.78$), also among paired SETs with opposite outcomes ($P = 0.7$) MR: not reported
El-Damen et al., 2021	Retrospective single center	NGS	April 2017–December 2018	United Arab Emirates	355 euploid SETs	Mitoscore: continuous variable		Mean ± SD 30.4 ± 10.8 (miscarriage), 29.3 ± 8.6 (implantation failure) versus 27.0 ± 8.9 (LB), $P = NS$
Wang et al., 2021a	Non-selection single center	NGS	April 2017–December 2019	China	337 euploid SETs	NGS-based relative mtDNA quantification		mtDNA relative content: median 0.00043, quartile 1 0.00018 quartile 3 0.00140 (miscarriage), median 0.00041, quartile 1 0.00002, quartile 3 0.00221 (implantation failure) versus median 0.00042, quartile 1 0.00006, quartile 3 0.00182 (LB), $P = NS$
Zhou et al., 2021	Non-selection single center	NGS	2016–2020	China, Single center	316 euploid SETs	NGS-based relative mtDNA quantification		No significant difference in the mtDNA content among groups: median 1.00×10^8, quartile 1 7.59×10^7, quartile 3 1.39×10^8 (miscarriage), and median 9.91×10^7, quartile 1 7.08×10^7, quartile 3 1.40×10^8 (implantation failure) versus median 1.01×10^8, quartile 1 7.37×10^7, quartile 3 1.32×10^8) (LB), $P = 0.999$
Heterosplasmic sites in mitochondrial DNA								
Lledo et al., 2018	Prospective non-selection single center	NGS	January 2017–December 2017	Spain	159 euploid SETs	Heteroplasmic sites in mtDNA: 1–2 Heteroplasmic sites in mtDNA >2	Heteroplasmic sites in mtDNA: none	OPR (undefined): 15/35, 42.8% (1–2), 1/5, 20.0% (>2) versus 49/119, 41.2% (control), $P = 0.6$ MR (undefined): 3/18, 12.5% (1–2), 0/1, 0% (>2) versus 4/53, 7.8% (control), $P = 0.53$
Cumulus cells transciptomics								
Parks et al., 2016	Prospective single center observational	SNP-array	–	USA	10 euploid SETs	Cumulus cells RNA sequencing expression analysis (transcriptomics)		306 significantly differentially expressed genes ($P < 0.05$; fold change ≥1.5) between embryos that resulted in LB versus those that did not. qRT–PCR validation conducted for APC, AXIN1, and GSK3B gene transcription relative to RPL19.
Green et al., 2018	Prospective single center observational on sibling oocytes	qPCR	January 2014–May 2014	USA	17 euploid DETs	Cumulus cells RNA sequencing expression analysis (transcriptomics)		132 differentially expressed genes between sibling embryos that resulted in a LB versus those that did not were identified ($P < 0.05$). However, after correcting for multiple testing, none of the genes remained significantly differentially expressed (FDR < 0.05).

(continued)

Table 2. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
Spent blastocyst media miRNomics								
Capalbo *et al.*, 2016b	Prospective single center observational	qPCR	–	Italy	53 euploid SETs	Spent Blastocyst Media (SBM) TaqMan Low-Density Array (TLDA) miRNA analysis (miRNomics)		2 differentially expressed miRNAs (miR-20a and miR-30c; $P < 0.05$) showed increased concentrations in SBM between embryos that resulted in LB versus those that did not+5 miRNAs (miR-220, miR-146b-3p, miR-512-3p, miR-34c, miR-375) were preferentially detected in SBM samples from embryos that resulted in LB
Cimadomo *et al.*, 2019a	Prospective multicenter observational	qPCR	September 2015–December 2017	Italy	221 euploid SETs	Custom protocol (Exiqon) qPCR analysis of 10 assays plus controls and calibrators (selected miRNA analysis)		miR-182-5p, miR-302a-3p, and miR-519d-3p showed higher detection rates in embryos that failed to implant+miR-302a-3p, miR-372-3p, miR-373-3p, and miR-518a-3p showed higher 'expression' in embryos that failed to implant. All differences were not significant after adjustments in a multivariate logistic regression analysis.
Combined trophectoderm biopsy and blastocoel fluid chromosomal analysis								
Magli *et al.*, 2019	Retrospective single center	aCGH	January 2015–December 2017	Italy	53 euploid SETs	DNA amplification from the blastocoel fluid	DNA amplification failure from the blastocoel fluid	LBR: 6/19, 31.5% (study) versus 23/34, 67.6% (control), $P = 0.01$ MR: 1/7, 14.3% (study) versus 3/26, 11.5% (control), $P = 0.99$
MATERNAL FEATURES								
Age at oocyte retrieval								
Guzman *et al.*, 2019	Retrospective single center	aCGH and SNP-array	January 2013–March 2016	Peru	482 euploid SETs	Women >35 years	Women ≤35 years	CPR (undefined): 190/315, 60.3% (study) versus 100/167, 59.9% (control), $P = 0.9$ MR (undefined): 11/201, 5.5% (study) versus 2/102, 2.0% (control), $P = 0.23$
Sekhon *et al.*, 2019	Retrospective single center	qPCR, aCGH, and NGS	January 2012–June 2017	USA	1135 euploid SETs	Maternal age: continuous variable		Mean 36.4 ± 3.8 years (no LB) versus 36.0 ± 4.1 (LB), $P = 0.07$ miscarriage: not reported
Boynukalin *et al.*, 2021	Retrospective single center	NGS	January 2016–July 2019	Turkey	1051 euploid SETs	Maternal age: continuous variable		median ± SE 36.1 ± 0.4 (miscarriage) versus 36.0 ± 0.2 years (LB), $P = 0.75$; adjusted-OR: 0.99, 95% CI 0.91–1.08, $P = 0.82$
Zhou *et al.*, 2021	Retrospective single center	NGS	2016–2020	China	316 euploid SETs	Maternal age: continuous variable		Mean ± SD: 31.6 ± 4.7 years (miscarriage), 33.2 ± 4.7 (implantation failure) versus 32.3 ± 4.7 years (LB), $P = 0.116$
Number of previous IVF attempts								
Boynukalin *et al.*, 2020	Retrospective single center	NGS	October 2015–January 2018	Turkey	707 euploid SETs	Number of previous: continuous		median 3, quartile 1 2—quartile 3 4 (no LB) versus median 2, quartile 1 1—quartile 3 4 (LB), $P = 0.95$ Miscarriage: not reported
Boynukalin *et al.*, 2021	Retrospective single center	NGS	January 2016–July 2019	Turkey	1051 euploid SETs	Number of previous: continuous		median ± SE 2.38 ± 0.21 (miscarriage) versus 2.55 ± 0.09 (LB), $P = 0.51$

(continued)

Table 2. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
Diminished ovarian reserve								
Katz-Jaffe *et al.*, 2013	Prospective single center observational	SNP-array	2007–2011	USA	Euploid ETs (absolute numbers cannot be retrieved)	Abnormal ovarian reserve (Day 2/3 FSH >10 mIU/ml and/or AMH ≤1 ng/ml)	Normal ovarian reserve	LBR: 78% (study) versus 70.9% (control), $P = 0.33$ MR: not reported
Jaswa *et al.*, 2021	Retrospective single center	aCGH, SNP-array, and NGS	2010–2019	USA	944 euploid SETs	DOR defined according to the Bologna criteria	No DOR	LBR: 55% (study) versus 57% (control), $P = 0.94$ MR: not reported
Adenomyosis								
Neal *et al.*, 2020	Prospective single center observational	NGS	April–December 2017	USA	638 euploid SETs	Women affected from adenomyosis	Women not affected from adenomyosis	LBR: 66/95, 69.5% (study) versus 361/543, 66.5% (control), $P = 0.57$ MR: 10/76, 13.2% (study) versus 42/407, 10.3% (control), $P = 0.43$
Arcuate uterus								
Surrey *et al.*, 2018	Retrospective single center	aCGH	January–December 2014	USA	437 euploid ETs (both SETs and DETs)	Women with a diagnosis of arcuate uterus	Women with normal uterine cavity	LBR: 57/83, 68.7% (study) versus 260/378, 68.7% (control), $P = 0.99$ MR: 4/61, 6.6% (study) versus 16/276, 5.8%, (control), $P = 0.77$
Inflammatory bowel disease								
Hernandez-Nieto *et al.*, 2020b	Retrospective propensity score matching-based single center	qPCR and NGS	January 2012–January 2018	USA	152 euploid SETs	Women affected from inflammatory bowel diseases (Chron's diseases or ulcerative colitis)	Women not affected from inflammatory bowel diseases	LBR: 17/38, 62.9% (study) versus 65/114, 73.0% (control), $P = 0.6$ MR: 2/19, 10.5% (study) versus 4/69, 5.8% (control), $P = 0.61$
BMI and body fat								
Sekhon *et al.*, 2019	Retrospective single center	qPCR, aCGH, and NGS	January 2012–June 2017	USA	1135 euploid SETs	BMI: continuous variable		Mean 23.8 ± 4.4 (no LB) versus 23.3 ± 4.0 (LB), $P = 0.05$ MR: not reported
Boynukalin *et al.*, 2020	Retrospective single center	NGS	October 2015–January 2018	Turkey	707 euploid SETs	BMI: continuous variable		median 27, quartile 1 24—quartile 3 29.2 (no LB) versus median 22.70, quartile 1 21.50—quartile 3 24.60 (LB),, $P < 0.01$; adjusted-OR: 0.79, 95% CI 0.73 0.85, $P < 0.01$ miscarriage: not reported
Boynukalin *et al.*, 2021	Retrospective single center	NGS	January 2016–July 2019	Turkey	1051 euploid SETs	BMI: continuous variable		median±SE 26.0 ± 0.5 (miscarriage) versus 24.4 ± 0.21 (LB), $P = 0.02$; adjusted-OR: 1.08, 95% CI 1.01–1.16, $P = 0.02$
Kim *et al.*, 2021	Prospective single center observational	qPCR and NGS	June 2016–January 2019	USA	Euploid ETs (absolute numbers cannot be retrieved)	BMI: <18.5 18.5–24.9 25–29.9 ≥30		LBR: 57% (<18.5), 70% (18.5–24.9), 72% (25–29.9), 68% (≥30), $P = NS$ MR: not reported

(continued)

Table 2. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
Kim *et al.*, 2021	Prospective single center observational	qPCR and NGS	June 2016–January 2019	USA	Euploid ETs (absolute numbers cannot be retrieved)	Body fat as determined by bioelectric impedance analysis (BIA): <25% 25–30.9% 31–39.9% ≥40%		LBR: 69% (<25%), 70% (25–30.9%), 71% (31–39.9%), 68% (≥40%), P = NS MR: not reported
Zhou *et al.*, 2021	Retrospective single center	NGS	2016–2020	China	316 euploid SETs	BMI: continuous variable		Mean ± SD: 21.0 ± 1.9 (miscarriage), 21.6 ± 2.4 (implantation failure) versus 21.5 ± 2.5 (LB), $P = 0.315$
					Basal AMH			
Morin *et al.*, 2018b	Retrospective single center	qPCR	2012–2016	USA	768 euploid ETs in women <38 years (both SETs and DETs)	AMH 1.1–4.5 ng/ml	AMH ≤0.5 ng/ml	LBR: 445/668, 66.6% (study) versus 63/101, 62.4% (control), $P = 0.47$ MR: 48/493, 9.7% (study) versus 12/75, 16.0% (control), $P < 0.01$
Wang *et al.*, 2019b	Retrospective single center	Not Reported	2014–2018	USA	389 euploid SETs	Basal AMH: <1 ng/ml 1–5 ng/ml >5 ng/ml		OPR (>12 gestational weeks): 37/68, 54.4% (<1 ng/ml), 123/235, 53.2% (1–5 ng/ml), 45/86, 53.2% (>5 ng/ml), $P = 0.95$ MR (<12 gestational weeks): 9/46, 19.5% (<1 ng/ml), 40/163, 24.5% (1–5 ng/ml), 14/59, 23.7% (>5 ng/ml), $P = 0.78$
Pipari *et al.*, 2021	Retrospective single center	aCGH	January 2015–December 2019	Spain	1673 euploid ETs (both SETs and DETs)	Basal AMH: <1 ng/ml 1–5 ng/ml ≥5 ng/ml		LBR: 249/475, 52.4% (<1), 540/1064, 50.8% (1–5), 69/134, 51.5% (>5), $P = 0.83$ MR: 36/285, 12.6% (<1), 81/621, 13.0% (1–5), 10/79, 12.7% (>5), $P = 0.98$
					Progesterone			
Kofinas *et al.*, 2015	Retrospective single center	aCGH	2010–2013	USA	213 euploid SETs	Serum progesterone levels the day of ET ≥20 ng/ml	Serum progesterone levels the day of ET <20 ng/ml	OPR (undefined) or LBR: 49% (study) versus 65% (control), $P = 0.02$; the OPR/LBR decreased at increasing serum progesterone levels (10–15 ng/ml, 15–20 ng/ml, 20–30 ng/ml, 30–40 ng/ml, and >40 ng/ml: 70%, 62%, 52%, 50%, and 33%) MR: not reported
Gaggiotti-Marre *et al.*, 2019	Retrospective single center	aCGH	January 2016–June 2017	Spain	244 euploid ETs (both SETs and DETs)	Serum progesterone levels the day prior to ET: Quartile 1 (≤8.06 ng/ml) Quartile 2 (8.07–10.64 ng/ml) Quartile 3 (10.65–13.13 ng/ml) Quartile 4 (>13.13 ng/ml)		LBR: 25/61, 41.0% (≤8.06 ng/ml), versus 33/61, 54.1% (8.07–10.64 ng/ml), 36/61, 59.0% (10.65–13.13 ng/ml), 40/61, 65.6% (>13.13 ng/ml), $P = 0.05$ MR: 12/37, 32.4% (≤8.06 ng/ml), versus 9/42, 21.4% (8.07–10.64 ng/ml), 4/40, 10.0% (10.65–13.13 ng/ml), 4/44, 9.1% (>13.13 ng/ml), $P = 0.02$
Boynukalin *et al.*, 2019	Prospective single center observational	NGS	March–August 2018	Turkey	168 euploid SETs	Serum progesterone levels the day of ET: Quartile 1 (<13.6 ng/ml) Quartile 2 (13.6–24.3 ng/ml) Quartile 3 (24.4–53.2 ng/ml) Quartile 4 (>53.2 ng/ml)		OPR (>12 gestational weeks): 11/42, 26.2% (<13.6 ng/ml), versus 32/43, 74.4% (13.6–24.3 ng/ml), 22/42, 52.4% (24.4–53.2 ng/ml), 34/41, 82.9% (>53.2 ng/ml), $P < 0.01$ MR (<12 gestational weeks): 4/15, 26.7% (<13.6 ng/ml), versus 2/34, 5.9% (13.6–24.3 ng/ml), 3/25, 12% (24.4–53.2 ng/ml), 0/34, 0% (>53.2 ng/ml), $P = 0.015$

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Table 2. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
Boynukalin *et al.*, 2020	Retrospective single center	NGS	October 2015–January 2018	Turkey	707 euploid SETs	Serum progesterone levels on the day of trigger: continuous variable		median 0.66 ng/ml, quartile 1 0.32—quartile 3 1.1 (no LB) versus median 0.62 ng/ml, quartile 1 0.31—quartile 3 0.88 (LB), $P = 0.26$ miscarriage: not reported
Boynukalin *et al.*, 2020	Retrospective single center	NGS	October 2015–January 2018	Turkey	707 euploid SETs	Serum progesterone levels on the day of progesterone initiation: continuous variable		median 0.13 ng/ml, quartile 1 0.085—quartile 3 0.25 (no LB) versus median 0.15 ng/ml, quartile 1 0.08—quartile 3 0.25 (LB), $P = 0.21$ miscarriage: not reported
Hernandez-Nieto *et al.*, 2020a	Retrospective single center	qPCR and NGS	September 2016–March 202	USA	4333 euploid SETs	Serum progesterone levels on the day of trigger >2 ng/ml	Serum progesterone levels on the day of trigger ≤2 ng/ml	LBR: 97/143, 67.8% (study) versus 3020/4190, 72.1% (control), $P = 0.65$ MR: 12/109, 11.0% (study) versus 429/3449, 12.4% (control), $P = 0.77$
Álvarez *et al.*, 2021	Prospective single center observational	NFS	November 2018–January 2020	Spain	574 euploid ETs (both SETs and DETs)	Low serum progesterone level on the day prior to ET <10.6 ng/ml, which were given subcutaneous progesterone and re-established to normal levels	Serum progesterone on day prior to ET >10.6 ng/ml	LBR: 115/220, 52.3% (study) versus 168/342, 49.1% (control), $P = 0.49$ MR: 14/130, 10.8% (study) versus 24/193, 12.4% (control), $P = 0.72$
Boynukalin *et al.*, 2021	Retrospective single center	NGS	January 2016–July 2019	Turkey	1051 euploid SETs	Serum progesterone levels on the day of progesterone initiation: continuous variable		Miscarriage: median ± SE 0.20 ± 0.02 (miscarriage) versus 0.27 ± 0.06 (LB), $P = 0.92$
Labarta *et al.*, 2021	Prospective single center observational	Not Reported	September 2017–November 2018	Spain	308 ETs (both SETs and DETs)	Serum progesterone levels the day of ET ≥8.8 ng/ml	Serum progesterone levels the day of ET <8.8 ng/ml	LBR: 53.1% (study) versus 34.3% (control), $P < 0.01$ MR: 11.7% (study) versus 19.0% (control), $P = 0.30$
Pardiñas *et al.*, 2021	Retrospective single center	Not Reported	January 2016–October 2018	Spain	1597 unmatched and 72 matched patients	Progesterone on the day of trigger ≥1.5 ng/ml	Progesterone on the day of trigger <1.5 ng/ml	LBR in unmatched patients: OR 1.08 (95% CI 0.65–1.75), $P =$ NS LBR in matched patients: OR 2.00 (95% CI 0.74–5.53), $P =$ NS MR: not reported
					Estradiol			
Irani *et al.*, 2020	Retrospective single center	aCGH and NGS	January 2013–December 2017	USA	930 SETs	Peak estradiol levels (pg/ml): <2000 2000–3000 >3000		LBR: No difference in the three groups, also when clustered according to maternal age MR: not reported
Boynukalin *et al.*, 2020	Retrospective single center	NGS	October 2015–January 2018	Turkey	707 euploid SETs	Serum estradiol levels on the day of progesterone initiation: continuous variable		median 319 pg/ml, quartile 1 232—quartile 3 442.5 (no LB) versus median 305 pg/ml, quartile 1 233—quartile 3 405 (LB), $P = 0.59$ miscarriage: not reported
Boynukalin *et al.*, 2021	Retrospective single center	NGS	January 2016–July 2019	Turkey	1051 euploid SETs	Serum estradiol levels on the day of progesterone initiation: continuous variable		median ± SE 355.7 pg/ml ± 40.35 (miscarriage) versus 325.1 pg/ml ± 0.06 (LB), $P = 0.99$

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Table 2. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
Romanski *et al.*, 2021	Retrospective single center	NGS	January 2013–December 2018	USA	635 euploid ETs (both SETs and DETs)	Median number of days from the estradiol level of >100 pg/ml before the LH surge in natural frozen ETs: >4 days	Median number of days from the estradiol level of >100 pg/ml before the LH surge in natural frozen ETs: ≤4 days	LBR: 202/316, 63.9% (study) versus 177/319, 55.5% (control), $P = 0.035$ MR: 14/216, 6.5% (study) versus 11/188, 5.9% (control), $P = 0.83$
					TSH			
Green *et al.*, 2015	Retrospective single center	Not Reported	February 2012–August 2014	USA	1599 euploid ETs (both SETs and DETs)	TSH 8 days after ET: <0.5 mIU/l 0.5–0.99 mIU/l 1–1.4 mIU/l 1.5–1.99 mIU/l 2–2.5 mIU/l >2.5 mIU/l		LBR: 18/28, 63% (<0.5 mIU/l), versus 64/96, 66.6% (0.5–0.99 mIU/l), 170/240, 70.8% (1–1.4 mIU/l), 249/372, 66.9% (1.5–1.99 mIU/l), 216/292, 73.9% (2–2.5 mIU/l), 400/571, 70.0% (>2.5 mIU/l), $P = 0.36$ MR: 0/18, 0% (<0.5 mIU/l), versus 0/64, 0% (0.5–0.99 mIU/l), 12/182, 6.6% (1–1.4 mIU/l), 30/279, 10.8% (1.5–1.99 mIU/l), 15/231, 6.5% (2–2.5 mIU/l), 29/429, 6.8% (>2.5 mIU/l), $P = 0.10$
					IGF-1, IGF-2, and IGFBP-1			
Irani *et al.*, 2018a	Retrospective single center	aCGH	–	USA	156 euploid ETs (not specified)	Serum IGF1 levels in cycle Day 10: continuous variable Serum IGF2 levels in cycle Day 10: continuous variable Serum IGFBP-1 levels in cycle Day 10: continuous variable		Serum IGF1 levels: 18.0 ± 1.1 (miscarriage) versus 14.6 ± 0.7 ng/mL (LB), $P = 0.03$ Serum IGF2 levels: 452.5 ± 13.2 (miscarriage) versus 471.1 ± 11.3 ng/mL (LB), $P =$ NS Serum IGFBP-1 levels: 28.6 ± 2.7 (miscarriage) versus 26.1 ± 1.4 ng/mL (LB), $P =$ NS
					Vitamin D			
Franasiak *et al.*, 2015a	Retrospective single center	qPCR	December 2012–December 2013	USA	529 euploid ETs (not specified)	Serum levels of 25-hydroxy vitamin D (25-OH D) drawn on day of ovulation trigger: <20 ng/mL (deficient) 20–29.9 ng/ml (insufficient)	Serum levels of 25-hydroxy vitamin D (25-OH D) drawn on day of ovulation trigger: ≥30 ng/mL (replete)	OPR (>12 gestational weeks): 131/206, 63.6% (deficient), 133/215, 61.9% (insufficient) versus 60/96, 62.5% (replete), $P =$ NS MR: 13/144, 9.0% (deficient), 18/151, 11.9% (insufficient) versus 4/64, 6.3% (replete), $P = 0.41$
					Drugs			
Green *et al.*, 2015	Retrospective single center	Not Reported	February 2012–August 2014	USA	1599 euploid ETs (both SETs and DETs)	Patients not taking levothyroxine	Patients taking levothyroxine	LBR: 705/1015, 69.5% (study) versus 408/584, 69.9% (control), $P = 0.86$ MR: not reported

(continued)

Table 2. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
Hernandez-Nieto *et al.*, 2017	Retrospective single center	qPCR and NGS	January 2012–March 2017	USA	2132 euploid SETs	Selective serotonin reuptake inhibitor (SSRI) exposed patients (at least 1 month before and throughout endometrial preparation for ET and continued after ET up to 12–14 gestational weeks	Selective serotonin reuptake inhibitor (SSRI) not exposed Patients	CPR: 58/97, 59.7% (study) versus 1186/2035, 58.2% (control), $P = 0.76$, OR 0.70 (95% CI 0.70–1.61) MR: not reported
Endometrial scratch								
Werner *et al.*, 2015	Retrospective single center	Not Reported	2010–2014	USA	290 euploid ETs (both SETs and DETs) in patients with 1 previous implantation failure after euploid ET	Endometrial scratch not performed	Endometrial scratch performed in a cycle before ET	Ongoing implantation rate (>9 gestational weeks): 38.5% (study) versus 42.6% (control), $P = 0.6$ MR: not reported
Endometrial compaction *(Decrease in the thickness of the endometrium from the end of the proliferative phase to the time of transfer)*								
Zilberberg *et al.*, 2020	Retrospective single center	NGS	February 2016–October 2018	Canada	234 euploid SETs	Endometrial compaction: ≥20% 15–20% 10–15% 5–10% <5%		OPR (>13 gestational weeks): 28/51, 54.9% (≥20%), versus 6/15, 40.0% (15–20%), 5/20, 25.0% (10–15%), 4/11, 36.4% (5–10%), 39/128, 30.5% (<5%), $P = 0.03$ MR: not reported
Riestenberg *et al.*, 2021b	Prospective single center observational	NGS	January–December 2018	USA	225 euploid SETs	<5% endometrial compaction	≥5% endometrial compaction	LBR: 124/216, 57.4% (study) versus 25/43, 58.1% (control), $P = 0.99$ MR: 17/147, 11.6% (study) versus 1/27, 3.7% (control), $P = 0.31$
Endometrial receptivity array (ERA) test: performed versus not performed								
Bergin *et al.*, 2021	Retrospective propensity score matched single center	Not Reported	January 2014–June 2019	USA	357 euploid ETs (both SETs and DETs). They correspond to >70% of all ETs performed in the study	ERA performed	ERA not performed	LBR: 49.6% (study—75.1% PGT-A cycles) versus 55.0% (control—72.8% PGT-A cycles), $P = 0.29$ MR: 13.4% (study—75.1% PGT-A cycles) versus 10.6% (control—72.8% PGT-A cycles), $P = 0.49$
Uterine fluid-derived extracellular vesicles transcriptomics								
Giacomini *et al.*, 2021	Prospective single center observational	NGS	–	Italy	42 euploid SETs	Uterine fluid-derived extracellular vesicles (UF-EVs) (collected on Day 7 after detection of a urinary LH surge in the month preceding ET) RNA sequencing expression analysis (transcriptomics)		161 genes were differentially 'expressed' between successful LBs and implantation failures + 14 transcripts selectively detected in UF-EVs of women with a LB and 5 in women with an implantation failure.

