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REVIEW

Should the flexibility enabled by performing a day-4 embryo transfer remain as a valid option in the IVF laboratory? A systematic review and network meta-analysis

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Abstract

Purpose The present systematic review and network meta-analysis aims to uniquely bring to literature data supporting the true place of the alternative practice of day-4 embryo transfer (D4 ET) in an IVF laboratory, beyond the one-dimensional option of facilitating a highly demanding program.

Methods A systematic search was conducted in the databases of PubMed/Medline, Embase, and Cochrane Central Library, resulting to six prospective along with nine retrospective cohort studies meeting eligibility criteria for inclusion. A comparison of D4 ET with day-2 (D2), day-3 (D3), and day-5 (D5) ET, respectively, was performed employing R statistics.

Results The sourced results indicate no statistically significant difference regarding clinical pregnancy rates, and ongoing pregnancy/live birth rates stemming from the comparison of D4 with D2, D4 with D3, and D4 with D5 ET, respectively. Additionally, no statistically significant difference could be established in respect to cancelation, and miscarriage rates, following the comparison of D4 with D3 and D4 with D5 ET. Interestingly, we report statistically significant lower preterm birth rates associated with D4 ET, in contrast with D5 ET (RR, 0.19; 95% CI, 0.05–0.67; p value = 0.01).

Conclusions The aforementioned results may serve as advocates buttressing the option of D4 ET as a valid candidate in the ET decision-making process. Possible limitations of the current study are the publication bias stemming from the retrospective nature of certain included studies, along with various deviations among studies' design, referring to number and quality of transferred embryos, or different culture conditions referring to studies of previous decades.

Keywords Cleavage embryo · Morula · Blastocyst · Embryo transfer · Day of embryo transfer · Clinical pregnancy

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Introduction

Embryo transfer (ET) represents the final step during an in vitro fertilization (IVF) procedure, towards achieving a pregnancy for an infertile couple. The appropriate day to perform an ET in order to ascertain an optimal outcome has been the topic of intense debate throughout the last decades. This has been driven by the numerous advances regarding the assisted reproduction technology (ART) field, namely cryopreservation employing embryo vitrification [1], culture media optimization [2], advances on preimplantation genetic diagnosis (PGD), and testing (PGT) [3], as well as the emergence of techniques focusing on the improvement of the endometrial receptivity [4]. All the novelties evolving may either allow for extension of embryo culture [5] or require extended culture in order for diagnosis to be performed [6]. Hitherto, no consensus seems to be reached on the definitive optimal ET timing [7], due to the uniqueness of each couple's management, rendering the issue of the optimal day for ET a vicious circle [7].

Traditionally, the ET process was carried out 1 or 2 days following