(continued)

Table 2. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
Endometrial microbiome at the time of transfer								
Franasiak *et al.*, 2016	Prospective single center observational	qPCR	–	USA	33 euploid SETs	Most distal 5-mm portion of the transfer catheter analyzed by NGS to assess the bacteria specific 16S ribosome gene, thereby allowing genus and species calls for microorganisms.		There was a total of 278 different genus calls present across patient samples (18 OP >8 gestational weeks versus 15 no-OP), although none reached enough statistical significance
Vaginal fluid microbiome at the time of transfer								
Bernabeu *et al.*, 2019	Prospective single center observational	NGS	April 2017–January 2018	Spain	31 euploid SETs	V3 V4 region of 16S rRNA amplified and sequenced in the vaginal fluid taken with dry swabs from the bottom of the rectouterine pouch just before ET		Greater but not significant ($P = 0.09$) alpha index of diversity in patients who did not obtain a positive pregnancy test compared to those who did. Also, the beta diversity was not significantly different.
PATERNAL FEATURES								
Age								
Boynukalin *et al.*, 2020	Retrospective single center	NGS	October 2015–January 2018	Turkey	707 euploid SETs	Male age: continuous variable		median 37, quartile 1 30—quartile 3 42 (no LB) versus median 37, quartile 1 30—quartile 3 43 (LB), $P = 0.528$ miscarriage: not reported
Boynukalin *et al.*, 2021	Retrospective single center	NGS	January 2016–July 2019	Turkey	1051 euploid SETs	Male age: continuous variable		Miscarriage: median ± SE 38.7 ± 0.6 (miscarriage) versus 38.7 ± 0.6 (LB), $P = 0.93$
Zhou *et al.*, 2021	Retrospective single center	NGS	2016–2020	China	316 euploid SETs	Male age: continuous variable		Mean±SD: 34.0 ± 4.7 years (miscarriage), 34.5 ± 5.2 years (implantation failure) versus 34.6 ± 6.1 years (LB), $P = 0.896$
Sperm DNA fragmentation								
Gat *et al.*, 2017	Retrospective single center	aCGH	January 2014–March 2016	USA	88 euploid ETs (both SETs and DETs)	DFI >15%	DFI ≤15%	OPR (>12 gestational weeks): 24/52, 46.2% (study) versus 15/36, 41.7% (control), $P = 0.83$ MR: 6/29, 24% (study) versus 2/17, 12% (control), $P = 0.69$
Irani *et al.*, 2018b	Retrospective single center	aCGH	January 2013–December 2016	USA	35 euploid SETs	DFI >15%	DFI ≤15%	LBR: 13/23, 52.5% (study) versus 6/12, 50.0% (control), $P = 0.7$ MR: 0/13, 0% (study) versus 0/6, 0% (control), $P = 0.99$
Green *et al.*, 2020	Prospective single center observational	qPCR and NGS	December 2014–June 2017	USA	180 euploid ETs (both SETs and DETs)	DFI >15%	DFI ≤15%	OPR (>9 gestational weeks): 72.6% (study) versus 65.9% (control), $P = 0.45$ MR: 8.8% (study) versus 4.2% (control), $P = 0.38$
CLINICAL or IVF LABORATORY FEATURES								
Ovarian stimulation or natural cycle for oocyte retrieval cycle								
Hong *et al.*, 2019	Prospective single center observational with historical control	SNP-array	April 2013–August 2015	USA	1646 euploid SETs	Modified natural cycle	OS	OPR (>8 gestational weeks): 48/79, 60.8% (study) versus 986/1567, 62.9% (control), $P = 0.72$ MR: not reported

(continued)

Table 2. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
Ovarian stimulation protocol for oocyte retrieval cycle								
Zhou *et al.*, 2021	Retrospective single center	NGS	2016–2020	China	316 euploid SETs	All other protocols	Antagonist protocol	LBR: 48/149, 32.2% (study) versus 57/167, 34.1% (control), $P = 0.72$ MR: 13/61, 21.3% (study) versus 13/70, 18.6% (control), $P = 0.83$
Gonadotropins dosage during ovarian stimulation for oocyte retrieval cycle								
Boynukalin *et al.*, 2020	Retrospective single center	NGS	October 2015–January 2018	Turkey	707 euploid SETs	Gn dosage: continuous variable		median 2235 IU, quartile 1 1662.5—quartile 3 3000 (no LB) versus median 2250 IU, quartile 1 1650—quartile 3 2850 (LB), $P = 0.93$ Miscarriage: not reported
Irani *et al.*, 2020	Retrospective single center	aCGH and NGS	January 2013–December 2017	USA	930 SETs	Gn dosage (IU): <4000 4000–6000 >6000		LBR: No difference in the three groups, also when clustered according to maternal age MR: not reported
Boynukalin *et al.*, 2021	Retrospective single center	NGS	January 2016–July 2019	Turkey	1051 euploid SETs	Gn dosage: continuous variable		median ± SE 2456.1 IU ± 87.8 (miscarriage) versus 2398.5 IU ± 40.9 (LB), $P = 0.37$
Zhou *et al.*, 2021	Retrospective single center	NGS	2016–2020	China	316 euploid SETs	Gn dosage: continuous variable		Mean ± SD: 2422.6 ± 449.3 IU (miscarriage), 2359.0 ± 738.0 IU (implantation failure) versus 2302.7 ± 778.9 IU (LB), $P = 0.599$
Oocytes retrieved after ovarian stimulation								
Barash *et al.*, 2017a	Retrospective single center	SNP-array	January 2013–January 2017	USA	651 euploid SETs	Oocytes retrieved: continuous variable		OP (>8 gestational weeks): OR 1, 95% CI 0.98–1.01, $P = 0.97$
Morin *et al.*, 2018b	Retrospective single center	qPCR	2012–2016	USA	768 euploid ETs in women <38 years (both SETs and DETs)	Oocytes retrieved ≤5	Oocytes retrieved >10	LBR: 80/108, 75.9% (study), versus 627/974, 64.3% (control), $P = 0.06$ MR: 6/86, 7.0% (study), versus 94/721, 13.0% (control), $P = 0.12$
Wu *et al.*, 2018	Retrospective single center	aCGH	January 2013–June 2017	China	683 euploid SETs	Oocytes retrieved ≤5	Oocyte retrieved >5	LBR: 21/59, 35.6% (study), versus 330/624, 52.9% (control), $P = 0.01$ MR: not reported
Boynukalin *et al.*, 2020	Retrospective single center	NGS	October 2015–January 2018	Turkey	707 euploid SETs	Oocytes retrieved: continuous variable		median 11, quartile 1 6—quartile 3 16.5 (no LB) versus median 11, quartile 1 7—quartile 3 16 (LB), $P = 0.69$ miscarriage: not reported
Irani *et al.*, 2020	Retrospective single center	aCGH and NGS	January 2013–December 2017	USA	930 SETs	Oocytes retrieved: <10 10–19 ≥20		LBR: No difference in the three groups, also when clustered according to maternal age MR: not reported
Boynukalin *et al.*, 2021	Retrospective single center	NGS	January 2016–July 2019	Turkey	1051 euploid SETs	Oocytes retrieved: continuous variable		median ± SE 12.2 ± 0.8 (miscarriage) versus 12.1 ± 0.3 (LB), $P = 0.31$
Fertilization method								
Bradley *et al.*, 2017b	Retrospective single center	aCGH and NGS	June 2013–August 2016	Australia	1072 2PN-derived euploid SETs	ICSI	IVF	CPR (>4 gestational weeks): 349/637, 54.8% (study) versus 224/435, 51.5% (control), $P = 0.29$ MR: not reported

(continued)

Table 2. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
Culture media								
Werner *et al.*, 2016	RCT single center on sibling zygotes	Not Reported	August 2013–March 2015	USA	126 paired euploid ETs (DET with 1 blastocyst from the control and 1 from the study group) + 42 euploid SETs	Continuous media (continuous culture medium, CSCM, Irvine Scientific)	Sequential media (Quinn's advantage cleavage Medium, Sage+Blast Assist, Origio)	OPR (>9 gestational weeks): 26/54, 48.1% (study) versus 31/60, 51.7% (control), $P = 0.85$ MR: not reported
Fabozzi *et al.*, 2021	Prospective single center on sibling oocytes	qPCR and NGS	April 2018–April 2019	Italy	81 euploid SETs	Continuous media (CSCM, Irvine Scientific)	Continuous media (Gems, Genea)	LBR: 14/34, 41.2% (study) versus 29/47, 61.7% (control), $P = 0.08$ MR: 2/16, 12.5% (study) versus 3/32, 9.4% (control), $P = 0.99$
Individual or group culture								
Glatthorn *et al.*, 2021	Prospective single center observational	NGS	August 2018–December 2019	USA	593 euploid SETs	Group culture	Individual culture	LBR: 90/144, 62.5% (study) versus 273/449, 60.8% (control), $P = 0.76$ MR: 2/92, 2.2% (study) versus 19/292, 6.5% (control), $P = 0.18$
Culture temperature								
Hong *et al.*, 2014	RCT single center on sibling oocytes	qPCR	February 2012–December 2012	USA	42 paired euploid ETs (DET with 1 blastocyst from the control and 1 from the study group) + 4 euploid SETs	Culture temperature 36 °C	Culture temperature 37 °C	LBR: 29/43, 67.4% (study) versus 33/45, 73.3% (control), $P = 0.28$ MR: not reported
Dynamic embryo culture								
Juneau *et al.*, 2020	RCT single center on sibling oocytes	Not Reported	June 2015–March 2017	USA	42 paired euploid ETs (DET with 1 blastocyst from the control and 1 from the study group) + 19 euploid SETs	Dynamic embryo culture system (NSSB-300, Nepagene: frequency of 42 Hz for 5 min every 60 min)	Static embryo culture system	LBR: 67.1% (study) versus 63.1% (control), $P = 0.14$ MR: similar in the two groups
Embryo selection based on static versus morphodynamic assessments								
Yang *et al.*, 2014	Prospective multicenter on sibling oocytes	aCGH	February–December 2012	USA	82 euploid ETs (34 SETs and 48 DETs)	Morphokinetics-based embryo selection	Static morphology-based embryo selection	LBR: 31/45, 68.9% (study) versus 15/37, 40.5% (control), $P = 0.019$ MR: 1/32, 3.2% (study) versus 2/17, 11.8% (control), $P = 0.273$
Rocafort *et al.*, 2018	Retrospective single center	NGS	October 2013–February 2016	Spain	81 euploid SETs	Eeva-based embryo selection (high, medium, and low groups)	Static morphology-based embryo selection	OPR (>12 gestational weeks): 15/20, 75% (High score), $P < 0.01$; versus 9/18, 50% (Medium score), $P = 0.38$; versus 2/6, 33.3% (Low Score) versus 13/37, 35.1% (static), $P = 0.99$ MR (<12 gestational weeks): 1/16, 6.3% (High score), $P = 0.99$; versus 1/10, 10.0% (Medium score), $P = 0.99$; versus 0/2, 0% (Low score) versus 0/13, 0% (static), $P = 0.99$

(continued)

Table 2. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
Gazzo *et al.*, 2020a	Retrospective single center	NGS	October 2016–June 2018	Peru	135 euploid SETs	KidscoreTM D5 algorithm	Static morphology-based embryo selection	OPR (undefined): 32/48, 66.7% (study) versus 42/86, 48.8% (control), $P = 0.037$ MR: not reported
Trophectoderm biopsy operator								
Capalbo *et al.*, 2016a	Retrospective multicenter	qPCR	April 2013–December 2014	Italy	494 euploid SETs	7 biopsy operators		LBR: Op. 1: 51/112, 45.5%; Op. 2: 41/91, 45.1%; Op. 3: 37/90, 41.1%; Op. 4: 31/64, 48.8%; Op. 5: 30/75, 40.0%; Op. 6: 16/34, 47.1%; Op. 7: 17/28, 60.7%; P = NS MR: Op. 1: 5/56, 8.9%; Op. 2: 5/46, 10.9%; Op. 3: 4/41, 9.8%; Op. 4: 3/34, 8.8%; Op. 5: 4/34, 11.8%; Op. 6: 2/18, 11.1%; Op. 7: 0/17, 0%; P = NS
Maggiulli *et al.*, 2019	Retrospective single center	qPCR and NGS	–	Italy	572 euploid SETs	7 biopsy operators		LBR: Op. 1: 73/182, 40.1%; Op. 2: 43/108, 39.8%; Op. 3: 33/106, 31.1%; Op. 4: 26/57, 45.6%; Op. 5: 26/53, 49.1%; Op. 6: 22/56, 39.3%; Op. 7: 4/10, 40.0%; P = NS MR: not reported
Trophectoderm biopsy number of cells								
Neal *et al.*, 2017	Retrospective single center	qPCR	January 2010–February 2014	USA	1147 euploid SETs	Relative DNA content in the biopsy sample (proxy of the cellularity) Quartile 1 (lowest) Quartile 2 Quartile 3 Quartile 4 (highest)		LBR: 163/264, 61.7% (quartile 1); 171/290, 59.0% (quartile 2); 172/282, 61.0% (quartile 3); 159/311, 51.1% (quartile 4); $P = 0.03$ MR: 25/188, 13.3% (quartile 1); 28/199, 14.1% (quartile 2); 29/201, 14.4% (quartile 3); 36/195, 18.5% (quartile 4); $P = 0.49$
Guzman *et al.*, 2019	Retrospective single center	aCGH and SNP-array	January 2013–March 2016	Peru	482 euploid SETs	Cellularity from validated biopsy operators (average 10)	Cellularity from validated biopsy operators (average 5)	CPR (undefined): 115/215, 53.4% (study) versus 175/267, 65.5% (control), $P < 0.01$ MR (undefined): 6/121, 5.0% (study) versus 7/182, 3.8% (control), $P = 0.77$
Time between biopsy and vitrification								
Chen *et al.*, 2017	Retrospective single center	aCGH	December 2012–May 2015	Taiwan	223 euploid SETs	Time between biopsy and vitrification ≥180 min	Time between biopsy and vitrification <180 min	LBR: 120/179, 67.0% (study) versus 22/44, 50.0% (control), $P = 0.04$ MR: 12/131, 9.2% (study) versus 2/24, 8.3% (control), $P = 0.13$
Maggiulli *et al.*, 2019	Retrospective single center	qPCR and NGS	–	Italy	572 euploid SETs	Time between biopsy and vitrification: ≤30 min 31–90 min >90 min		LBR: 92/251, 36.7% (31–90 min), N = 81/204, 39.7% (>90 min) versus 56/117, 47.9% (≤30 min), $P = 0.12$ MR: not reported
Xiong *et al.*, 2021a	Retrospective single center	NGS	January 2015–December 2019	China	79 euploid SETs	Time between biopsy and vitrification: <60 min 60–120 min >120 min		OPR (undefined): 8/17, 47.1% (60–120 min), 7/19, 36.8% (>120 min) versus 23/43, 53.5% (<60 min), $P = 0.48$ MR (undefined): 1/9, 11.1% (60–120 min), 3/10, 30.0% (>120 min) versus 5/29, 17.2% (<60 min), $P = 0.54$

(continued)

Table 2. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
Blastocyst re-biopsy								
Taylor *et al.*, 2014b	Retrospective single center	aCGH	January 2009–April 2013	USA	87 euploid ETs (both SETs and DETs)	Two biopsy and vitrification-warming cycles	One biopsy and vitrification-warming cycle	OPR (undefined): 0/2, 0% (study) versus 49/85, 57.6% (control), $P = 0.19$ MR: not reported
Neal *et al.*, 2019	Retrospective single center	NGS	June 2016–October 2018	USA	3578 euploid SETs	Two biopsy and vitrification-warming cycles	One biopsy and vitrification-warming cycle	OPR (8 gestational weeks): 18/36, 50.0% (study) versus 2366/3542, 66.8% (control), $P = 0.05$ MR (<8 gestational weeks): 5/23, 21.7% (study) versus 256/2622, 9.8% (control), $P = 0.07$
Biopsy and second vitrification-warming of previously vitrified untested blastocysts								
Taylor *et al.*, 2014b	Retrospective single center	aCGH	January 2009–April 2013	USA	94 euploid ETs (both SETs and DETs)	One biopsy and two cryopreservation cycles	One biopsy and vitrification-warming cycle	OPR (undefined): 5/9, 55.6% (study) versus 49/85, 57.6% (control), $P = 0.99$ MR: not reported
Neal *et al.*, 2019	Retrospective single center	NGS	June 2016–October 2018	USA	3697 euploid SETs	One biopsy and two cryopreservation cycles	One biopsy and vitrification-warming cycle	OPR (8 gestational weeks): 98/155, 62.3% (study) versus 2366/3542, 66.8% (control), $P = 0.38$ MR (<8 gestational weeks): 18/116, 15.5% (study) versus 256/2622, 9.8% (control), $P = 0.06$
Fresh or vitrified-warmed transfer								
Ma *et al.*, 2016	Prospective single center observational	aCGH and NGS	–	Taiwan	21 euploid ETs (8 fresh SETs, 4 vitrified SETs, and 9 vitrified DETs)	Vitrified-warmed ET (both SETs and DETs)	Fresh ET (all SETs)	OPR (>8 gestational weeks): 7/13, 53.8% (study) versus 5/8, 62.5% (control), $P = 0.99$ MR (<8 gestational weeks): 3/10, 30% (study) versus 2/7, 28.6% (control), $P = 0.99$
Transfer difficulty								
Alvarez *et al.*, 2019	Retrospective single center	aCGH	April 2014–December 2016	Spain	370 euploid ETs (307 SETs and 63 DETs)	Difficult ET (Wallace stylet/tenaculum)	Easy ET (i.e. direct/outer sheath)	LBR: 34/84, 40.5% (study) versus 156/286, 54.5% (control), $P = 0.03$ MR: 12/46, 26.1% (study) versus 39/195, 20.0% (control), $P = 0.42$
Different transfer operators								
Guzman *et al.*, 2019	Retrospective single center	aCGH and SNP-array	January 2013–March 2016	Peru	482 euploid SETs	8 physicians		CPR (undefined): Physician 1: 42/73, 57%, Physician 2: 30/82, 37%, Physician 3: 38/75, 51%, Physician 4: 8/12, 67%, Physician 5: 21/42, 50%, Physician 6: 5/11, 45%, Physician 7: 44/76, 58%, Physician 8: 15/24, 62%, P = NS from a multivariable logistic regression analysis
Endometrial preparation protocol for vitrified-warmed transfer								
Wang *et al.*, 2019c	Retrospective single center	Not Reported	2014–2018	USA	389 euploid SETs	Hormone replacement	(Modified) natural cycle	OPR (>8 gestational weeks): 75/175, 42.9% (study) versus 130/214, 60.7% (control), $P < 0.01$ MR: not reported

(continued)

Table 2. (continued)

Article	Study design	CCT technique	Period of observation	Country	Population	Study group	Control group	Results
Follicular phase length prior to LH surge in natural vitrified-warmed transfer cycles								
Romanski *et al.*, 2021	Retrospective single center	Not Reported	January 2013–December 2018	USA	783 euploid ETs (both SETs and DETs)	Follicular phase length prior to LH surge >15 days in natural vitrified-warmed ETs	Follicular phase length prior to LH surge ≤15 days in natural vitrified-warmed ETs	LBR: 257/420, 61.2% (study) versus 212/363, 58.4% (control), $P = 0.46$ MR: 19/276, 6.9% (study) versus 12/224, 5.4% (control), $P = 0.58$
Progesterone and estradiol administration during endometrial preparation for vitrified-warmed transfer								
Asoglu *et al.*, 2019	Retrospective single center	aCGH and NGS	January 2015–March 2018	Turkey	767 euploid SETs	Daily vaginal progesterone plus intramuscular hydroxyprogesterone caproate	Daily intramuscular progesterone	LBR: 80/159, 50.3% (study) versus 315/608, 51.8% (control), $P = 0.74$ MR: 18/98, 18.4% (study) versus 47/362, 12.9% (control), $P = 0.19$
Sekhon *et al.*, 2019	Retrospective single center	qPCR, aCGH, and NGS	January 2012–June 2017	USA	1135 SETs	Route of progesterone administration: Vaginal or oral Intramuscular Both		LBR: 330/678, 48.7% (intramuscular), 58/150, 65.3% (both) versus 139/302, 46.0% (vaginal or oral), $P < 0.01$ MR: not reported
Sekhon *et al.*, 2019	Retrospective single center	qPCR, aCGH, and NGS	January 2012–June 2017	USA	1135 SETs	Days of oestrogen administration: continuous variable		Mean 17.4 days ± 2.8 (no LB) versus 17.5 days ± 3.1 (LB), $P = 0.51$ miscarriage: not reported
Sekhon *et al.*, 2019	Retrospective single center	qPCR, aCGH, and NGS	January 2012–June 2017	USA	1135 SETs	Cumulative dose of oral oestrogen: continuous variable		Mean 93.8 ± 19.5 mg (no LB) versus 92.8 ± 18 mg (LB), $P = 0.38$ miscarriage: not reported
Different IVF centers in multicenter studies								
Capalbo *et al.*, 2014	Retrospective multicenter	aCGH	January 2009–August 2013	Italy, USA	168 euploid ETs (both SETs and DETs)	2 IVF centers		LBR: IVF center 1: 42/82, 51.2%; IVF center 2: 51/86, 59.3%; $P = 0.35$ MR: IVF center 1: 2/44, 4.5%; IVF center 2: 6/57, 10.5%; $P = 0.46$
Capalbo *et al.*, 2016a	Retrospective multicenter	qPCR	April 2013–December 2014	Italy	494 euploid SETs	3 IVF centers		LBR: IVF center 1: 190/432, 44.0%; IVF center 2: 16/34, 47.1%; IVF center 3: 17/28, 60.7%; $P = 0.22$ MR: IVF center 1: 21/211, 9.9%; IVF center 2: 2/18, 11.1%; IVF center 3: 0/17, 0%; $P = 0.8$
Cimadomo *et al.*, 2018b	Retrospective multicenter	qPCR	June 2016–August 2017	Italy	962 euploid SETs	2 IVF centers		LBR: IVF center 1: 287/719, 39.9%; IVF center 2: 103/243, 42.4%; $P = 0.50$ MR: not reported
Rienzi *et al.*, 2019	Retrospective multicenter	qPCR, aCGH, and NGS	September 2017–June 2018 (validation phase)	Italy, Spain	319 euploid SETs	3 IVF centers		LBR: IVF center 1: 34/74, 45.9%; IVF center 2: 68/168, 40.5%; IVF center 3: 35/77, 45.5%; $P = 0.64$ MR: not reported

Grade A, B, or C is defined according to Gardner and Schoolcraft's criteria.
CCT, comprehensive chromosome testing; aCGH, array comparative genomic hybridization; qPCR, quantitative polymerase chain reaction; SNP-array, single nucleotide polymorphisms array; NGS, next generation sequencing; SET, single embryo transfer; DET; double embryo transfer; LBR, live birth rate; MR, miscarriage rate; OPR, ongoing pregnancy rate; CPR, clinical pregnancy rate; PN, pronuclei; MN, multinucleation; tPNf, time of PN fading; t(n), time of (n) cells; tM, time of morula formation; tSB, time of starting blastulation; tB, time of blastocyst formation; DOR, diminished ovarian reserve; BMI, body mass index; DFI, DNA fragmentation index; ERA, endometrial receptivity array; Gn, gonadotrophins; OS, ovarian stimulation; AMH, anti-Mullerian hormone; TSH, thyroid stimulating hormone; mtDNA, mitochondrial DNA; LH, luteinizing hormone; FSH, follicle stimulating hormone; IGF, insulin growth factor; IGFBP, IGF binding protein.

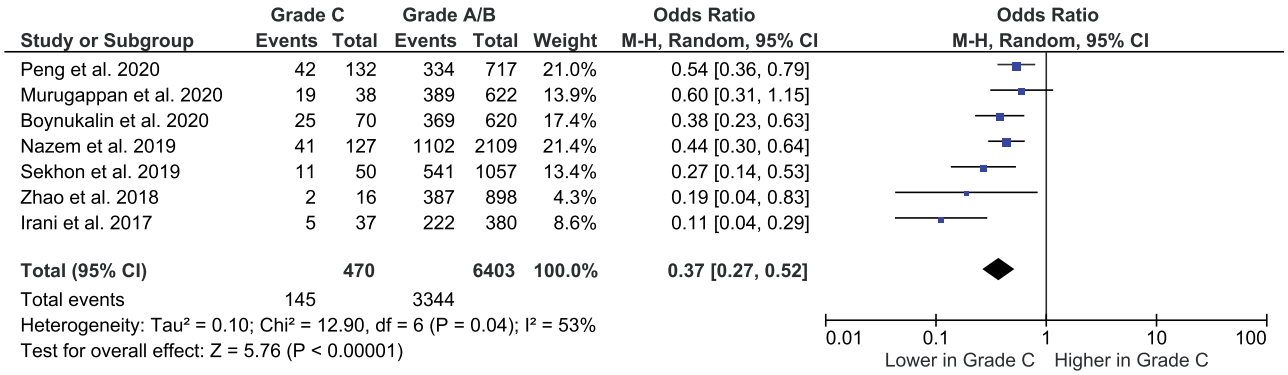

Figure 2. Grade C inner cell mass (ICM) was associated with a lower live birth rate per euploid transfer than Grade A/B ICM.

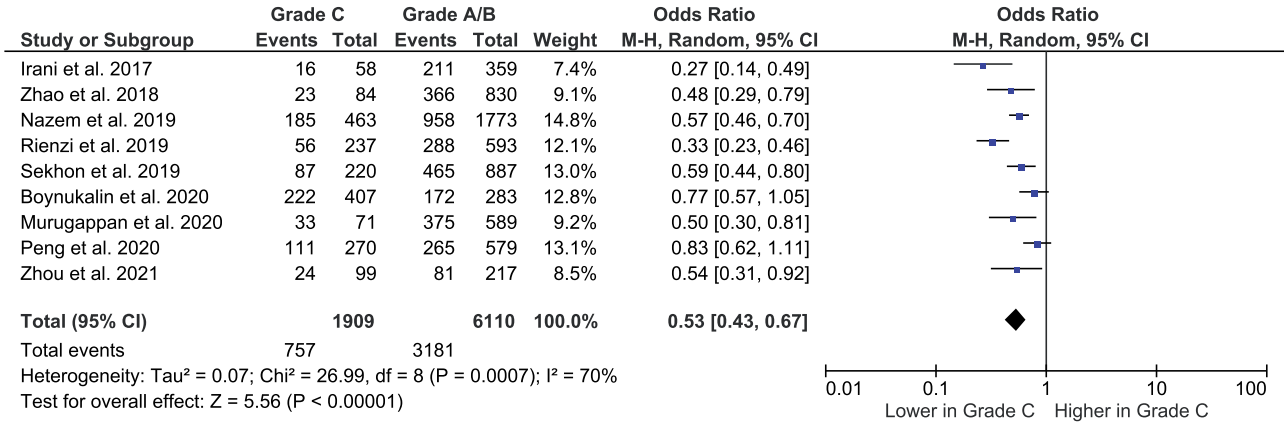

Figure 3. Grade C trophectoderm (TE) was associated with a lower live birth rate per euploid transfer than Grade A/B TE.

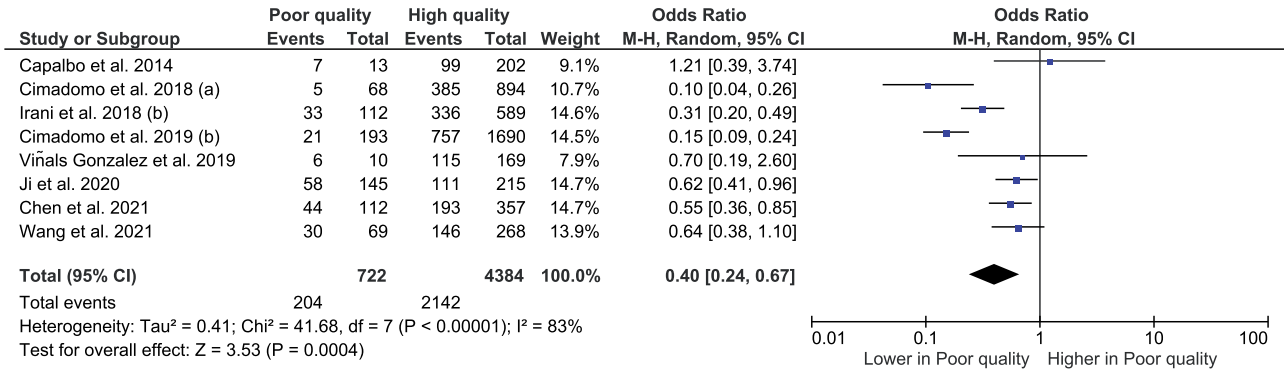

Figure 4. Poor-quality blastocysts (<BB) were associated with a lower live birth rate per euploid transfer than high-quality blastocysts.

Kimelman *et al.*, 2019; Sekhon *et al.*, 2019; Whitney *et al.*, 2019; Boynukalin *et al.*, 2020; Peng *et al.*, 2020; Sardana *et al.*, 2020; Ji *et al.*, 2021; Wang *et al.*, 2021a; Zhou *et al.*, 2021; Chen *et al.*, 2022) (Table 1). One study instead reported only OPR and MR based on a 12 gestational weeks threshold and could not be meta-analyzed (Moutos *et al.*, 2021) (Table 2).

In our meta-analysis, Day 6–7 blastocysts (N = 4627 overall) were associated with a significantly lower LBR per euploid SET than Day 5 blastocysts (N = 6716 overall) with an OR 0.56, 95% CI 0.49–0.63, $I^2 = 47\%$, $P < 0.01$ (Fig. 5). The MR per clinical pregnancy (N = 1753 from Day 6–7 blastocysts and N = 3062 from day5) was also significantly higher for the former group (OR 1.49, 95% CI 1.25–1.76, $I^2 = 0\%$, $P < 0.01$) (Supplementary Fig. S4).

Mono-pronuclear zygotes, multinucleation in Day 2, and number of cells in day3

Fertilization is generally assessed through microscopic evaluation of the inseminated oocyte at 16–18 hpi. The presence of 2 pronuclei (2 PN) outlines normal fertilization with equal genomic contribution from the oocyte and the sperm. In cases where 1PN

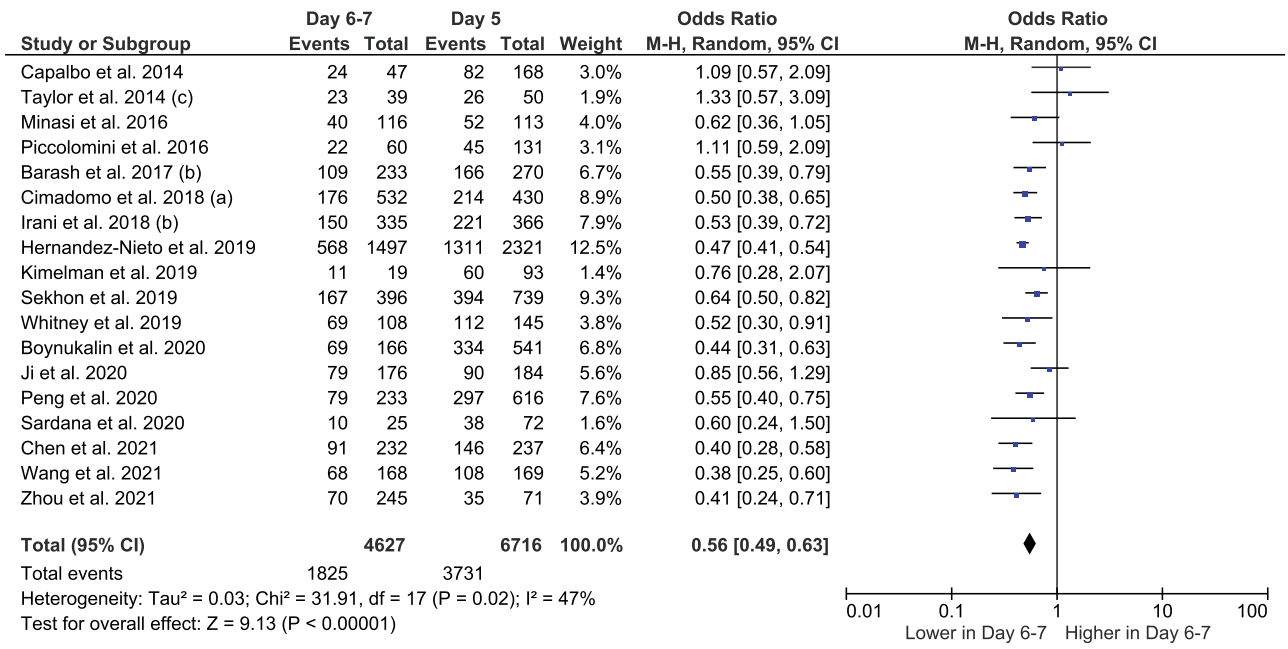

Figure 5. Day 6–7 blastocysts were associated with a lower live birth rate per euploid transfer than Day 5 blastocysts.

or >2PN are displayed, the zygote is considered to have abnormal contributions from the oocyte or multiple genomic contributions from both gametes. However, standard microscopic PN assessment is imperfectly associated with the ploidy level, as genetic studies showed that around 1% of 2PN zygotes produce embryos with abnormal ploidy levels, while 50% of 1PN and 10% of 3PN-derived embryos are diploid (Grau *et al.*, 2015; Capalbo *et al.*, 2017a; Mateo *et al.*, 2017). This imprecision in microscopic ploidy detection is mainly due to asynchronous appearance of PN, leading to false positives (e.g. 1PN detected whilst the second appears at earlier or later stages) and false negatives (e.g. 2PN detected whilst additional ones appear at earlier or later stages). Because abnormal ploidy level is associated with implantation failure, miscarriage, molar pregnancy, and overall negative reproductive outcomes (Staessen and Van Steirteghem, 1997), failure to identify its presence can impact the expected success rates. Although most of current PGT technologies allow the detection of chromosomal abnormalities, they fail to distinguish ploidy levels when chromosomes are represented in an equal copy number. The development and integration of ploidy level assessment and biparental inheritance confirmation in current PGT strategies offer the possibility to reduce the uncertainty regarding the impact of altered embryo chromosomal constitution and improve (although marginally) the overall outcome of euploid SET. Several case reports have shown healthy LBs obtained after the transfer of one PN-derived blastocysts biopsied, analyzed via PGT-A for chromosomal testing plus genome-wide haplotyping, SNP-array, NGS, or short tandem repeats analyses for ploidy assessment, and diagnosed as euploid-diploid (Bradley *et al.*, 2017b; Capalbo *et al.*, 2017a; Destouni *et al.*, 2018). However, only Bradley *et al.* has reported the clinical outcomes resulted from the transfer of 1072 2PN- versus 26 1PN-derived blastocysts that were carried out at their center. In particular, the former group of embryos resulted in a 53.5% clinical pregnancy rate (CPR) (>4 gestational weeks), versus 34.6% for the latter. This difference did not reach statistical significance ($P = 0.07$) (Table 2).

Blastomere multinucleation is a common nuclear abnormality observed in early human embryos and other mammals (Daughtry *et al.*, 2019). During mitosis, embryonic blastomeres undergo duplication of the chromosomes prior to cellular division. If this process progresses normally, each blastomere contains one nucleus. When either chromosomal segregation or cellular cleavage fail, the ensuing cells may possess either no nucleus or more than one. Especially during the first and second mitotic divisions, between 17% and 74% of embryos are expected to show multinucleation (Hardy *et al.*, 1993). A study describing the outcomes of 74 euploid SETs reported a lower OPR (>12 gestational weeks) for embryos showing multinucleation on day2 compared to a control group not displaying the feature (33% versus 76%) (Balakier *et al.*, 2016) (Table 2).

A single study assessed a putative association between the number of blastomeres counted on day3 and LBR and MR following 297 euploid blastocyst SETs. Embryos containing fewer than eight blastomeres at 68 ± 1 hpi resulted in a significantly lower LBR (Pons *et al.*, 2019) (Table 2).

Abnormal cleavage patterns and morula compaction

Direct unequal cleavage (DUC), namely the division of one blastomere directly into three cells, and reverse cleavage (RC), namely the fusion of two blastomeres into one (Apter *et al.*, 2020), are the most frequent abnormal cleavage events in human embryos with a reported prevalence of ≥10% (Ozbek *et al.*, 2021). Notably, lower blastulation rates but higher euploidy rates were reported among blastocysts obtained after these events. A single study reported a lower LBR per single euploid blastocyst transfer after DUC and/or RC compared to controls, with no difference in MR (Ozbek *et al.*, 2021) (Table 2).

Abnormal cleavage patterns are often related with partial compaction at the morula stage, namely the exclusion or extrusion of some blastomeres from the embryo proper (Coticchio *et al.*, 2019, 2021a,b; Lagalla *et al.*, 2020). Partial compaction is more common than full compaction in human embryos, but no statistically significant difference was observed in aneuploidy rates and OPR per euploid SET between the two groups of embryos (Lagalla *et al.*, 2020) (Table 2).

Blastocyst expansion dynamics

Blastocyst spontaneous collapse, namely a reduction of blastocyst volume associated with its detachment from the zona pellucida (ZP) (Cimadomo *et al.*, 2022a), and consistently detectable only through time-lapse microscopy (TLM), appears indicative of lower euploidy rates (Vinals Gonzalez *et al.*, 2018; Gazzo *et al.*, 2020b), as well as lower OPR per euploid SET (Gazzo *et al.*, 2020b) (Table 2).

A recent study adopted artificial intelligence (AI) and TLM to track the expansion dynamics of human blastocysts throughout the 10 h from its initiation (Huang *et al.*, 2021). Faster and greater expansion dynamics were reported to be more typical of euploid and reproductively competent embryos than aneuploid and reproductively incompetent embryos (Table 2), thereby suggesting this as a potential embryo selection parameter.

Timings of preimplantation development

TLM allows the continuous monitoring of preimplantation development of embryos and the measurement of specific timepoints. Various timings are recorded, mainly following ESHRE guidelines (Ciray *et al.*, 2014; Apter *et al.*, 2020), e.g. time of pronuclear fading (tPNf) or cleavage times at all stages (t2, t3, t4, etcetera). Then, the length of the first, second, and third cell cycle (CC1, CC2 and CC3), or the duration of blastocyst expansion, can be inferred from the raw data. Clearly, several studies across the years have investigated whether these timings could predict embryonic competence: yet, large heterogeneity exists in terms of patient populations, clinical and laboratory practice, and analysis method, thereby limiting the generalizability of the evidence. Regarding chromosomal constitution, 58 studies and over 40 000 embryos were recently meta-analyzed to assess a putative association between ploidy status morphokinetic features detected through TLM (Bamford *et al.*, 2022): t8, t9, and time of initiation of expansion (tEB) were reported to be longer in aneuploid blastocysts, along with the fragmentation grade, persisting multinucleation at the four-cell stage, and blastocyst contractions. Nonetheless, because of the heterogeneity of the results and the low quality of the evidence, the authors suggested that further investigations were required. In the present review, we aimed at assessing the prediction of morphokinetics assessment on the reproductive competence of euploid blastocysts, and five papers that investigated this association were retrieved (Table 2). Nonetheless, a meta-analysis was not feasible because of the heterogeneity in the parameters and the clinical outcomes examined across the studies. Unsurprisingly, also the results were diverging. Specifically, a randomized controlled trial (RCT) with sibling MII oocytes assessed the efficiency of embryo selection based on PGT-A with or without TLM (Yang *et al.*, 2014). It showed better OPR with the former strategy, but a sub-analysis in the TLM arm did not unveil any specific timing associated with OPR and MR after euploid SET. A recent multicenter study instead clustered 830 transferred euploid blastocysts in two groups according to the time of morula formationI (tM) as $<$ or $\geq$80 h and reported a higher LBR with faster embryos (Rienzi *et al.*, 2019). In a retrospective study, early blastulation on Day 4 led to an OPR per euploid SET of >70%, which was significantly higher than the control embryos (Hung *et al.*, 2018). In another investigation including 129 euploid SETs, the duration of blastulation, i.e. time of full blastocyst (tB)—time of starting blastulation (tSB), was shorter in implanting embryos versus non-implanting ones (Mumusoglu *et al.*, 2017). Lastly, a recent retrospective study (McQueen *et al.*, 2021), investigated tPNf, t2, t3, t4, t8, tM, and tB based on the outcome of 192 euploid SETs, and showed no difference in the morphokinetics of embryos resulting in euploid miscarriage compared with those resulting in live birth.

Additional molecular analyses

mtDNA score on a trophectoderm biopsy

The amount of mitochondrial DNA (mtDNA) in embryonic cells has been hypothesized as a determinant of embryonic competence. Mitochondria are crucial components of the cell and the site of oxidative phosphorylation that produces ATP to be spent for energy release in metabolic processes across the whole organism. Moreover, mitochondria derive from the oocyte and, since oocyte quality is a driver of early embryo development, it is reasonable to presume that mitochondria may have an impact on embryonic competence. In fact, it has been proposed that elevated mtDNA levels are symptomatic of inefficient energy production and defective homeostasis in the embryo (Fragouli and Wells, 2015), in line with the 'quiet embryo hypothesis' outlined by Leese's group which suggests that reproductively competent embryos are metabolically silent (Leese, 2002; Leese *et al.*, 2007, 2008). Nevertheless, these theoretical assumptions have lately been both questioned and revised. Firstly, Leese *et al.* (2019, 2022) themselves updated the 'quiet embryo hypothesis' in view of the 'Goldilocks effect' which pictures a trend among biological systems to suffer from both the extreme situations of 'too much' and 'too little', metabolic activity in this case, and prefer the 'just right' condition, namely an optimum range, which is a concept that in the Swedish language is conveyed by the term 'Lagom'. Possibly, human embryos can tolerate slight changes in their metabolism in response to stressors, while extreme perturbations can irreversibly shift the metabolism towards a fatal pessimum range. Moreover, a 'one size fits all' perspective with respect to embryo metabolism is erroneous because 'each single embryo is a unique as each individual animal or person, with an exclusive genotype manifesting as a distinctive phenotype' and with its own optimal 'quite zone' of metabolic activity (Leese *et al.*, 2022). Secondly, human embryos rely only partially upon oxidative metabolism for energy production purposes, while being heavily dependent upon glycolysis to this end (Gardner and Wale, 2013). In summary, this background questions the analysis of mtDNA as a reliable embryo selection tool in the first place, which had been the conclusion achieved after almost 5 years of publications on this topic.

Thirteen studies were retrieved, although different methodologies for mtDNA quantitation and thresholds for clinical relevance were employed (Diez-Juan *et al.*, 2015; Fragouli *et al.*, 2015, 2017; Ravichandran *et al.*, 2017; Treff *et al.*, 2017; Victor *et al.*, 2017; Lledo *et al.*, 2018; Lee *et al.*, 2019b; Boynukalin *et al.*, 2020; Scott *et al.*, 2020; El-Damen *et al.*, 2021; Wang *et al.*, 2021a; Zhou *et al.*, 2021) (Table 2). Initial pilot studies reported that the ratio between mtDNA and nuclear DNA reads (mtDNA: nDNA) after whole genome amplification was associated with OPR, identifying also thresholds beyond which no pregnancy was ever reported (Diez-Juan *et al.*, 2015; Fragouli and Wells, 2015; Fragouli *et al.*, 2017; Ravichandran *et al.*, 2017). Their evidence was supported by two additional clinical studies (Lledo *et al.*, 2018; Boynukalin *et al.*, 2020). On the contrary, several more studies failed to confirm this association (Lee *et al.*, 2019b; El-Damen *et al.*, 2021; Wang *et al.*, 2021a; Zhou *et al.*, 2021), even when assessing multiple consecutive transfers with opposite outcomes from the same patient (Victor *et al.*, 2017; Scott *et al.*, 2020), or double ETs with one implanted and one non-implanted euploid blastocyst (Treff *et al.*, 2017). Unfortunately, the heterogeneity in study designs, experimental group characteristics, analytical methodologies, and

outcome measures, prevents a direct comparison across studies and a real appreciation of the impact of mtDNA levels on embryo reproductive competence. Moreover, normalization of the results is an issue; in fact, mtDNA levels in euploid blastocysts may be related to other features, such as the day of biopsy or TE quality. Lastly, the prevalence of embryos with exceedingly high mtDNA: nDNA ratios, beyond the threshold of 'normality', were relatively infrequent in the non-selection studies. They represented only 4–12% of the euploid blastocysts transferred (Fragouli *et al.*, 2017; Lledo *et al.*, 2018), suggesting a limited prevalence of this phenomenon among euploid embryos.

Cumulus cells or spent media molecular analyses

Some authors attempted to complement PGT-A analysis with additional molecular analyses conducted on routinely discarded material, such as cumulus cells or spent blastocyst media (SBM).

Two studies conducted transcriptomic analyses on cumulus cells retrieved from oocytes that developed into euploid blastocysts that implanted versus those that did not implant (Parks *et al.*, 2016; Green *et al.*, 2018) (Table 2). One study analyzed five cases per group, while the other analyzed 17 double ETs of sibling blastocysts, and the two studies produced opposing results. Both reported several differentially expressed genes, but no difference was statistically significant enough to represent a valuable biomarker of blastocyst competence.

Two studies from a single group assessed miRNAs in the SBM of euploid implanted versus non-implanted blastocysts (Capalbo *et al.*, 2016b; Cimadomo *et al.*, 2019a) (Table 2). Because of their role as powerful messengers in the blastocyst-endometrium dialogue and their high stability despite chemo-physical environmental insults, miRNAs in the SBM may represent an intriguing opportunity of non-invasive and easy-to-manage biomarkers of implantation. However, the results presented major shortcomings. Briefly, miR-20a and miR-30c were found to be more expressed in the SBM of implanted blastocysts in a first single center miRNomic study of 53 euploid SETs (Capalbo *et al.*, 2016b), but a second multicenter study, where a custom plate and protocol were designed for the analysis of 10 miRNAs in the SBM of 221 euploid SETs, did not confirm this evidence. Although higher amplification rates were reported for miR-182-5p, miR-302a-3p, and miR-519d-3p along with higher abundance levels of miR-302a-3p, miR-372-3p, miR-373-3p, and miR-518a-3p from the SBM of non-implanted euploid blastocysts, when the data were adjusted for blastocyst quality and day of biopsy, these associations were no longer significant (Cimadomo *et al.*, 2019a).

Recently, several investigations focused on the possibility of conducting PGT-A on SBM, aiming to set up a workflow to conduct non-invasive aneuploidy testing (Leaver and Wells, 2020). Two studies reported the outcomes after the SET of blastocysts reported as euploid by TE biopsy PGT-A analysis but as either euploid or aneuploid by the SBM specimen (Rubio *et al.*, 2019; Yeung *et al.*, 2019) (Table 1). In our meta-analysis, SBM reported as aneuploid (N = 19 overall) or euploid (N = 24 overall) were associated with a similar LBR (OR 0.38, 95% CI 0.07–2.06, $I^2 = 33\%$, $P = 0.26$) (Fig. 6) and MR per clinical pregnancy (N = 10 from aneuploid SBM and N = 14 from euploid; OR 4.05, 95% CI 0.35–46.15, $I^2 = 32\%$, $P = 0.26$) (Supplementary Fig. S5).

A study adopted a similar design but complementing TE analysis with the result of amplification of DNA (i.e. either amplification success or failure) from the blastocoel fluid collected via blastocentesis (Magli *et al.*, 2019) (Table 2). Intriguingly, in 53 euploid SETs, the detection of DNA in the blastocoel was associated with a significantly lower LBR (31.5% versus 67.6%), but a similar MR. The authors hypothesized that this inexpensive analysis may serve as a biomarker of embryo reproductive fitness, as it indirectly unveils the consequences of apoptosis or necrosis of embryonic cells that release DNA in the blastocoel fluid acting as a reservoir. However, more data are needed to confirm this hypothesis.

Maternal features

The maternal features potentially associated with euploid blastocysts' reproductive competence were clustered as age at oocyte retrieval, number of previous IVF attempts, cause of infertility, body mass index (BMI) and body fat, hormones, drugs, and endometrial and uterine features.

Age at oocyte retrieval

It is well established that embryo aneuploidy is associated with increasing maternal age (Harton *et al.*, 2013; Irani *et al.*, 2019), in both the fertile and infertile populations (Taylor *et al.*, 2014a) as well as among women with repeated implantation failure (RIF) and recurrent pregnancy loss (RPL) (Rubio *et al.*, 2009; Liu *et al.*, 2020; Tong *et al.*, 2021). The preponderance of data shows better outcomes following PGT-A in women of advanced maternal age (AMA) (Lee *et al.*, 2015, 2019a; Ubaldi *et al.*, 2015; Phuong *et al.*, 2019; Sacchi *et al.*, 2019), in a setting with fewer embryos transferred (Lee *et al.*, 2019a; Phuong *et al.*, 2019) and fewer multiple gestations (Ubaldi *et al.*, 2015; Phuong *et al.*, 2019). The data regarding LBR for women <35 years following PGT-A is somewhat more mixed with the majority still suggesting a higher LBR compared with older women (Debrock *et al.*, 2010; Lee *et al.*, 2015, 2019a; Ubaldi *et al.*, 2015; Phuong *et al.*, 2019; Sacchi *et al.*, 2019). As the detrimental effect of increasing maternal age can be offset by testing for aneuploidies, the logical next question is whether age still impacts the implantation of euploid embryos. Several studies have suggested that PGT-A with euploid ET acts as an equalizer between younger and older women regarding implantation success (Barash *et al.*, 2017b; Irani *et al.*, 2019; Lee *et al.*, 2019a; Boynukalin *et al.*, 2020; Tong *et al.*, 2021) (Table 1). This evidence was corroborated also by three studies that assessed a putative impact of maternal age, investigated as a continuous variable (Sekhon *et al.*, 2019; Boynukalin *et al.*, 2021; Zhou *et al.*, 2021) or according to the 35 years threshold (Guzman *et al.*, 2019) (Table 2). Conversely, a large retrospective study published in 2020 evaluated >8000 SETs and suggested that age may in fact still impact LBRs (Reig *et al.*, 2020), supporting the data reported in a 2013 multicenter retrospective analysis of 343 euploid SETs clustered among women younger or older than 38 years (Harton *et al.*, 2013) (Table 1).

In our meta-analysis, women ≥38 years at oocyte retrieval (N = 3175 overall) had a significantly lower LBR in both euploid SETs and DETs than younger women (N = 7563 overall) with an OR 0.87, 95% CI 0.75–1.00, $I^2 = 31\%$, $P = 0.05$ (Fig. 7). The MR per clinical pregnancy in the two groups (N = 1631 women ≥38 years at oocyte retrieval and N = 4623 women <38 years) was not significantly different (OR 1.17, 95% CI 0.99–1.38, $I^2 = 0\%$, $P = 0.07$) (Supplementary Fig. S6).

Taken together, these results point towards a subtle decrease in implantation with increasing age which is most clinically relevant when comparing the oldest to the youngest women. The cause of this decrease with AMA is unclear but may relate to non-chromosomal oocyte quality factors, *de novo* mutations or copy number variants, or acquired uterine factors.

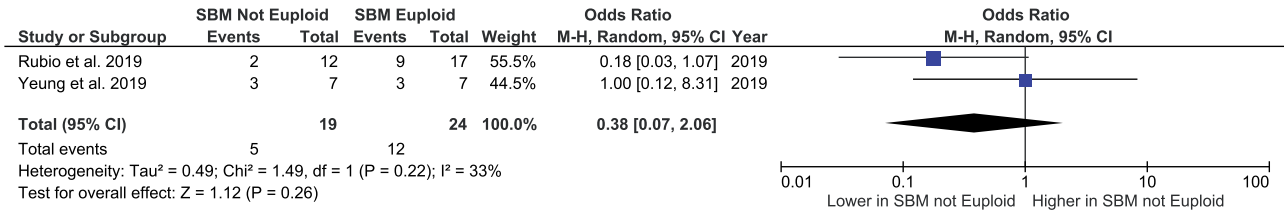

Figure 6. **Blastocysts reported as euploid on both the trophectoderm biopsy and the spent blastocyst media (SBM) showed similar live birth rates to blastocysts reported as euploid on the trophectoderm biopsy but aneuploid on the SBM.**

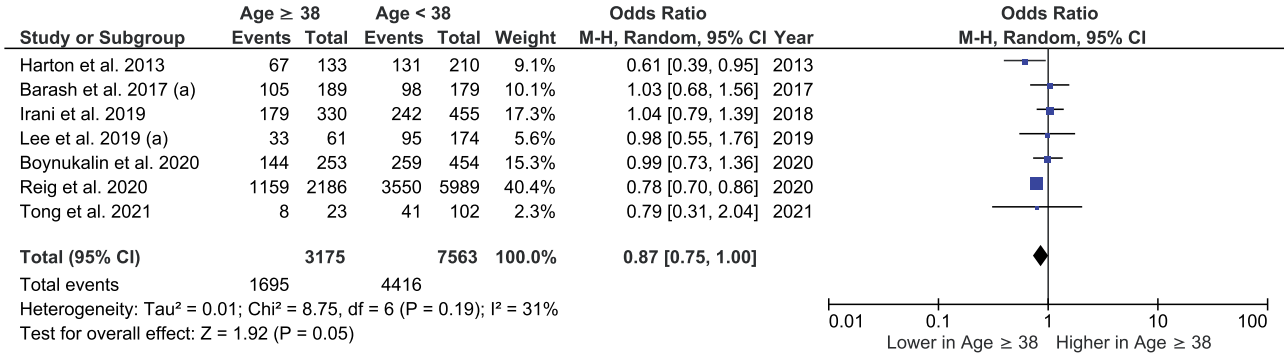

Figure 7. **Women ≥38 years were subject to lower live birth rates per euploid transfer than women younger than 38.**

Number of previous IVF attempts

Only two studies from the same group aimed to assess whether the number of previous IVF attempts was associated with clinical outcomes after euploid blastocyst transfer. No difference was reported in rates of implantation failure, miscarriage, or live birth (Boynukalin *et al.*, 2020, 2021) (Table 2).

Cause of infertility

Unexplained

The first challenge in achieving a live birth during a PGT-A cycle is the production of euploid embryos suitable for ET. Patients may become disappointed or frustrated following a stimulation cycle yielding no euploid embryos. For cases where euploid embryos were obtained, however, four studies attempted to assess whether a diagnosis of infertility was associated with outcomes after their transfer or not (Table 1). They were all retrospective single center studies (Taylor *et al.*, 2014a; Boynukalin *et al.*, 2020, 2021), except for an analysis that used the 2014 SART-CORS data (Meng *et al.*, 2021). Although the studies were concordant in excluding an impact on MR, two of them reported higher LBRs in cases of unexplained infertility.

In our meta-analysis, women with a clear diagnosis of infertility (N = 2590 overall) and women with unexplained infertility (N = 627 overall) showed similar a LBR in both euploid SETs and DETs with an OR 0.62, 95% CI 0.35–1.10, $I^2 = 78\%$, $P = 0.1$ (Fig. 8). The MR per clinical pregnancy (N = 1701 from infertile women and N = 541 from women with unexplained infertility) was also similar (OR 0.93, 95% CI 0.71–1.23, $I^2 = 0\%$, $P = 0.63$) (Supplementary Fig. S7).

Nevertheless, this analysis clustered all different infertility causes into a single group, preventing an appreciation of the impact on clinical outcomes of each individual diagnosis.

Polycystic ovary syndrome

A small retrospective case–control study suggested that the presence of polycystic ovary syndrome (PCOS) worsens the outcomes of euploid SET (Luo *et al.*, 2017). Specifically, 67 women with PCOS as per the Rotterdam criteria were compared with 201 women with any other infertility diagnosis in a 1:3 ratio. All women were lean (BMI 18–25), undergoing preimplantation genetic testing for structural chromosomal rearrangements (PGT-SR) as either they or their partner had a diagnosed translocation, and the pairs were matched based on age, BMI, and embryo grade. Although this data suggests a detrimental effect of PCOS, the study group included only lean women with PCOS to control for the impact of obesity on reproductive outcomes. Lean PCOS is a unique entity and unfortunately, these findings are not generalizable to the overall PCOS population. Three more studies investigating LBR and/or MR in both euploid SETs and DETs in PCOS versus non-PCOS women reported no significant difference (Boynukalin *et al.*, 2020, 2021; Meng *et al.*, 2021) (Table 1).

In our meta-analysis, women affected (N = 383 overall) and not affected by PCOS (N = 2921 overall) showed similar LBRs in both euploid SETs and DETs with an OR 0.87, 95% CI 0.70–1.08, $I^2 = 0\%$, $P = 0.2$ (Fig. 9). Their MRs per clinical pregnancy (N = 228 from PCOS women and N = 1968 from non-PCOS) were also similar (OR 1.47, 95% CI 0.85–2.54, $I^2 = 49\%$, $P = 0.17$) (Supplementary Fig. S8).

Diminished ovarian reserve

Although the data regarding an association between diminished ovarian reserve (DOR) and aneuploidy rates are contrasting, the use of PGT-A in this group decreases the MR and the time to live birth (Katz-Jaffe *et al.*, 2013; Morin *et al.*, 2018a,b; Jaswa *et al.*, 2021). Three studies reported LBR and/or MR in women with DOR versus those without DOR after PGT-A and claimed equivalent outcomes across groups (Boynukalin *et al.*, 2020, 2021; Meng *et al.*, 2021) (Table 1). In our meta-analysis, women with DOR (N = 513

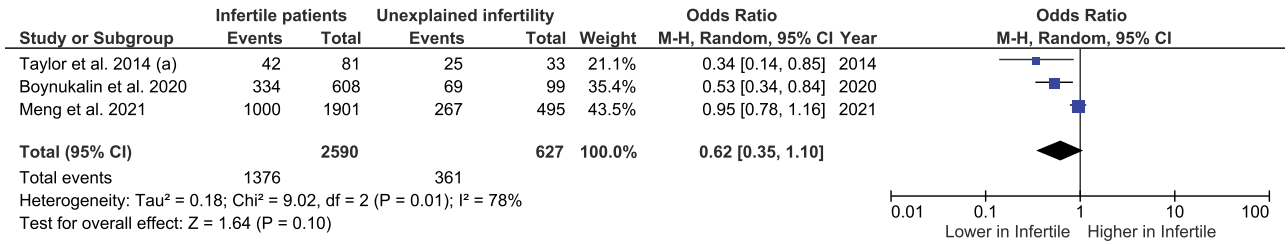

Figure 8. **Women with a diagnosis of infertility showed similar live birth rates per euploid transfer to women with idiopathic infertility.**

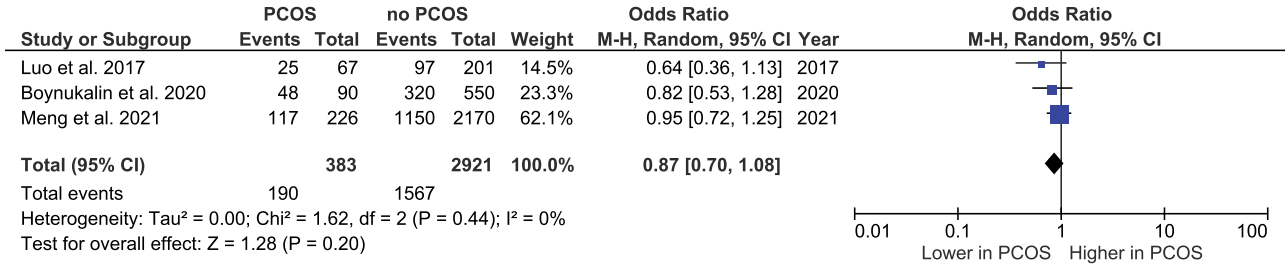

Figure 9. **Women affected by polycystic ovarian syndrome (PCOS) showed similar live birth rates per euploid transfer to women not affected by PCOS.**

overall) and women without DOR (N = 2500) showed similar a LBR in both euploid SETs and DETs with an OR 0.90, 95% CI 0.74–1.09, $I^2 = 0\%$, $P = 0.28$ (Fig. 10). The MR per clinical pregnancy (N = 328 from DOR women and N = 1723 from non-DOR) was also similar (OR 0.95, 95% CI 0.68–1.34, $I^2 = 0\%$, $P = 0.78$) (Supplementary Fig. S9).

Two more studies that supported this conclusion were retrieved from the literature. However, their absolute numbers could not be accessed, and they had to be excluded from the meta-analysis (Katz-Jaffe *et al.*, 2013; Jaswa *et al.*, 2021) (Table 2).

Endometriosis

Endometriosis is a relatively frequent cause of infertility. In a multicenter case–control study, where enrolled women were diagnosed with endometriosis through ultrasound or surgical inspection and age-matched in a 1:2 ratio with controls, the presence of the pathology did not appear to influence outcomes following euploid SET (Vaiarelli *et al.*, 2021). Similar results were shown in another investigation (Bishop *et al.*, 2021) comparing vitrified-warmed euploid SET outcomes in women with surgically proven endometriosis versus women undergoing IVF for non-endometrial factors (PGT-M for single gene defects, male factor infertility). Three more studies excluded an impact of endometriosis on euploid blastocyst implantation (Boynukalin *et al.*, 2020, 2021; Meng *et al.*, 2021) (Table 1).

In our meta-analysis, women affected (N = 350 overall) and women not affected by endometriosis (N = 3607 overall) showed similar LBRs in both euploid SETs and DETs with an OR 1.11, 95% CI 0.87–1.40, $I^2 = 0\%$, $P = 0.40$ (Fig. 11). The MR per clinical pregnancy (N = 196 in women affected and N = 2390 in women not affected from endometriosis) was also similar (OR 0.79, 95% CI 0.51–1.24, $I^2 = 0\%$, $P = 0.31$) (Supplementary Fig. S10).

Adenomyosis

Adenomyosis is also thought to impact reproductive outcomes, yet asymptomatic adenomyosis, incidentally diagnosed during ultrasound monitoring, did not involve worse results following euploid SET in the only study that investigated this topic (Neal *et al.*, 2020) (Table 2). Specifically, 648 women undergoing endometrial preparation prior to vitrified-warmed SET underwent sonographic evaluation the day prior to transfer. There were 99 women (15.3%) were diagnosed with adenomyosis based on presence of any of its seven sonographic markers. The MR and LBR were not different between those with and without adenomyosis. Of note, while this study suggests that asymptomatic and incidentally found adenomyosis is not a concerning diagnosis, it does not address the potential impact of symptomatic adenomyosis which may be a separate and more severe disease.

Tubal factor

Three studies investigated whether LBR and/or MR were impaired by a diagnosis of tubal factor infertility in the context of PGT-A. No difference was reported (Boynukalin *et al.*, 2020, 2021; Meng *et al.*, 2021) (Table 1).

In our meta-analysis, women affected from tubal factor infertility (N = 172 overall) and women not affected by it (N = 2841 overall) showed similar LBRs in both euploid SETs and DETs with an OR 0.88, 95% CI 0.64–1.20, $I^2 = 0\%$, $P = 0.40$ (Fig. 12). The MR per clinical pregnancy (N = 85 in women affected and N = 1966 in women not affected from tubal factor) was also similar (OR 0.150, 95% CI 0.87–2.60, $I^2 = 0\%$, $P = 0.15$) (Supplementary Fig. S11).

Arcuate uterus

Arcuate uterus is the most common congenital uterine anomaly, and it has been debated whether it may impact reproductive outcomes. Only a retrospective cohort study compared LBRs following euploid ET in women with and without an arcuate uterus (Surrey *et al.*, 2018) (Table 2). Arcuate uterus was defined as a perpendicular depth of 4 mm to <10 mm from the level of the cornua and myometrial angle >90°, diagnosed on 3D ultrasound and confirmed via hysteroscopy. No difference was reported.

Inflammatory bowel disease

Although not a gynecologic condition, inflammatory bowel disease (IBD) can severely alter the pelvis. Among a cohort of women with both infertility and IBD in the only report retrieved from the

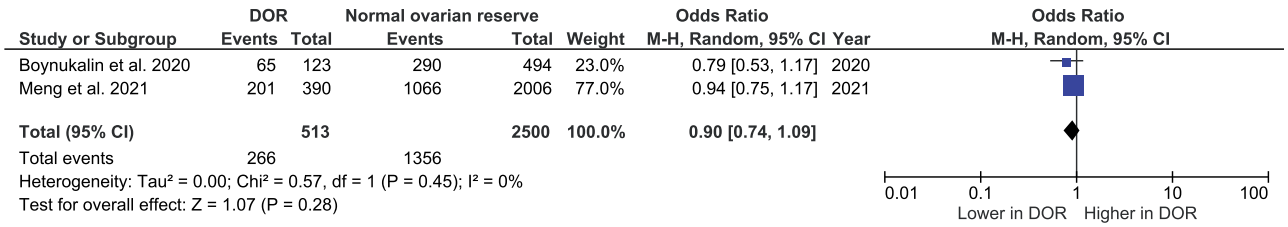

Figure 10. Women with diminished ovarian reserve (DOR) showed similar live birth rates per euploid transfer to women without DOR.

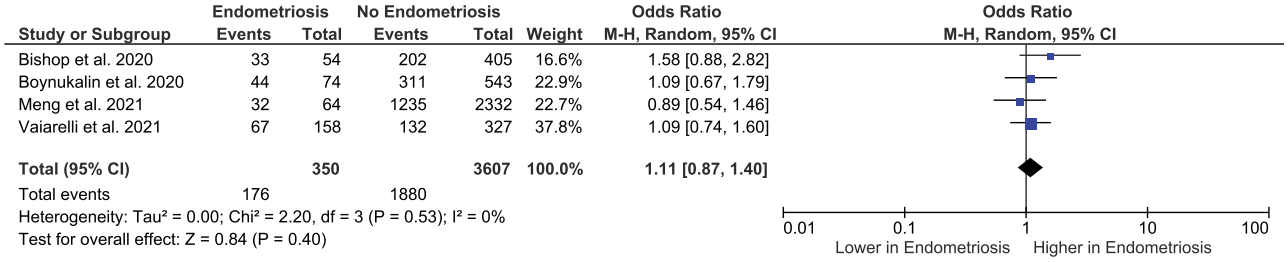

Figure 11. Women affected by endometriosis showed similar live birth rates per euploid transfer to women not affected by endometriosis.

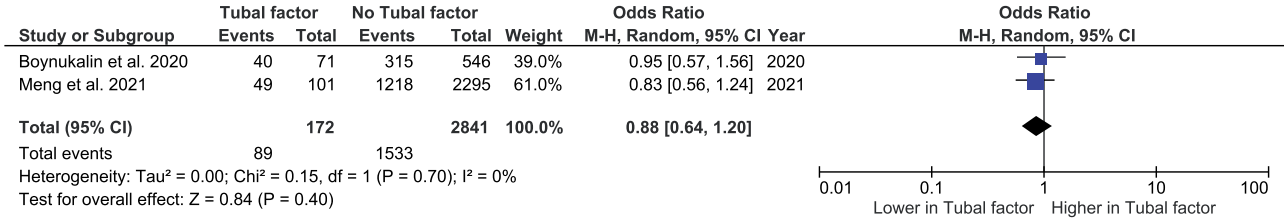

Figure 12. Women affected by tubal factor showed similar live birth rates per euploid transfer to women not affected by tubal factor.

literature (Table 2), the MR and LBR were not different following vitrified-warmed euploid SET when compared with other infertile controls (Hernandez-Nieto *et al.*, 2020b). A diagnosis of ulcerative colitis versus Crohn's disease also did not impact the outcomes.

Repeated implantation failure

Two specific poor prognosis conditions with the potential to impact ET outcomes have been studied in more detail: RIF and RPL. Lately, we have learned that true RIF is rare, with a cumulative 95% of women achieving an ongoing pregnancy within their third euploid transfer (Pirtea *et al.*, 2020). Additionally, it has been established that the use of PGT-A improves implantation rates per transfer while lowering the MR in poor prognosis patients, including in a subset of women with apparent RIF (Fragouli *et al.*, 2010; Greco *et al.*, 2014; Lee *et al.*, 2019a; Sato *et al.*, 2019; Deng *et al.*, 2020a). Still, the impact of RIF on future outcomes after euploid ETs remains uncertain with two studies excluding an association (Greco *et al.*, 2014; Zhou *et al.*, 2021), and one study claiming an incremental decrease in LBR with an increasing number of prior implantation failures that culminates in a statistically significant difference with ≥3 implantation failures (Cimadomo *et al.*, 2021a) (Table 1). However, only 16% of the latter study group had previously undergone IVF with PGT-A, and therefore it is unknown how many of the prior unsuccessful transfers involved non-euploid embryos. Thus, the authors recommended replication of their study with a more tightly defined RIF population.

In our meta-analysis, women with RIF (N = 310 overall) showed a lower LBR per euploid SET than women with no RIF (N = 1672 overall), with an OR 0.72, 95% CI 0.55–0.93, $I^2 = 0\%$, $P = 0.01$ (Fig. 13). However, the MR per clinical pregnancy (N = 143 from RIF women and N = 849 from non-RIF) was similar (OR 1.17, 95% CI 0.68–2.01, $I^2 = 0\%$, $P = 0.58$) in the two groups (Supplementary Fig. S12).

Recurrent pregnancy loss

For some patients, implantation is not the primary barrier to LB, but rather they suffer from RPL, which is generally defined as the loss of two or more clinically recognized pregnancies. Patients with RPL are thought to have a larger proportion of aneuploid blastocysts, particularly younger women who have a lower baseline risk of aneuploidy (Kort *et al.*, 2018; Liu *et al.*, 2020). Consequently, the data supports the use of PGT-A for decreasing the MR in women with RPL (Lei *et al.*, 2019; Sato *et al.*, 2019). When investigating in detail the literature, an inverse relationship appears between an increasing number of prior miscarriages and the likelihood of LB, but whether this association stands for everyone with RPL remains uncertain (Wang *et al.*, 2019a; Boynukalin *et al.*, 2020, 2021; Liu *et al.*, 2020; Ni *et al.*, 2020; Cimadomo *et al.*, 2021a). Four studies could be included in this meta-analysis; two of them showed a significant association (Boynukalin *et al.*, 2020; Liu *et al.*, 2020) while the other two did not (Cimadomo *et al.*, 2021a; Zhou *et al.*, 2021) (Table 1).

In our meta-analysis, women with RPL (N = 436 overall) showed a similar LBR in both euploid SETs and DETs as women

with no RPL (N = 2457 overall), with an OR 0.75, 95% CI 0.50–1.12, $I^2 = 69\%$, $P = 0.16$ (Fig. 14). The MR per clinical pregnancy (N = 138 from RPL women and N = 968 from non-RPL) was also similar (OR 1.97, 95% CI 0.89–4.36, $I^2 = 58\%$, $P = 0.10$) (Supplementary Fig. S13).

Body mass index and body fat

Several studies have examined the impact of BMI on clinical outcomes following vitrified-warmed euploid blastocyst transfers. In two studies, the patients were categorized according to their BMI as normal weight, overweight, or obese and it was possible to conduct a meta-analysis (Cozzolino *et al.*, 2020b; Meng *et al.*, 2021) (Table 1), while in another large study a more thorough classification was adopted, that included also the body fat outlined via bioelectric impedance analysis (BIA). Unfortunately, the absolute numbers could not be retrieved from that paper (Kim *et al.*, 2021) (Table 2). Also, several other studies have assessed a putative impact of maternal BMI by reporting it as a continuous variable (Sekhon *et al.*, 2019; Boynukalin *et al.*, 2020, 2021; Zhou *et al.*, 2021) (Table 2). In general, a higher BMI was associated with a lower LBR (Sekhon *et al.*, 2019; Boynukalin *et al.*, 2020, 2021; Cozzolino *et al.*, 2020b; Meng *et al.*, 2021) and a higher MR (Cozzolino *et al.*, 2020b;Boynukalin *et al.*, 2021), although these associations were not supported by all reports (Kim *et al.*, 2021; Zhou *et al.*, 2021).

In our meta-analysis, obese women (BMI ≥30) (N = 554 overall) had a significantly lower LBR in both euploid SETs and DETs than non-obese women (BMI <30) (N = 5948 overall), with an OR 0.66, 95% CI 0.55–0.79, $I^2 = 0\%$, $P < 0.01$ (Fig. 15). Also, the MR per clinical pregnancy (N = 283 from obese women and N = 3296 from non-obese) was significantly higher in the obese women (OR 1.80, 95% CI 1.08–2.99, $I^2 = 52\%$, $P = 0.02$) (Supplementary Fig. S14).

Further studies with larger cohorts of obese women are needed to corroborate these findings, especially since a common critique is that the analyses did not control for infertility diagnoses that could be related to BMI through structural, endometrial, or hormonal pathways (Ginsburg and George, 2021).

Hormones

Basal anti-Müllerian hormone

With the nearly ubiquitous use of AMH as a marker of ovarian reserve, questions have arisen regarding its impact on PGT-A outcomes (Morin *et al.*, 2018b; Wang *et al.*, 2019b; Pipari *et al.*, 2021) (Table 2). Two studies clustered the patients in three to six groups according to basal AMH levels (Wang *et al.*, 2019b; Pipari *et al.*, 2021). Both analyses showed no association between the levels of AMH and the outcomes after euploid blastocyst transfer, but they could not be meta-analyzed because the LBR was accessible only for one study. Another study including 768 euploid SETs and DETs in women <38 years compared clinical outcomes resulting from women with AMH levels of ≤0.5 ng/ml or 1.1–4.5 ng/ml. No difference in LBRs was reported, although a significantly higher MR was recorded in the latter group (Morin *et al.*, 2018b). More and larger studies are needed to assess this factor.

Progesterone

Several groups investigated progesterone levels throughout the IVF journey and its putative impact on reproductive outcomes. Three papers assessed its levels the day of trigger, either as a continuous variable (Boynukalin *et al.*, 2020) or by categorization based on a 1.5 or 2 ng/ml threshold (Hernandez-Nieto *et al.*, 2020a; Pardiñas *et al.*, 2021). No association was reported with either LBR or MR (Table 2). Two papers from the same group assessed its levels on the day of progesterone initiation during endometrial preparation for ET, and again no association was reported with either LBR or MR (Boynukalin *et al.*, 2020, 2021) (Table 2). One paper assessed the serum progesterone level on the day prior to euploid SET, clustering the patients in four quartiles (≤8.06 ng/ml, 8.07–10.64 ng/ml, 10.65–13.13 ng/ml, and >13.13 ng/ml), and showed a lower LBR and higher MR in lower quartiles, especially below 10.65 ng/ml (Gaggiotti-Marre *et al.*, 2019) (Table 2). In a follow-up study, the same authors showed that when the women with progesterone levels <10.6 ng/ml on the day prior to euploid SET were given subcutaneous

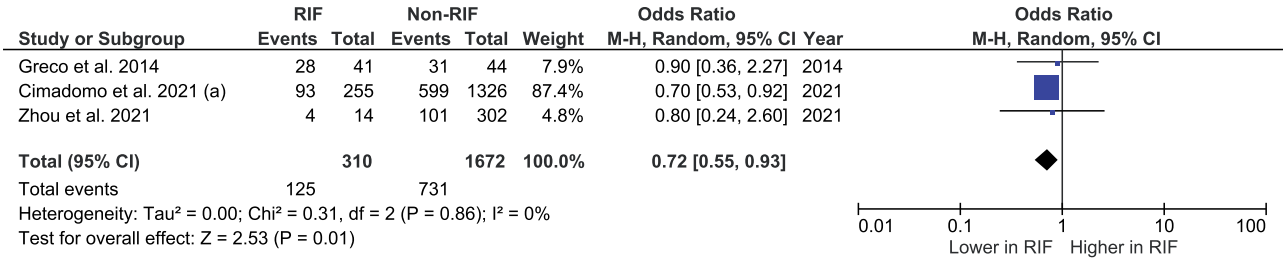

Figure 13. Women with previous repeated implantation failure (RIF) showed lower live birth rates per euploid transfer than women without RIF.

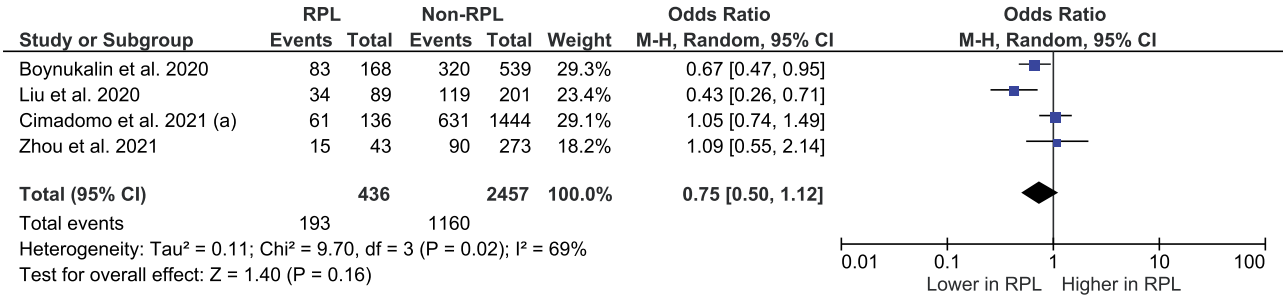

Figure 14. Women with previous recurrent pregnancy loss (RPL) showed similar live birth rate per euploid transfer to women without RPL.

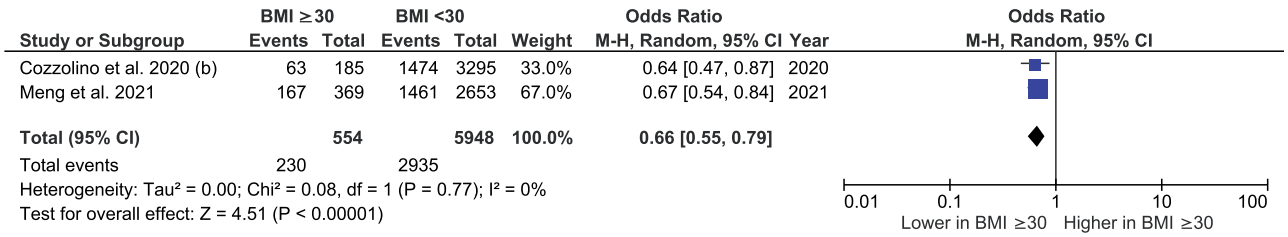

Figure 15. Obese women (body mass index (BMI) >30) showed lower live birth rates per euploid transfer than non-obese women.

progesterone to re-establish normal levels, the same outcomes as women with progesterone levels >10.65 ng/ml were achieved (Álvarez *et al.*, 2021) (Table 2). Lastly, three other papers investigated the association between serum progesterone levels on the day of euploid ET and the related outcomes. Unfortunately, the clustering strategies were too variable: one adopted a 20 ng/ml threshold and reported lower OPRs and LBRs with increasing progesterone levels (Kofinas *et al.*, 2015); one clustered the patients according to quartiles (<13.6 ng/ml, 13.6–24.3 ng/ml, 24.4–53.2 ng/ml, >53.2 ng/ml) and reported largely different OPRs (>12 gestational weeks) and MRs across the groups (Boynukalin *et al.*, 2019); one instead used a 8.8 ng/ml threshold and reported a higher LBR in the case of higher progesterone levels but no difference in the MR (Labarta *et al.*, 2021) (Table 2). The potential impact of progesterone levels on the day of ET certainly deserves further attention from future investigations.

Estradiol

One study investigated the outcomes following euploid SET in relation to estradiol peak levels during ovarian stimulation and clustered the patients into three groups (<2000 pg/ml, 2000–3000 pg/ml, and >3000 pg/ml); no difference was shown in LBR, while MR was not reported (Irani *et al.*, 2020) (Table 2).

According to another study involving a subset of 635 euploid embryos transferred during natural cycles, the length of estradiol exposure may impact the LBR (Romanski *et al.*, 2021). In fact, among the subjects divided based on the length of exposure to estradiol (i.e. >100 pg/ml prior to luteinizing hormone (LH) surge for ≤4 or >4 days), the LBR was lower in case of shorter exposure (Table 2). Lastly, two studies investigated the putative impact of estradiol levels on the day of progesterone initiation on the outcomes euploid SETs (Boynukalin *et al.*, 2020, 2021) (Table 2). In both studies, no association was reported.

Thyroid stimulating hormone

TSH levels are closely monitored during preconception and early pregnancy as TSH >2.5 mIU/l has been associated with poor reproductive outcomes (Stagnaro-Green *et al.*, 2011). For women whose TSH falls within the desired range of <2.5 mIU/l, there appears to be no difference in outcomes following euploid ET (Green *et al.*, 2015). A total of 1599 women who underwent both euploid SETs and DETs following PGT-A at a single institution between 2012 and 2014 were stratified by their TSH levels 8 days after transfer. The groups, divided into 0.5 mIU/l increments of TSH, were similar in age, baseline FSH, AFC, peak oestradiol, and endometrial thickness. Within this range of low-normal TSH, there were no differences in LBR and MR (Table 2).

Insulin growth factor 1 and 2, and insulin growth factor binding protein 1

In a recent study, among 156 women who became pregnant following a natural cycle vitrified-warmed euploid ETs, 23% who experienced a miscarriage had higher than normal follicular IGF-1 levels (18.0 versus 14.7 ng/ml, $P = 0.03$) (Irani *et al.*, 2018a) (Table 2). No differences were shown for IGF-2 and IGF-BP1.

Vitamin D

A retrospective cohort study evaluated OPR based on vitamin D levels at the time of oocyte trigger in 529 euploid ET cycles (Franasiak *et al.*, 2015a) (Table 2). All embryos underwent PGT-A with qPCR and were transferred in either fresh or frozen cycles. Vitamin D levels were divided into tiers: <20 ng/ml, deficient; 20–29.9 ng/ml, insufficient; and ≥30 ng/ml, replete. Notably, only 18.4% of the cohort was Vitamin D replete with older average age of women in the replete category (36.4 years versus 35.1 years in the insufficient and 34.5 years in the deficient categories, $P < 0.01$). The authors found no difference in OPR according to Vitamin D levels. A receiver operating characteristics (ROC) curve evaluating the relationship between Vitamin D level and OPR had an area under the curve (AUC) of 0.502 indicating an almost complete lack of relationship between the two variables. A letter to the editor argued that timing of Vitamin D measurement could add significant bias to these results, given the seasonal differences in sunlight exposure (Sertoglu *et al.*, 2015); the authors responded that season at the time of ET was included in their multivariate analysis (Franasiak *et al.*, 2015b), although Vitamin D levels were measured at the time of oocyte trigger, and not at the time of ET. While these time points are proximate in fresh cycles, the authors did not specify how many transfers were fresh versus vitrified, nor the length of time between oocyte retrieval and ET in the vitrified-warmed ETs. Overall, these results suggest a lack of association between Vitamin D levels and IVF, but further studies looking at Vitamin D levels at time of ET and considering seasonality of Vitamin D measurement are warranted.

Drugs

Levothyroxine

In the previously mentioned study by Green *et al.* (2015), there was no difference in LBR between women who required thyroid hormone supplementation to stay within the desired TSH range and women those who did not require supplementation (Table 2).

Selective serotonin reuptake inhibitor

The commonly prescribed selective serotonin re-uptake inhibitors (SSRIs) were studied for their impact on euploid SET outcomes (Hernandez-Nieto *et al.*, 2017). Specifically, self-reported SSRI exposure (defined as regular use of an SSRI for at least one month prior to ET until finishing at the clinic at 12–14 weeks gestation) resulted in no difference in the CPR (Table 2). If confirmed, these results are reassuring, and suggest that patients can safely take medication to help combat the psychological downside of infertility without adversely impacting their treatment outcomes.

Endometrial features or interventions

Endometrial scratch

Endometrial scratch is an attempt to improve endometrial receptivity by inducing endometrial damage and locally recruiting cytokines and growth factors. While relatively small studies have suggested improvements, a large multicenter trial demonstrated no benefit in non-PGT cycles (Lensen *et al.*, 2019). In a retrospective study, 39 women who failed their first euploid transfer and underwent single pass endometrial scratch in the cycle preceding their second transfer were compared to 251 women who underwent their second transfer without interventions (both SETs and DETs were performed, with no statistical difference between the number of embryos transferred between groups) (Werner *et al.*, 2015) (Table 2). The decision whether to perform endometrial scratch was based on physician preference. There was no difference in the euploid embryo OPR (>9 gestational weeks) between the groups. The authors hypothesize that differences in the technique could add bias to their results but stand by the conclusion that this practice does not improve outcomes.

Endometrial compaction

In the estrogen dominant proliferative phase, the endometrium thickens while after ovulation or with exposure to progesterone, a secretory transformation occurs and the endometrial thickness plateaus or even compacts. Endometrial compaction, defined as a decrease in the thickness of the endometrium from the end of the proliferative phase to the time of transfer, may improve pregnancy rates following euploid SET (Zilberberg *et al.*, 2020) (Table 2). In women undergoing vitrified-warmed ETs, those with any amount of endometrial compaction (5–20%) demonstrated a significantly higher OPR than those without compaction. Nevertheless, these results are limited by the inconsistency in transvaginal ultrasound measurement of the endometrial thickness prior to the start of progesterone versus transabdominal measurement on the day of transfer. Another similar prospective observational study found no association between LBR and endometrial compaction dynamics from the end of the estrogen phase to the day prior to the SET (Riestenberg *et al.*, 2021b) (Table 2). This study used sequential transvaginal ultrasound measurements to control for differences in the sonographic technique. They found that a minority of women (16.6%) experienced compaction, while a majority were found to have endometrial expansion (58.7%). Even so, the LBRs and MRs were not different between groups.

Endometrial receptivity analysis test

The relationship between the evolving endometrium and the growing embryo is vital for implantation, placentation, and ultimately live birth. This relationship is complex, influenced by variations in gene expression leading to a unique combination of enzymes, biomarkers, and implantation factors from both the endometrial decidua and the invading trophoblast (Lague *et al.*, 2010; Teklenburg *et al.*, 2010; Xiong *et al.*, 2012; Brosens *et al.*, 2014; Kang *et al.*, 2014; Herington *et al.*, 2016; Wetendorf *et al.*, 2017; Xu *et al.*, 2019). The intricacy of these interactions is not yet fully understood, and aberrations are thought to contribute to implantation failure. Implantation failure is thought to be due, at least in part, to a failure to properly synchronize the embryo to the endometrium, specifically a patient's unique WOI (Valdes *et al.*, 2017). To this end, the endometrial receptivity analysis (ERA) was designed to determine this personalized window by analyzing endometrial gene expression during a mock ET. Some studies have analyzed the impact of ERA on outcomes following vitrified-warmed euploid blastocyst transfers. Specifically, three studies compared the outcomes in patients performing the ERA versus patients not performing the ERA (Neves *et al.*, 2019; Cozzolino *et al.*, 2020a; Riestenberg *et al.*, 2021a) (Table 1).

In our meta-analysis, transfers conducted after the ERA test (N = 190 overall) resulted in similar a LBR per euploid SETs and DETs as the control transfers (N = 397 overall), with an OR 0.89, 95% CI 0.59–1.35, $I^2 = 0\%$, $P = 0.58$ (Fig. 16). The MR per clinical pregnancy (N = 113 after ERA test and N = 137 in the control) was also similar (OR 1.06, 95% CI 0.48–2.34, $I^2 = 0\%$, $P = 0.88$) (Supplementary Fig. S15).

Four studies sub-analyzed the data according to the result of the ERA test, by comparing patients with a receptive endometrium who underwent a conventional ET versus patients with a non-receptive endometrium who underwent a personalized-ET (Tan *et al.*, 2018; Neves *et al.*, 2019; Barrenetxea *et al.*, 2021; Riestenberg *et al.*, 2021a) (Table 1).

In our meta-analysis, transfers conducted in ERA non-receptive patients who underwent personalized ETs (N = 151 overall) resulted in a similar LBR per euploid SETs and DETs as the patients who were ERA receptive (N = 141 overall), with an OR 1.01, 95% CI 0.43–2.41, $I^2 = 58\%$, $P = 0.97$ (Fig. 17). The MR per clinical pregnancy (N = 96 in the personalized ET group and N = 76 in the ERA receptive one) was also similar between the two groups (OR 1.95, 95% CI 0.2–18.66, $I^2 = 76\%$, $P = 0.58$) (Supplementary Fig. S16).

One last study was not included in the meta-analysis (Bergin *et al.*, 2021) because, although >70% of the transfers analyzed were conducted after PGT-A, the absolute numbers could not be retrieved from the paper. Also in this case, both the MR and LBR were similar, with or without ERA test (Table 2).

Uterine fluid-derived extracellular vesicles transcriptomics

An interesting study analyzed by RNA sequencing the uterine fluid-derived extracellular vesicles (UF-EVs) collected on Day 7 after detection of a urinary LH surge in the month preceding 42 euploid SETs. The authors reported 161 genes which were differentially 'expressed' between ETs resulting in successful live births versus implantation failures, with 14 transcripts selectively detected in UF-EVs of women with a live birth and 5 transcripts detected in women with an implantation failure (Giacomini *et al.*, 2021) (Table 2). This study was comprehensive and full of interesting details about a poorly explored source of information, which could be potentially relevant in decoding the blastocyst-endometrial dialogue during the WOI.

Endometrial and vaginal microbiome

The unique microbiome of the reproductive tract is not fully characterized but may offer an opportunity for intervention (Franasiak and Scott, 2017). In this context, a study analyzed the most distal 5-mm portion of the transfer catheter by next generation sequencing (NGS) to assess the bacteria-specific 16S ribosome gene, thereby allowing genus and species calls for endometrial microorganisms. There were 33 euploid SETs included (18 resulting in an ongoing pregnancy and 15 not resulting in a pregnancy) and 278 different genus calls were reported, although none reached sufficient statistical significance (Franasiak *et al.*, 2016) (Table 2). Another study amplified and sequenced the V3 V4 region of 16S rRNA in the vaginal fluid taken with dry swabs from the bottom of the rectouterine pouch just before 31 euploid SETs with opposing outcomes. A greater, but not significantly different, alpha index of diversity was reported in patients who did not obtain a positive pregnancy test compared to those

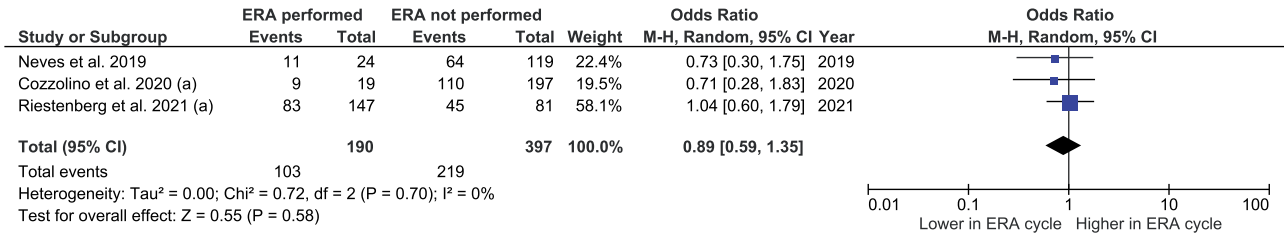

Figure 16. **Euploid blastocyst transfers performed after the endometrial receptivity array (ERA) test showed similar live birth rate to those without the ERA test.**

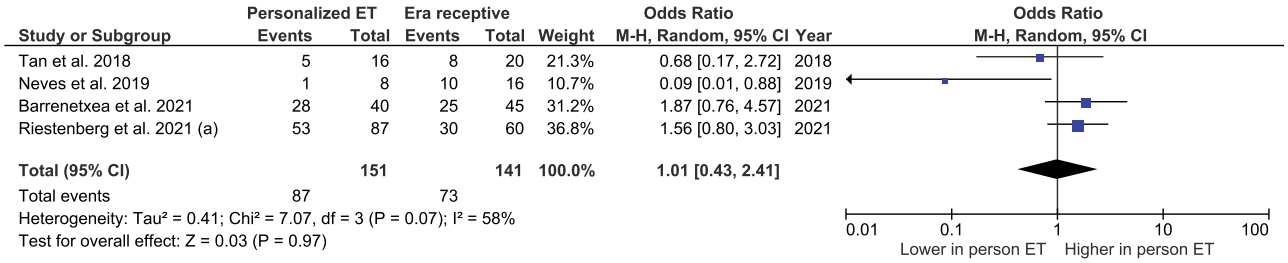

Figure 17. **Personalized embryo transfers (ET) of euploid blastocysts after a report of 'non-receptive endometrium' by the endometrial receptivity array (ERA) test showed similar live birth rates to standard ETs performed after a report of 'receptive endometrium' by the ERA test.**

who did. Also, the beta diversity was not significantly different (Bernabeu *et al.*, 2019) (Table 2). Future studies, with a larger sample size, are required to provide more details on this field of investigation.

Paternal features

The paternal features investigated in the literature for a potential association with the reproductive competence of euploid blastocysts were age, severe male factor (SMF), and sperm DNA fragmentation.

Age

Delayed parenthood and advanced paternal age (APA) are becoming more prevalent in developed countries. While many studies focus on the implications of AMA to IVF, there is a paucity of data on the impact of APA. In fact, there is not even consensus regarding its definition or age cut-off. Two papers from our literature review could be meta-analyzed (Tiegs *et al.*, 2017; Hanson *et al.*, 2020) (Table 1). Both studies reported inferior embryological outcomes in cases of APA, where a lower chance of identifying at least one euploid blastocyst was found compared with controls of younger paternal age. However, APA (here defined as ≥40 years) did not affect the MR, nor the LBR per euploid SET.

In our meta-analysis, transfers conducted in APA couples (N = 1199 overall) showed similar LBRs per euploid SET as non-APA couples (N = 3143 overall) with an OR 0.95, 95% CI 0.83–1.09, $I^2 = 0\%$, $P = 0.45$ (Fig. 18). The MR per clinical pregnancy (N = 905 in APA patients and N = 2391 in non-APA) was also similar for the two groups (OR 1.16, 95% CI 0.90–1.49, $I^2 = 0\%$, $P = 0.25$) (Supplementary Fig. S17).

Three other studies investigated a putative association between paternal age (analyzed as a continuous variable) and euploid SET outcomes (Boynukalin *et al.*, 2020, 2021; Zhou *et al.*, 2021) (Table 2). Similar to the previous studies, no association between APA and either LBR or MR was reported.

Severe male factor

The definition of male factor infertility was variable across the seven papers retrieved from our systematic search, being: (i) sperm concentration <15 million/ml plus motility <40% plus morphology <4%, cryptozoospermia, or surgical sperm retrieval (Mazzilli *et al.*, 2017), (ii) motility <40%, morphology <3%, sperm count <20 million/ml, and total motile count <13 millions/ml (Denomme *et al.*, 2018), (iii) sperm concentration <0.1 million/ml (Tarozzi *et al.*, 2019), or even (iv) undefined (Boynukalin *et al.*, 2020, 2021; Meng *et al.*, 2021; Zhou *et al.*, 2021) (Table 1). Regardless of the definition, none of these papers reported an association between male factor infertility and LBR after euploid SETs and DETs, and only one paper reported a higher MR in euploid SETs and DETs for cases affected by severe male factor (14.7% versus 2.2%) (Denomme *et al.*, 2018).

In our meta-analysis, transfers conducted in couples with sever male factor (SMF) (N = 962 overall) showed a similar LBR per euploid SET/DET for non-SMF couples (N = 3697 overall) with an OR 0.96, 95% CI 0.83–1.11, $I^2 = 0\%$, $P = 0.58$ (Fig. 19). The MR per clinical pregnancy (N = 602 in SMF patients and N = 2255 in non-SMF) was also similar in the two groups (OR 0.89, 95% CI 0.54–1.45, $I^2 = 49\%$, $P = 0.64$) (Supplementary Fig. S18).

Sperm DNA fragmentation

Sperm DNA fragmentation refers to damaged DNA that impairs the genomic integrity of spermatozoa. It can be caused by apoptosis, DNA strand breaks during remodeling, oxygen radicals during transport, endogenous caspases or endonucleases, or occur as a result of radiation, chemotherapy or environmental toxins (Sakkas and Alvarez, 2010). In this review, two retrospective (Gat *et al.*, 2017; Irani *et al.*, 2018b) and one prospective studies (Green *et al.*, 2020) were retrieved on this topic (Table 2); they reported the outcomes after euploid SETs and DETs by clustering the results according to a 15% threshold for the sperm DNA fragmentation index. None of them showed an association with either the MR or LBR, but they could not be included in the meta-

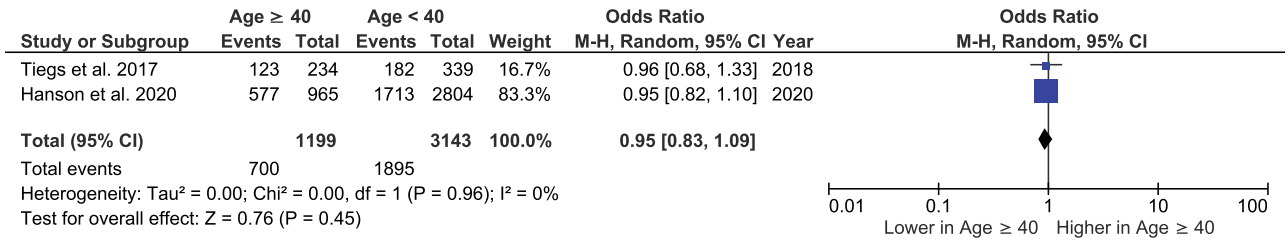

Figure 18. **Advanced paternal age (≥40 years) is associated with a similar live birth rate per euploid blastocyst transfer to paternal age <40 years.**

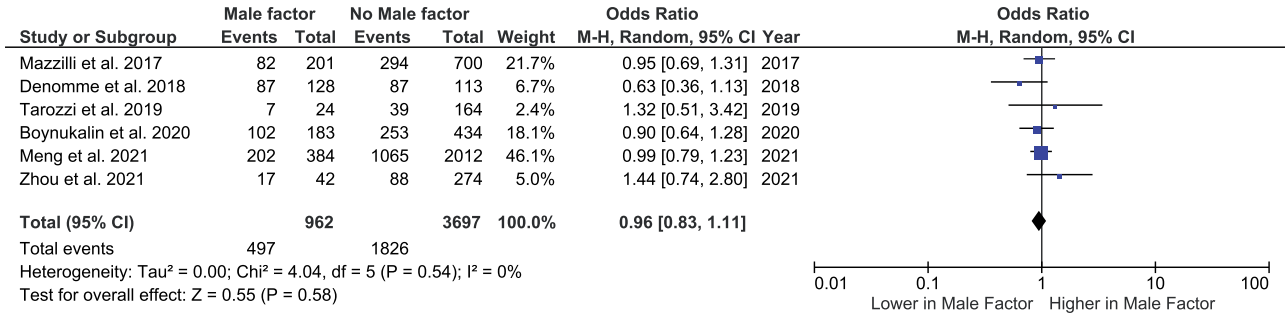

Figure 19. **The live birth rate per euploid blastocyst transfer was independent of severe male factor infertility.**

analysis as two of the three studies reported only the OPR based on a 9–12 gestational weeks threshold.

Clinical and laboratory features

A putative impact of clinical and/or laboratory features on embryonic competence has always represented a matter of concern. Euploid blastocyst ETs provide a relatively unbiased setting to assess this possibility. Hereafter, we summarized the results obtained for all the procedures performed along an IVF treatment in a stepwise order: ovarian stimulation, oocyte vitrification, fertilization method, embryo culture, TE biopsy, and ET. The performance across different IVF centers involved in multicenter studies was also assessed for its putative impact.

Ovarian stimulation for the oocyte retrieval cycle

Stimulation protocols for the oocyte retrieval cycle can differ by the cycle type, gonadotropin dose, stimulation length, and type of ovulation trigger. The debate on whether altering these stimulation parameters may influence the embryo euploidy status and embryo competence dates back over one decade and it will require additional large-scale investigations to be clarified (Rubio *et al.*, 2010; Massie *et al.*, 2011).

Natural cycle versus ovarian stimulation for the oocyte retrieval cycle

An American study compared ET outcomes after euploid blastocysts were obtained from natural cycles with a dual hCG and GnRH-agonist trigger with their historical control of euploid blastocysts obtained after ovarian stimulation for the oocyte retrieval cycle (Hong *et al.*, 2019) (Table 2). No difference between the two groups was shown in either the aneuploidy rates or in the OPR (>8 gestational weeks) after SET. More studies investigating this topic are certainly needed.

Protocol of ovarian stimulation for the oocyte retrieval cycle

A single study reported the MR and LBR after euploid SETs of embryos produced after different ovarian stimulation protocols administrated for oocyte retrieval cycle (Zhou *et al.*, 2021) (Table 2). No association was reported, but, also in this case, more investigations are encouraged.

Gonadotrophins dosage used in the oocyte retrieval cycle

Several groups tested a putative association between euploid ET outcomes, and the total dosage of gonadotrophins (Gn) administered during the ovarian stimulation for the oocyte retrieval cycle. Two papers could be meta-analyzed by clustering their results into two groups according to a 3000 IU threshold (Barash *et al.*, 2017a; Wu *et al.*, 2018) (Table 1). Unfortunately, both assessed the LBR but not the MR.

In our meta-analysis, transfers conducted after the ovarian stimulation for the oocyte retrieval cycles used ≥3000 IU used (N = 311 overall) showed similar LBRs per euploid SET as cycles that used <3000 IU (N = 740 overall), with an OR 1.04, 95% CI 0.76–1.42, $I^2 = 0\%$, $P = 0.83$ (Fig. 20).

One paper could not be included in the meta-analysis because the population was divided into Gn dosage ranges incompatible with the previous studies (<4000 IU, 4000–6000 IU, and >6000 IU groups). No difference in the LBR per SET was reported between the two groups (Irani *et al.*, 2020) (Table 2). Three more studies investigated the Gn total dosage as a continuous variable (Boynukalin *et al.*, 2020, 2021; Zhou *et al.*, 2021) (Table 2). Again, no associations between Gn dosage and LBR or MR were reported following euploid SETs.

Number of oocytes retrieved after ovarian stimulation

Several studies investigated a putative association between the number of oocytes retrieved after ovarian stimulation and the outcomes after euploid ETs. None of them could be meta-analyzed because we could not identify similar thresholds to cluster the results, namely: (i) ≤5 versus >5 (Wu *et al.*, 2018),

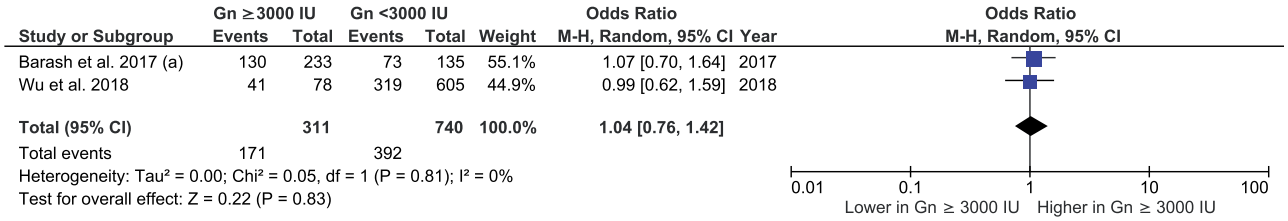

Figure 20. The live birth rate per euploid blastocyst transfer was no different whether the total gonadotrophins (Gn) dosage was ≥3000 IU or <3000 IU in the fresh ovarian stimulation cycle.

(ii) ≤5 versus >10 (Morin *et al.*, 2018b), (iii) <10, 10–19, and ≥20 (Irani *et al.*, 2020), or (iv) the number of oocytes as a continuous variable (Barash *et al.*, 2017a; Boynukalin *et al.*, 2020, 2021) (Table 2). Among these studies, only one reported a significant improvement in LBR per SET in good responders (defined as >5 oocytes retrieved, 52.6% versus 35.6% in poor responders) (Wu *et al.*, 2018), while the outcomes were comparable across all of the other papers.

Double ovarian stimulation in the same ovarian cycle

Ovarian stimulation protocols can also differ regarding the phase of the ovarian cycle in which they are started. DuoStim (double stimulation in the same ovarian cycles) takes advantage of the multiple waves arising during folliculogenesis in humans (Baerwald *et al.*, 2012) and it has been adopted to increase the oocyte yield in a short timeframe (about 15 days). Interestingly, embryological outcomes from cohorts of follicles collected after one or two stimulations appear no different (Cimadomo *et al.*, 2018d). In our review, two prospective studies from the same group were retrieved. One single center (Ubaldi *et al.*, 2016) and one multicenter (Vaiarelli *et al.*, 2020) study compared the MR and LBR of euploid blastocysts obtained from DuoStim cycles (one versus two stimulations), with both reporting comparable outcomes (Table 1).

In our meta-analysis, transfers conducted with embryos obtained from luteal phase stimulation (LPS) (N = 215 overall) showed a similar LBR per euploid SET as embryos obtained from follicular phase stimulation (FPS) (N = 189 overall) with an OR 1.21, 95% CI 0.82–1.80, $I^2 = 0\%$, $P = 0.33$ (Fig. 21). The MR per clinical pregnancy (N = 124 from embryos obtained from LPS and N = 100 from FPS) was also similar across the two groups (OR 0.90, 95% CI 0.43–1.91, $I^2 = 0\%$, $P = 0.79$) (Supplementary Fig. S19).

Trigger for ovulation

Near the end of ovarian stimulation, a final ovulation trigger shot is typically administered 35–36 h prior to oocyte retrieval. This injection matures oocytes to complete the first meiotic division and reach the MII stage to become ready for fertilization. Our review retrieved three studies investigating whether the use of the GnRH-agonist or hCG for trigger affected the outcomes after euploid SET (Makhijani *et al.*, 2020; Tan *et al.*, 2020; Cimadomo *et al.*, 2021c) (Table 1). In general, using a GnRH-agonist trigger reduced the likelihood of ovarian hyperstimulation syndrome (OHSS) by decreasing the production of vasoactive substances (i.e. vascular endothelial growth factor) with no impact on the clinical outcomes.

In our meta-analysis, transfers conducted in cycles where hCG was employed (N = 803 overall) showed similar LBRs per euploid SET as in cycles where GnRH-agonist was used (N = 1216 overall) with an OR 0.86, 95% CI 0.55–1.35, $I^2 = 71\%$, $P = 0.52$ (Fig. 22). The MR per clinical pregnancy (N = 123 after hCG trigger and N = 197 after GnRH-agonist trigger) was also similar in the two groups (OR 1.43, 95% CI 0.76–2.68, $I^2 = 0\%$, $P = 0.26$) (Supplementary Fig. S20).

Oocyte vitrification

Cryopreservation, especially via vitrification, was a game-changing technique in IVF. It implied a plethora of benefits for patient management, treatment strategy, and safety. Vitrification is less efficient for oocytes than for blastocysts, however, oocyte cryopreservation is more suitable for fertility preservation purposes as it ensures women's reproductive autonomy without committing to a specific partner (Rienzi and Ubaldi, 2015; Rienzi *et al.*, 2017). In some cases, oocyte vitrification can be even suggested to poor prognosis patients for oocyte accumulation purposes (Cobo *et al.*, 2012) or used in oocyte donation cycles (Rienzi *et al.*, 2020). Two groups assessed whether this procedure may impact the clinical outcomes in the context of euploid embryo transfers (Table 1). In particular, a RCT on sibling oocytes, half vitrified and warmed the same day and half processed fresh (Forman *et al.*, 2012), and a retrospective case–control study, where maternal age-matched couples using fresh oocytes were compared to couples using vitrified-warmed oocytes (Goldman *et al.*, 2015), were published. No difference in clinical outcomes was reported.

In our meta-analysis, transfers conducted with embryos obtained from vitrified-warmed oocytes (N = 45 overall) showed similar LBRs per euploid SETs and DETs as from fresh oocytes (N = 86 overall) with an OR 1.21, 95% CI 0.58–2.53, $I^2 = 0\%$, $P = 0.61$ (Fig. 23).

Fertilization method

ICSI has been recommended during PGT cycles to ensure monospermic fertilization and to minimize the risk of DNA contamination from sperm attached to the ZP or residual cumulus cells (Thornhill *et al.*, 2005). Despite this recommendation, the use of conventional IVF has been lately explored. Similar euploidy rates were reported for IVF and ICSI in PGT-M cycles with both blastomere (Feldman *et al.*, 2017; Sahin *et al.*, 2017) and TE (Palmerola *et al.*, 2019) biopsies. A recent prospective RCT in sibling oocytes also confirmed that similar euploidy outcomes may be obtained by ICSI and by conventional IVF (De Munck *et al.*, 2020). However, only one study reported clinical pregnancy rate (>4 gestational weeks) after euploid SETs in cycles that used ICSI versus conventional IVF (Bradley *et al.*, 2017b). No difference was shown in this study (Table 2).

In context of ICSI and PGT-A, there was only one study that investigated whether the timings of oocyte denudation and ICSI itself, as well as the overall interval between induction of ovulation and ICSI, were associated with the reproductive competence of euploid blastocysts. No association was reported with all outcomes, including the cumulative live birth rate (Maggiulli *et al.*, 2020).

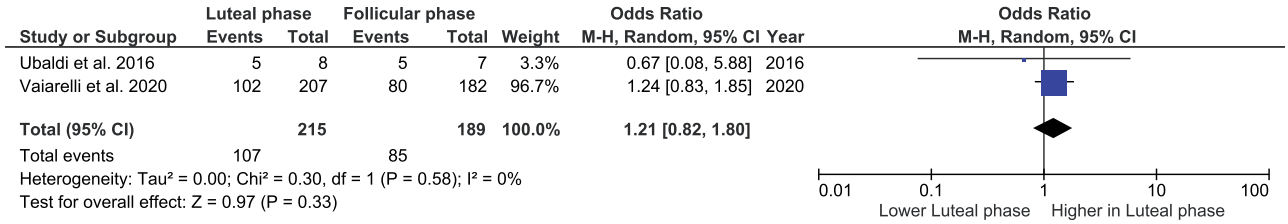

Figure 21. The live birth rate per euploid blastocyst transfer was no different whether the double stimulation protocol for the fresh cycle was started in the luteal or follicular phase.

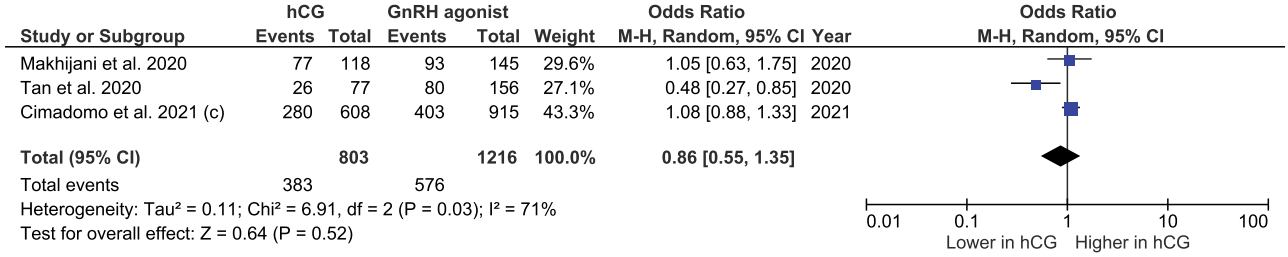

Figure 22. The live birth rate per euploid blastocyst transfer was no different whether the ovulation trigger adopted at the end of ovarian stimulation in the fresh cycle was hCG or GnRH-agonist.

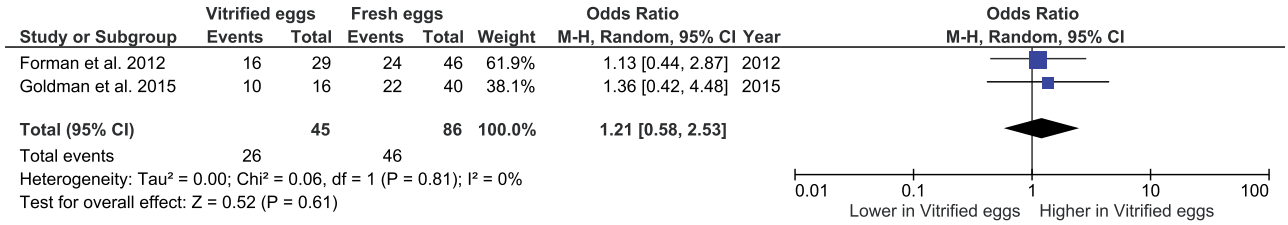

Figure 23. The live birth rate per euploid blastocyst transfer was similar regardless of whether fresh or vitrified-warmed oocytes were used for embryogenesis.

Embryo culture

Culture strategies vary between IVF laboratories and a wide range of variables (e.g. culture media, incubator, temperature, oxygen concentrations, single or sequential media, group, or individual culture) may impact both embryological and clinical outcomes (Wale and Gardner, 2016). It is not surprising that some authors tried to assess potential impacts of these parameters on embryo reproductive competence in the context of euploid ETs.

Culture media

Some studies compared continuous media (blastocyst culture in the same media with or without change-over) to sequential ones (culture in two different media with a changeover in day3) reporting either comparable (Werner *et al.*, 2016; Cimadomo *et al.*, 2018c) or different euploidy rates (Deng *et al.*, 2020b) at the blastocyst stage. Two studies could be meta-analyzed for MR and LBR outcomes after euploid ETs, namely a prospective study that used different media according to the day of the week oocyte retrieval was conducted on (Cimadomo *et al.*, 2018c), and a retrospective study (Deng *et al.*, 2020b) (Table 1).

In our meta-analysis, transfers conducted after embryo culture in a continuous media (N = 632 overall) showed a similar LBR per euploid SET as culture in sequential media (N = 374 overall), with an OR 0.93, 95% CI 0.71–1.21, $I^2 = 0\%$, $P = 0.58$ (Fig. 24). The MR per clinical pregnancy (N = 320 from embryos obtained with a continuous media and N = 192 with sequential media) was also similar between the two groups (OR 1.71, 95% CI 0.96–3.04, $I^2 = 0\%$, $P = 0.07$) (Supplementary Fig. S21).

A RCT on sibling zygotes cultured in either a continuous or sequential media was also retrieved from the literature. It showed no association between culture strategy and clinical outcomes after euploid SET however, only the OPR (>9 gestational weeks) was reported (Werner *et al.*, 2016) (Table 2). Lastly, one study compared the clinical outcomes after 81 euploid SETs from embryos cultured in two different media, both continuous. Even in this case, no association was documented in the LBR and MR between the groups (Fabozzi *et al.*, 2021) (Table 2).

Individual or group culture

Only one study reported the MR and LBR after euploid SETs by comparing individual embryo culture to group culture (Glatthorn *et al.*, 2021) (Table 2). No difference was shown between the two types of cultures.

Culture temperature

Only one study reported the LBR after euploid ET in two groups clustered according to the embryo incubation temperature (37 °C versus 36 °C from ICSI onwards) (Table 2) (Hong *et al.*, 2014). Specifically, sibling oocytes were split into the two groups and 42 double ETs of euploid blastocysts from both study arms were

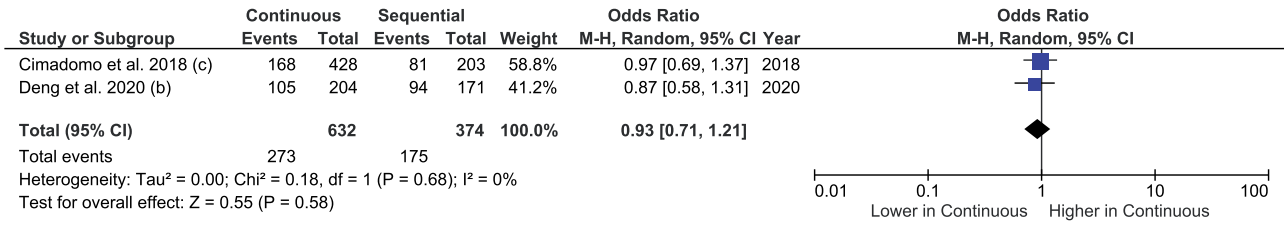

Figure 24. The live birth rate per euploid blastocyst transfer was similar regardless of whether continuous or sequential media were used for embryo culture.

conducted. Additionally, four euploid SETs of euploid blastocysts from either one or the other study arm were carried out. The LBR was similar across the two groups.

Dynamic versus static culture

Only one study compared the outcomes from sibling oocytes cultured on either a standard system or a dynamic microvibration platform (NSSB-300, Nepagene: frequency of 42 Hz for 5 min every 60 min) which is supposed to better mimic *in vivo* conditions (Table 2) (Juneau *et al.*, 2020). Following 42 paired euploid double ETs and 19 euploid SETs, no difference was shown between the two groups across all outcomes investigated, including euploidy and LBR.

Embryo selection based on static or morphodynamic criteria

Time lapse parameters can be adopted in an attempt to improve embryo selection in the context of PGT-A cycles and euploid ET. Three papers that investigated whether morphodynamic embryo assessment (and indirectly also embryo culture in undisturbed time lapse incubators) improved the outcomes versus static embryo assessment (Yang *et al.*, 2014; Rocafort *et al.*, 2018; Gazzo *et al.*, 2020a) were retrieved from the literature, one prospective and two retrospective studies (Table 2). Unfortunately, their data could not be meta-analyzed since only one reported LBR, and two limited their reports to OPR; nonetheless, all these studies showed higher LBRs or OPRs per SET and DET with morphokinetics-based embryo selection. In two studies, the operator's choice was further powered with dedicated software, namely Eeva and KidscoreTM D5 algorithm (Rocafort *et al.*, 2018; Gazzo *et al.*, 2020a). With the growing implementation of artificial intelligence-powered tools for the analysis of IVF time-lapse videos, this preliminary evidence certainly encourages further studies.

Trophectoderm biopsy

In the last decade, TE biopsy has gradually started to replace blastomere biopsy (Dahdouh *et al.*, 2015a; Rosenwaks *et al.*, 2018; Kokkali *et al.*, 2020). This shift was driven by the accumulating evidence supporting its safety and clinical reliability (Scott *et al.*, 2012, 2013; Capalbo *et al.*, 2016a; Cimadomo *et al.*, 2016; Tiegs *et al.*, 2020). Nevertheless, good training, constant operator monitoring, and protocol validation are essential for preventing unexpected impact on clinical outcomes.

Protocol for TE biopsy

Four blastocyst biopsy protocols have been described, three entailing ZP drilling at either Day 3 (de Boer *et al.*, 2004; McArthur *et al.*, 2005) or the morula or blastocyst stage plus artificial hatching (Veiga *et al.*, 1997), and one entailing simultaneous ZP drilling plus TE biopsy (Capalbo *et al.*, 2014) (reviewed by ESHRE in its recent good practice recommendations; Kokkali *et al.*, 2020). The day3 hatching-based and the simultaneous ZP opening plus TE biopsy protocols are the mostly used worldwide, and three studies (a RCT, a retrospective matched case–control and a retrospective observational study) investigated whether an impact on MR and LBR after euploid blastocyst transfer could be possible due to the biopsy technique employed (Zhao *et al.*, 2019; Rubino *et al.*, 2020; Xiong *et al.*, 2021b) (Table 1).

In our meta-analysis, transfers conducted after a simultaneous ZP opening and biopsy protocol (N = 950 overall) showed higher LBRs per euploid SET than transfers of embryos biopsied after day3 hatching (N = 950 overall), with an OR 1.41, 95% CI 1.18–1.69, $I^2 = 0\%$, $P < 0.01$ (Fig. 25). However, the MR per clinical pregnancy (N = 620 from embryo biopsied with a simultaneous ZP opening and biopsy protocol and N = 529 from embryos biopsied after day3 hatching) was similar (OR 1.00, 95% CI 0.68–1.49, $I^2 = 0\%$, $P = 0.99$) (Supplementary Fig. S22).

It should be noted that the resulting differences in clinical outcomes may in part be due not only to the procedure of ZP opening, but also to factors intrinsic to the technique (e.g. Day 3 hatching requires the embryo to be exposed to suboptimal temperatures as well as laser pulsing sessions twice).

Operators for TE biopsy

There is still limited knowledge about the reproducibility and consistency among TE biopsy practitioners across different IVF laboratories. Therefore, the risk that less skilled embryologists may affect its technical or clinical outcomes is not negligible. From a technical standpoint, a study involving 42 fertility clinics referring to a single genetic laboratory for PGT-A purposes in oocyte donation cycles, unveiled significantly different technical outcomes for ten clinics (Munne *et al.*, 2017). Similarly, another study across six IVF clinics and in non-donor PGT-A cycles reported statistically significant differences in the rate of inconclusive diagnoses, which increased from 1.5% in the clinics with the largest volumes to 4.5% in the clinics with the lowest ones (Cimadomo *et al.*, 2018b). From a clinical standpoint, two retrospective studies (one multicenter and one single center) investigated whether clinical outcomes differed across several equally trained qualified biopsy practitioners. No difference was reported for all metrics including the LBR (Capalbo *et al.*, 2016a; Maggiulli *et al.*, 2019) (Table 2). The same group then investigated whether equally trained qualified operators performing ICSI, denudation, vitrification, and warming affected the clinical outcomes after vitrified-warmed euploid SETs. Also, for these procedures, no association was reported (Cimadomo *et al.*, 2018a; Maggiulli *et al.*, 2020).

Number of cells biopsied

The number of TE cells removed during a biopsy is critical. Each operator's goal is to obtain good-quality molecular analyses, that would allow a conclusive diagnosis, while minimizing a putative impact on embryo competence and viability. Both these purposes

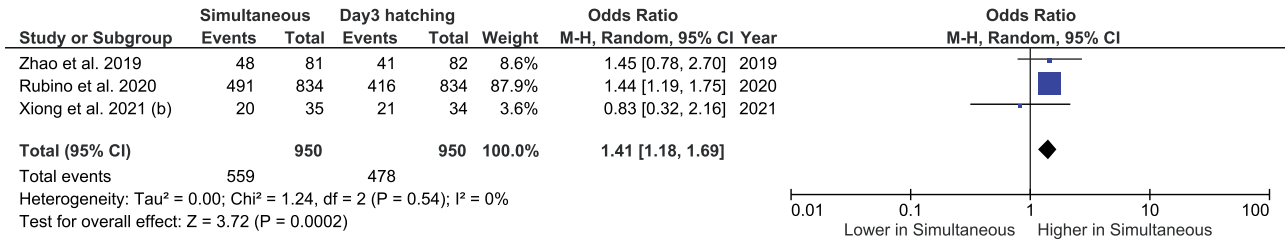

Figure 25. The live birth rate per euploid blastocyst transfer was higher when the simultaneous zona pellucida opening and trophectoderm biopsy protocol was used rather than the day3 hatching based protocol.

may be fulfilled by defining the ideal blastocyst expansion stage to retrieve at least seven to eight cells, which is a reasonable cellularity to achieve good molecular analyses (Capalbo *et al.*, 2016a; Cimadomo *et al.*, 2018b), thereby also limiting overall embryo biomass reduction. In two studies, the removal of a larger (estimated) number of cells was associated with worse implantation after euploid SETs (Neal *et al.*, 2017; Guzman *et al.*, 2019) (Table 2). Specifically, the highest quartile in a range 1–20 TE cells in an American study, and the group that averaged 10 TE cells versus 5 TE cells in a Peruvian study, showed lower implantation. These data emphasize the importance of obtaining appropriately sized TE biopsies to suitably balance good technical outcomes and the invasiveness of the technique.

Time between biopsy and vitrification

Three studies reported on a putative impact of the time elapsing between TE biopsy and vitrification on the outcomes after warming (Chen *et al.*, 2017; Maggiulli *et al.*, 2019; Xiong *et al.*, 2021a) (Table 2). However, their data could not be meta-analyzed mainly because different ranges of time to cluster the results were defined. Some authors suggested a trend towards better OPRs or LBRs per SET if blastocyst vitrification was performed before 30–60 min from biopsy (Maggiulli *et al.*, 2019; Xiong *et al.*, 2021a), whereas others claimed that >180 min is the optimal timing for vitrification after biopsy, showing higher full re-expansion rate after biopsy and improved LBR per SET after warming (Chen *et al.*, 2017). In summary, the production of more data focused on this stage of the biopsy procedure are highly encouraged.

Re-biopsy and re-vitrification of blatocysts

Typically, a single biopsy and vitrification-warming cycles is required for PGT. However, in case of inconclusive diagnoses, two biopsy and vitrification-warming cycles are needed. Five papers reported the outcomes of re-biopsied euploid blastocysts versus embryos biopsied and vitrified only once. However, only three of these studies could be meta-analyzed (Bradley *et al.*, 2017a; Cimadomo *et al.*, 2018b; Aluko *et al.*, 2021) (Table 1) since the other two limited their reports to OPR (Taylor *et al.*, 2014b; Neal *et al.*, 2019) (Table 2). The data are controversial, ranging from no impact to a limited but significant impact.

In our meta-analysis, transfers conducted after a re-biopsy and re-vitrification (N = 86 overall) showed a similar LBR per euploid SET as embryos biopsied once (N = 6896 overall) with an OR 0.68, 95% CI 0.43–1.07, $I^2 = 4\%$, $P = 0.10$ (Fig. 26). The MR per clinical pregnancy (N = 34 from re-biopsied embryos and N = 3789 from embryos biopsied once) was also similar in the two groups (OR 0.77, 95% CI 0.23–2.51, $I^2 = 0\%$, $P = 0.66$) (Supplementary Fig. S23).

To limit a putative impact of multiple manipulations, it is critical to ensure operators' expertise in conducting biopsy and tubing, as well as in choosing the most suitable stage to start the biopsy procedure.

Biopsy and second vitrification-warming of previously untested vitrified blastocysts

When untested vitrified blastocysts are warmed to be biopsied due to a deferred clinical or personal choice, two vitrification-warming cycles and a single biopsy might be needed. Also in this case, two of the four studies retrieved could not be meta-analyzed due to incompatible differences in the outcome measures adopted (Taylor *et al.*, 2014b; Neal *et al.*, 2019) (Table 2). The other two studies reported both the MR and LBR and were meta-analyzed (Bradley *et al.*, 2017a; Aluko *et al.*, 2021) (Table 1). In our meta-analysis, transfers conducted after a single biopsy but two vitrification-warmings (N = 121 overall) showed a lower LBR per euploid SET than embryos biopsied and vitrified only once (N = 4071 overall) with an OR 0.41, 95% CI 0.22–0.77, $I^2 = 50\%$, $P < 0.01$ (Fig. 27). However, the MR per clinical pregnancy (N = 47 from embryos biopsied once but vitrified twice and N = 2410 from embryos biopsied and vitrified only once) was similar in the two groups (OR 2.14, 95% CI 0.99–4.62, $I^2 = 0\%$, $P = 0.05$) (Supplementary Fig. S24).

Worse outcomes were reported in the group subject to additional manipulations, although this result would require dedicated adjustments according to the protocols adopted, operators' expertise, blastocyst day of biopsy and quality, as well as patient prognosis. Therefore, more larger studies are strongly recommended.

Embryo transfer

Given that no known adjustment in stimulation protocol or trigger influences LBR after euploid ET, attention is turned towards optimizing ET and endometrial preparation.

Fresh versus vitrified-warmed embryo transfer

Evidently, the application of most PGT-A techniques on TE biopsies would not be possible without blastocyst cryopreservation. Blastocyst biopsy and vitrification are indeed both essential and equally critical in the routine activity of a clinic offering PGT (Maggiulli *et al.*, 2019). Nevertheless, when a limited turn-around time can be guaranteed between TE biopsy and diagnosis, some authors have also assessed a putative difference between fresh ET and conventional vitrified-warmed ET after obtaining the results of PGT-A. Specifically, a retrospective study (Rodriguez-Purata *et al.*, 2016) and a RCT (Coates *et al.*, 2017) (Table 1) were retrieved.

In our meta-analysis, vitrified-warmed transfers (N = 489 overall) showed a higher LBR per euploid SET and DET than rapid fresh transfers (N = 362 overall) with an OR 1.56, 95% CI 1.05–2.33, $I^2 = 23\%$, $P = 0.03$ (Fig. 28).

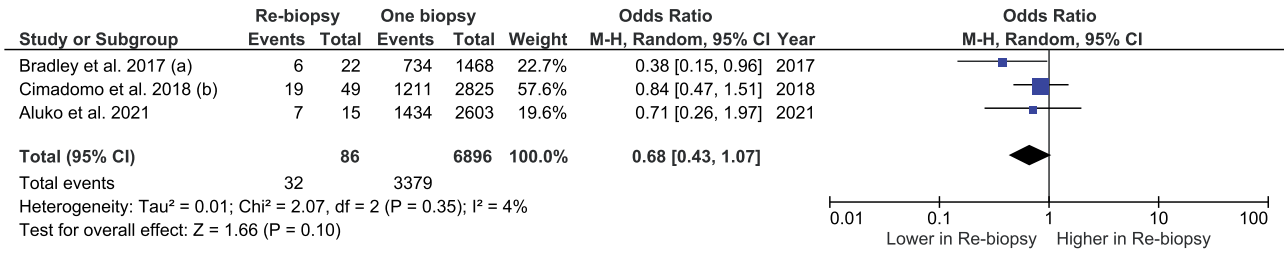

Figure 26. **The live birth rate per euploid blastocyst transfer was similar between blastocysts re-biopsied and re-vitrified and blastocysts biopsied and vitrified only once.**

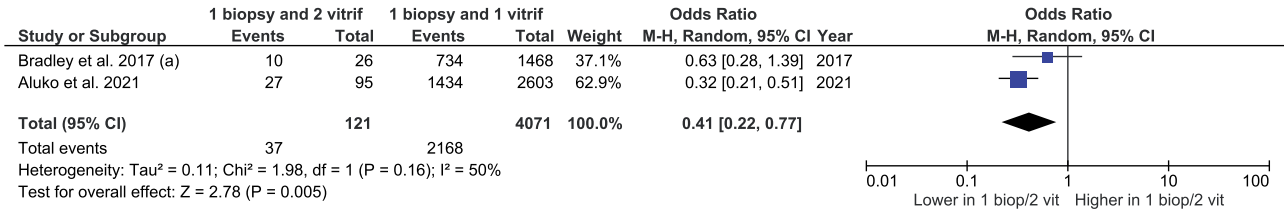

Figure 27. **The live birth rate per euploid blastocyst transfer was lower when blastocysts were vitrified twice (though biopsied only once) then when blastocysts were vitrified (and biopsied) only once.**

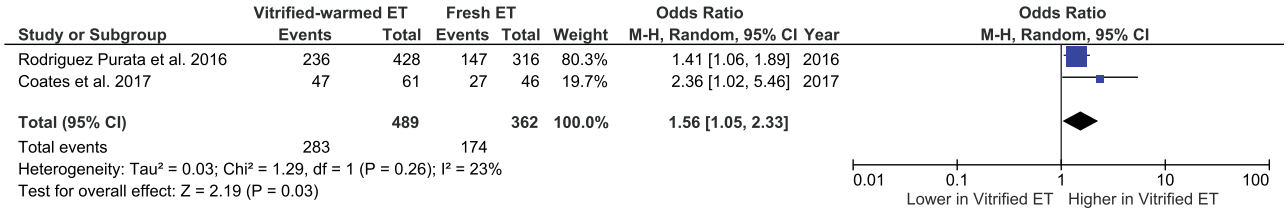

Figure 28. **The live birth rate per euploid blastocyst transfer was higher after a vitrified-warmed embryo transfer (ET) than after a fresh ET.**

Also, a prospective single center study reported no difference, although it accounted for only 8 fresh and 13 vitrified-warmed ETs (Ma *et al.*, 2016) (Table 2).

Embryo transfer difficulty

Our review retrieved one study that found a decreased LBR after difficult (Wallace stylet or tenaculum required) compared to easy (direct or outer sheath required) euploid ETs; however, this association was not significant after adjusting for confounders (Alvarez *et al.*, 2019) (Table 2). This is another aspect that requires further investigation.

Different embryo transfer operators

According to two studies from the same group, the operators conducting the embryo transfers can impact the clinical outcomes (Cirillo *et al.*, 2020, 2022). This evidence put the human factor during the ET procedure under the spotlight. Nevertheless, we retrieved only one study that reported CPR after euploid SETs according to the physician who performed the procedures (Guzman *et al.*, 2019) (Table 2). Although variable outcomes were reported, after adjusting for confounders, no significant association could be confirmed.

Endometrial preparation protocol for vitrified-warmed transfer

The endometrial preparation protocols currently in use are modified natural cycle (MNC) or hormone replacement therapy (HRT) with exogenous estrogen and progesterone. Our review retrieved three studies focused on this practice that could be meta-analyzed, a RCT and two retrospective ones (Greco *et al.*, 2016; Melnick *et al.*, 2017; Zhou *et al.*, 2021) (Table 1). The RCT compared MNC to HRT in 236 patients undergoing vitrified-warmed euploid SET and showed comparable outcomes (Greco *et al.*, 2016). This evidence was confirmed by a retrospective analysis of 316 euploid SETs (Zhou *et al.*, 2021), while a smaller report of 113 euploid SETs in anovulatory women claimed significantly lower outcomes in the HRT group, although they did not report the cycle cancelation rate in the natural cycle arm (Melnick *et al.*, 2017), which is notoriously more frequent.

In our meta-analysis, transfers conducted after HRT (N = 368 overall) showed similar LBRs per euploid transfer as those conducted after a MNC (N = 283 overall) with an OR 0.73, 95% CI 0.41–1.30, $I^2 = 66\%$, $P = 0.29$ (Fig. 29). The MR per clinical pregnancy (N = 167 after HRT and N = 144 after MNC) was also similar (OR 1.57, 95% CI 0.79–3.09, $I^2 = 0\%$, $P = 0.20$) between the two groups (Supplementary Fig. S25).

Lastly, another retrospective analysis of 389 euploid SETs reported a higher OPR (>8 gestational weeks) in the natural cycle group compared to an HRT group (Wang *et al.*, 2019c) (Table 2). In summary, further investigations, also including gestational and perinatal outcomes in both study arms, are recommended to shed light on a practice that significantly affects the flexibility in the management of an IVF treatment.

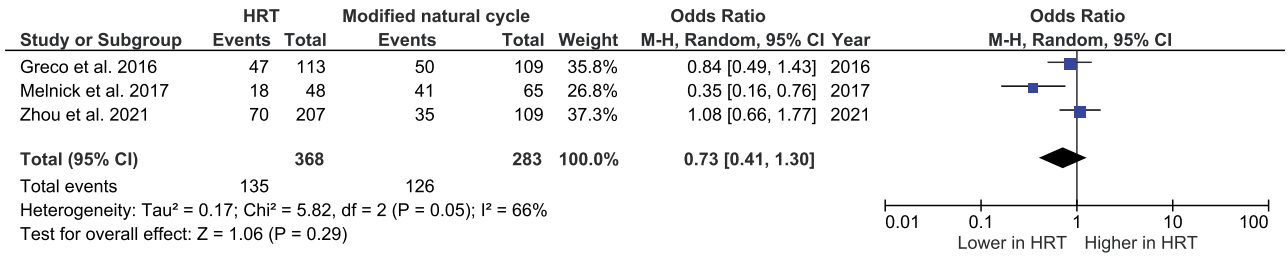

Figure 29. The live birth rate per euploid blastocyst transfer was similar when either hormone replacement therapy (HRT) or modified natural cycle was used as the endometrial preparation protocol.

Follicular phase length prior to LH surge in natural vitrified-warmed transfer cycles

A single study reported the MR and LBR according to the follicular phase length prior to the LH surge in the context of a natural cycle for endometrial preparation for vitrified-warmed euploid SETs and DETs. Specifically, the authors showed no difference LBR or MR whether the follicular phase was shorter or longer than 15 days (Romanski *et al.*, 2021) (Table 2).

Progesterone and estradiol during endometrial preparation for vitrified-warmed transfer

A study explored different approaches to luteal phase support, comparing daily administration of intramuscular progesterone (100 mg/day) versus a daily vaginal gel (90 mg twice daily) plus weekly intramuscular progesterone (250 mg/week) administration, in the context of euploid SETs. No difference was found in the MR or LBR (Asoglu *et al.*, 2019) (Table 2). Another study, instead, reported higher LBRs when vaginal/oral and intramuscular routes of progesterone administration were both adopted rather than only the former or only latter. They investigated also whether varying durations and cumulative dose of estrogen administration prior to euploid ET impacted the LBR. In this case, no difference was found (Sekhon *et al.*, 2019) (Table 2).

Different IVF centers in multicenter studies

Standardization is critical in ART and, theoretically, euploid ET should minimize the differences between IVF centers in the outcomes per transfer, beyond the confounders that this review aims at outlining. Among the multicenter studies retrieved from our systematic search, though, only four clearly stated the outcomes at each center involved (Capalbo *et al.*, 2014, 2016a; Cimadomo *et al.*, 2018b; Rienzi *et al.*, 2019). No differences were shown in the MR (when reported) and LBR after SETs and DETs (Table 2). We encourage all future multicenter studies to always state whether different outcomes are reported across the clinics. This evidence is critical to testify whether and to what extent PGT outcomes are reproducible.

Risk of bias and level of evidence

The risk of bias within the meta-analyzed studies is illustrated in Supplementary Tables S1 and S2. Publication bias was evaluated for risk factors in which at least eight papers were included. According to trim and fill analysis, no publication bias was observed in these categories (Supplementary Figs S26–S28).

Almost all features were characterized by very low level of evidence because the analysis was largely based on retrospective studies. The only features for which at least one RCT could be included, and that are therefore characterized by low level of evidence, are fresh or vitrified-warmed transfer, oocyte vitrification, the endometrial preparation protocol for vitrified-warmed transfer, and the trophectoderm biopsy protocol.

Discussion

The transfer of euploid blastocysts in an apparently receptive uterine environment offers the highest chance of embryo implantation with currently available IVF technologies and strategies. Yet, many euploid blastocysts either fail to implant or result in a miscarriage before the mid gestation. In this systematic review and meta-analysis, we scrutinized all possible causes of negative reproductive outcomes in the context of euploid blastocyst transfers, by categorizing them as embryonic, maternal, paternal, clinical, or laboratory features. The aim of this quest was to identify all relevant features that may influence IVF clinical outcomes, define the level of evidence of their impact on treatment, and unveil areas of investigation still poorly (or not) explored, which would require future efforts in academic and clinical research.

Embryo static morphological assessment still represents a valuable tool for embryo selection, also in the context of euploid blastocyst transfer. In fact, poor-quality ICM and TE, either considered individually or together, were consistently found to be highly associated with a lower LBR per transfer across all the papers included in our analysis. Moreover, a grade C TE was also consistently associated with a higher MR, presumably because the embryonic annexes (like the placenta) arise from this section of the blastocyst. Nevertheless, this evidence is subject to at least two putative downsides: (i) poor-quality blastocysts are presumably transferred to poor prognosis patients and/or as a last choice in women who have not become pregnant with better-quality blastocysts, and (ii) embryo morphological grading is poorly reproducible across different IVF centers (Khosravi *et al.*, 2019; Cimadomo *et al.*, 2021b). In this regard, the implementation of artificial intelligence-powered software to standardize embryo assessment might provide measurable definitions of embryo quality.

Slower embryo development is associated with poorer blastocyst morphology, as well as worse reproductive outcomes *per se* (Cimadomo *et al.*, 2022b), as reported as early as 1984 on untested cleavage stage embryos (Edwards *et al.*, 1984), therefore it is not surprising that consistently lower LBRs and MRs were reported also for Day 6–7 blastocysts versus Day 5 blastocysts. A delayed blastulation is a plausible consequence of multiple minor functional flaws met by the embryo which, although permissive throughout the *in vitro* preimplantation period, prevent an effective implantation process and/or subsequent viable gestation. Although the same limitations outlined for the association between blastocyst morphology and embryo reproductive competence also apply to the embryo developmental rate, time-lapse microscopy, and morphodynamic data on these parameters are

collected with higher throughput and increased quality, improving the overall generalizability of the evidence. In fact, the presence of multinucleation on Day 2, number of blastomeres on Day 3, abnormal cleavage patterns (e.g. direct unequal cleavage, reverse cleavage, time of morula formation, spontaneous blastocyst collapse, blastocyst expansion dynamics (i.e. blastocyst area increase per hour), and duration of blastulation), were all suggested for their association with euploid blastocyst implantation, although mainly in single center retrospective studies. In summary, despite the clear limitations affecting their design, all these studies suggest an association between irregularities in the cellular divisions prior to blastocyst development and the reproductive fitness of the resulting euploid embryos. From an academic perspective, further research is desirable to shed light on the cellular and molecular mechanisms regulating these mechanisms. From a clinical perspective, the view of the ESHRE time lapse technology group is that 'the combination of PGT-A with morphokinetic analysis may help in selecting the embryo with the highest implantation potential' and 'the promise that time lapse technology may evolve into a full-blown embryo selection modality, once combined with AI and non-invasive analytical approaches, is compelling' (Apter *et al.*, 2020). Future studies may unveil putative improvements derived from artificial intelligence and time lapse technology in the context of PGT-A cycles. Yet, these data are desirable with a prospective or non-selection design, and with both study arms adopting undisturbed incubators.

Euploid blastocyst transfer also represents the least biased scenario to test any putative additional non-invasive or invasive molecular analyses, which in the future may replace or complement PGT-A for embryo selection purposes. Nevertheless, none of the strategies investigated to date have reached enough reliability, concordance, reproducibility and/or clinical value to this end. The analysis of mtDNA: nDNA ratio was the object of several investigations throughout the past decade. However, the initial enthusiasm was soon curbed by the evidence that, possibly due to a lack of standardization in data normalization, it provided no additional predictive power to euploidy. Transcriptomic analyses of cumulus cells might be further investigated in terms of blastocyst development prediction (Scarica *et al.*, 2019). However, a putative long-term effect in the peri-implantation period derived from an unbalanced gene expression in cumulus cells cannot be currently supported. Spent blastocyst media (SBM) miRNomic analysis has shown promising associations with euploid blastocyst reproductive incompetence. This evidence is in line with the 'implantation checkpoint' hypothesis that portrays the human endometrium as a biosensor of embryo quality engaged in positive/negative selection (Brosens *et al.*, 2022). Yet, also in this case, the predictive power of embryo quality and developmental rate were *per se* were more relevant than miRNA analysis (Cimadomo *et al.*, 2019a). Lately, non-invasive preimplantation genetic testing for aneuploidies (niPGT-A) from SBM is under intense investigation, but its replacement of conventional PGT-A in clinical practice cannot be supported yet. Two proof of concept studies have hypothesized that blastocysts diagnosed as euploid in both TE biopsy and SBM would be more competent than blastocysts whose SBM turns out to be aneuploid (Rubio *et al.*, 2019; Yeung *et al.*, 2019), however they were not powered studies nor specifically designed to address this possibility. Following the same line of reasoning, a single-center pilot study reported blastocoel fluid positive DNA amplification as being associated with a lower implantation in the context of euploid blastocyst transfer (Magli *et al.*, 2019). Therefore, the authors proposed that the blastocoel as well can provide additional molecular information to pinpoint less competent blastocysts. Nevertheless, more data from larger studies are certainly needed to draw any conclusion on this topic.

Maternal age at oocyte retrieval represents a barrier to successful reproductive outcomes that might be largely leveled out by transferring euploid blastocysts. Still, a slight but significant decrease in LBR was reported for older women receiving euploid blastocyst transfers, suggesting a yet unknown effect of aging on oocyte and/or uterine competence (Nelson *et al.*, 2013; Bebbere *et al.*, 2022). Interestingly, the comparisons between women affected from a known cause of infertility, regardless of its nature, versus idiopathic patients resulted in similar outcomes after euploid blastocyst transfers. Although from limited data, this trend was also suggested for cases involving the presence of adenomyosis, arcuate uterus, and inflammatory bowel diseases. Regarding RIF and RPL, the evidence produced in this meta-analysis are in line with Macklon and Brosens' theory (Macklon and Brosens, 2014) portraying these two phenomena as the consequence of a hyper-selective or hyper-receptive endometrium, respectively. In fact, patients with RIF displayed significantly suboptimal implantation rates also when euploid blastocysts are transferred, while the LBR after euploid transfers was comparable between women with and without RPL. Notably, women with RPL experienced a slightly higher MR also after euploid transfers compared to women with no RPL, and this difference that, although not statistically significant, invites further investigations on the causes of miscarriage in the context of euploid pregnancies (Colley *et al.*, 2019). Regarding RIF, it has been recently shown that implantation failure recurrence after the transfer of three euploid blastocysts is infrequent (<10%), thus suggesting that future research on the diagnosis and treatment of this phenomenon should follow a stricter definition of the study population.

Although BMI is an unrefined biomarker of maternal nutritional homeostasis and one study excluded an association between BMI or body fat with the clinical outcomes after PGT-A (Kim *et al.*, 2021), two large meta-analyzed studies were concordant in reporting obesity (BMI >30) as being significantly associated with both lower LBR and higher MR after euploid ET. Therefore, we cannot disregard the putative relevance of a nutritional and lifestyle support in the management of infertility (Fabozzi *et al.*, 2022), especially in case of previous adverse reproductive outcomes. This feature is in part actionable, and the time invested in intervening on it before euploid ET might elicit a more favorable prognosis. Future studies on enhanced metrics to assess nutritional homeostasis and/or on the management of nutritional imbalances are highly recommended.

Serum progesterone levels were investigated at the time of the ovulation trigger, prior to the start of progesterone supplementation, as well as on the day prior to and on the day of euploid blastocyst transfer. A meta-analysis was not feasible because this feature was mainly investigated as a continuous variable, or the cut-off levels were heterogenous across the studies. Nevertheless, three studies suggested that progesterone levels on the day of ET are associated with LBR per ET (Kofinas *et al.*, 2015, 2016; Boynukalin *et al.*, 2019; Labarta *et al.*, 2021). Moreover, one group reported that low serum progesterone level (<10.6 ng/ml) on the day prior to ET is associated with both a lower LBR and a higher MR after euploid ET (Gaggiotti-Marre *et al.*, 2019). However, this suboptimal scenario can be rescued through the administration of subcutaneous progesterone to re-establish normal levels (Álvarez *et al.*, 2021). Further investigation is advisable on this topic. With respect to other hormones (AMH, estradiol, TSH, IGF, vitamin D), the evidence to date is minimal and it points towards a limited or no association between hormonal levels and the

outcomes after euploid ET. Similarly, two studies reported that the use of drugs, specifically levothyroxine and SSRI, were not associated with the chance of euploid blastocyst implantation.

Endometrial evaluation represents another black box in our understanding of the causes of implantation failure, especially in the context of euploid ET. Across the years, three endometrial evaluation approaches explored the association between their target parameter and euploid blastocyst implantation. The first one involved the observation of endometrial compaction, a parameter defined as a decrease in the thickness of the endometrium from the end of the proliferative phase to the time of ET; however, besides the inconsistency in its definition and evaluation, the two studies published to date showed opposite results. A second approach involved intervention through endometrial scratching (or endometrial disruption), although no benefit was reported. A third approach, and perhaps the most used for endometrial evaluation to date, was diagnostic and operational. It involved the analysis of endometrial gene expression (i.e. ERA test) and subsequent adjustment of transfer date (i.e. personalized ET) in case a non-receptive endometrium was detected during the presumed window of implantation. Despite the biologic plausibility of this latter methodology aimed at optimizing the synchronicity between embryo and endometrium, it did not improve outcomes for vitrified-warmed euploid ET neither in the general population of infertile women, nor in patients with RIF. This may indicate that the window of receptivity is relatively wide for most IVF patients (Bartels *et al.*, 2019). However, the population of patients tested was variable across the studies because of the criteria employed for proposing ERA testing: i.e. (i) any patient, (ii) patients with ≥1 previous failure, or (iii) patients with moderate/severe RIF. Most importantly, a non-selection study, which would show whether an ERA-diagnosed non-receptive endometrium is more prone to cause implantation failure after euploid blastocyst ET, is still missing. Moreover, recent data suggested that when ERA test was adopted in both non-PGT and PGT-A cycles, overall chance of reproductive success was impacted with lower cumulative live birth rates compared to controls (Cozzolino *et al.*, 2022). In summary, although larger datasets are required to draw clear conclusions on this topic, it is undeniable that more academic research may unveil other endometrial characteristics associated with reproductive fitness in the future. To this end, it is certainly helpful to minimize the potential embryonic causes of implantation failure and miscarriage by studying putative endometrial issues in the context of euploid ET (Hernandez-Vargas *et al.*, 2020). In fact, the data produced on uterine fluid derived extracellular vesicles transcriptomics, as well as the endometrial and vaginal microbiome, represent valuable experience and intriguing future perspectives.

Advanced paternal age, severe male factor and sperm DNA fragmentation were all assessed for a putative association with reproductive competence of euploid blastocysts. No impact was reported. Perhaps, a paternally driven impairment is exerted mainly on the fertilization and blastulation processes, as well as in the post-natal period. In fact, the prevalence of paternal meiotic aneuploidies is less than 10% at the blastocyst stage (Bonus *et al.*, 2022), and neither advanced paternal age nor severe male factor and high DNA fragmentation appear to impact either the euploidy rate, LBR, or MR in the context of PGT-A cycles. Nevertheless, more studies are required, especially in view of a recent study that showed improved LBR among older couples when hyaluronic acid binding or selection was conducted prior to ICSI, thereby putting sperm DNA damage under the spotlight again (West *et al.*, 2022). Germline *de novo* mutations increase with paternal aging (about 1.3 additional mutations per year versus 0.4 with maternal aging), indicating that the accumulation of mutations in sperm as a cause of genetic diseases and as an evolution driver in the long run (Goldmann *et al.*, 2019). Likewise, sperm were proposed as propagators of epigenetic defects associated with conditions such as obesity (Donkin *et al.*, 2016; Koch, 2016). In summary, future research in the context of advanced paternal age and severe male factor is certainly desirable.

Ovarian stimulation is a cornerstone of IVF and its tailoring (in terms of protocols and dosage) based on patients' characteristics is essential to achieve success. According to the Poseidon group, success in ovarian stimulation is defined as 'the ability to retrieve the number of oocytes necessary to obtain at least one euploid embryo for transfer in each patient' (Alviggi *et al.*, 2016). To this end, a higher dosage and/or oocyte or embryo accumulation strategies might be useful to compensate the natural decline in ovarian reserve and oocyte quality typical of advancing maternal age and to treat patients showing poor response to ovarian stimulation. In order to maintain treatment safety, protocols entailing GnRH antagonist analogue as pituitary suppressants, GnRH-agonist triggers and cycle segmentation have been introduced, since they are functional to minimize complications, such as ovarian hyperstimulation syndrome. Reassuringly, our meta-analysis showed no association between ovarian stimulation characteristics and the reproductive competence of the euploid blastocysts obtained, thereby supporting (when needed) its maximal exploitation, with the aim of identifying a transferable blastocyst in the shortest possible timeframe.

A putative impact of IVF-related manipulations and culture conditions on the competence of gametes and embryos has always been a matter of concern. The blastulation rate certainly represents a strong, clinically valid, and user-friendly key performance indicator for quality control purposes in IVF laboratories (Hammond and Morbeck, 2019). In fact, this metric unveiled both biological (e.g. severe male factor, advanced maternal age) (Maggiulli *et al.*, 2020) and technical (e.g. poor culture conditions, oocyte cryopreservation) (Forman *et al.*, 2012; Goldman *et al.*, 2015; Wale and Gardner, 2016) insults on embryo developmental competence. Nevertheless, although subject to a larger number of confounders (e.g. uterine environment and post-IVF issues) and a longer turn-around time, the LBR and MR after euploid blastocyst transfer might also be used to unveil putative negative effects on embryo viability. Based on these two indicators, our meta-analysis showed no imputable impact from oocyte vitrification, fertilization method and embryo culture on clinical outcomes. Conversely, TE biopsy-related features might affect reproductive outcomes after euploid ET. Specifically, day3 assisted hatching-based TE biopsies were associated with lower LBRs compared with the simultaneous ZP opening and TE biopsy protocol, perhaps due to the hampering of blastocyst expansion dynamics imputable to the former approach, or to the increase in time the embryo is exposed to suboptimal conditions for manipulations. Some authors suggested that an increased number of cells in the TE biopsy may also cause poorer reproductive outcomes. In addition, multiple vitrification-warming cycles and embryo re-biopsy cannot be overlooked, because they can also cause lower LBRs per euploid ET. Nevertheless, this trend may be partially imputable to poorer blastocyst morphology and the associated inferior prognosis of the patients involved, rather than to the additional procedures themselves. In fact, the vitrification of artificially collapsed blastocysts involves slightly higher cryosurvival rates after warming compared to re-expanded embryos, perhaps due to a better equilibration with the cryoprotectants

(Cimadomo *et al.*, 2018a). Therefore, post-biopsy cryopreservation should be preferably started shortly after (Maggiulli *et al.*, 2019). More data are required also on this important practice. In general, well-equipped laboratories, properly trained and constantly monitored operators are essential to minimize any putative impact of IVF-related manipulations on gametes and embryo viability. Based on the current body of evidence, when seven to eight cells are retrieved from a fully expanded blastocyst by experienced operators, TE biopsy is a safe procedure (Scott *et al.*, 2013; Capalbo *et al.*, 2016a; Neal *et al.*, 2017; Maggiulli *et al.*, 2019; Tiegs *et al.*, 2020). Still, an efficient interaction between IVF clinics and genetic laboratories is a fruitful policy to attain high-quality and reproducible technical/clinical outcomes. A mutual improvement can be achieved only by comparing molecular data and clinical outcomes with the protocols and the operators that put them into practice. This exercise is useful to distinguish between sources of biological and technical variability, so as to acknowledge the former and minimize the latter. For instance, new developments in PGT-A, such as the incorporation of genotyping data in addition to quantitative chromosome analysis, will represent a better approach to monitor biopsy outcomes and provide effective troubleshooting.

Finally, ET-related features were reported to be only marginally, or not, associated with the outcomes after PGT-A. No influence of transfer difficulty or operators was reported after adjusting for confounders, yet more data are desirable on this aspect. The adoption of hormone replacement therapy or modified natural cycle for endometrial preparation have elicited comparable outcomes. However, the choice of protocol requires review of the pros and cons of each, including gestational and perinatal outcomes. Indeed, while it is still questionable which protocol is more effective for endometrial preparation (Groenewoud *et al.*, 2017), the absence of the corpus luteum with the hormone replacement therapy approach has been suggested to increase the risk for gestational complications, especially hypertensive disorders like preeclampsia (Singh *et al.*, 2020). Specifically, the corpus luteum, before placentation, produces oestrogens, progesterone, as well as vasoactive products such as relaxin, vascular endothelial growth factor, and angiogenic metabolites of estrogen, whose deficiency may lead to an increased risk of abnormal maternal cardiovascular adaptation to pregnancy and abnormal early placentation (Johnson *et al.*, 1991; Conrad and Baker, 2013). More rigorous RCTs are warranted because hormone replacement therapy has clear logistic advantages such as scheduling flexibility (Singh *et al.*, 2020). Significantly higher LBRs were reported here with a vitrified-warmed ET approach than after fresh ET following the results of PGT-A. Nevertheless, this conclusion is partially biased because fresh ET in the context of PGT-A inevitably requires that the procedure be postponed according to the turn-around time between biopsy and diagnosis. In fresh PGT-A cycles, this delay may in turn expose fully developed embryo to unnecessarily longer culture and may affect the blastocyst-endometrial synchrony, ultimately causing a slightly lower LBR.

Conclusions

The main known causes of failed implantation after euploid blastocyst transfer can be summarized as follows:

- Maternal aging and obesity. This evidence advocates for future systematic investigations of the mechanisms involved in reproductive aging beyond *de novo* chromosomal abnormalities, and how the lifestyle (including nutritional aspects assessed via finer biomarkers other than BMI) may accelerate or exacerbate their consequences.
- Issues in endometrial receptivity or selectivity toward implanting embryos and the embryo-endometrial dialogue. Intense academic research is suggested on these topics, to better unveil the players involved in these processes, describe their interactions, and build enough solid knowledge, that can be ultimately converted into clinically valuable tools. Clearly, an appropriate workflow encompassing technical, pre-clinical and clinical validation should be followed to this end.
- Reduced blastocyst quality assessed via either static or dynamic assessments. Nevertheless, standardization is eagerly needed to overcome the subjectivity and limited reproducibility of these evaluations. In this regard, automation and artificial intelligence represent valuable future perspectives.
- Excessive or poor embryo manipulations. The importance of reducing excessive manipulations and proper training of the operators qualified to perform any invasive procedure cannot be overlooked; indeed, poor practice and limited standardization are at the roots of poorer outcomes and significant inter-center variability. Also in this case, automation is an intriguing future perspective. Likewise, we shall invest in developing non-invasive embryo selection strategies to limit the need for invasive procedures; yet a careful validation process and a prompt definition of the positive and negative predictive values of any novel strategy is essential before their clinical implementation in IVF.

Importantly, the associations outlined in the present manuscript have mostly issued from retrospective studies, therefore the level of evidence is low or very low, and all putative causations and clinical gains still require verification. For instance, even though some blastocyst morphological and morphodynamic features are associated with euploid embryo implantation, a true definition of the extent of this association requires RCTs. In addition, some of the meta-analyses rely upon a limited number of studies or studies with a limited sample size, and the comprehensive chromosome testing techniques adopted for PGT-A purposes has changed across the years 2010s from arrays (aCGH and SNP-array) or qPCR in the first half to NGS (either whole genome amplification-based or targeted) in the second half (Tables 1 and 2). This can cloud the benefit of a systematic review approach due to different specificity and sensitivity across these diagnostic approaches, especially if leveraging intermediate copy numbers (ICN) in an to attempt at report alleged mosaicism. For this reason, we pre-emptively excluded studies where alleged mosaicism was reported or 'mosaic' embryos were transferred in the second half of 2010s.

Lastly, some of the present findings represent 'prognosis without promise', namely the poorer outcomes of some euploid blastocysts outlined is not clinically actionable, like those of women older than 38 years.

Future investigations are therefore invited to either confirm or refute the current levels of evidence, as well as to unveil novel features to ultimately crack the riddle behind the black box of implantation.

Supplementary data

Supplementary data are available at *Human Reproduction Update* online.

Data availability

All data are included in the manuscript and its supplementary material.

Authors' roles

DC, ACo, MP, SC, FI, JH, LG, and AV were involved in the literature search, data extraction and data synthesis. Discrepancies were resolved by LR, CA, EF, FMU, and ACa. The risk of bias and the quality of the studies included in this meta-analysis were evaluated independently by DC and ACo. ACo conducted data analysis. The manuscript was drafted by DC, ACo, MP, SC, FI, JH, and LG. All authors contributed to the discussion of the evidence.

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The authors have no conflicts of interested related to this review.

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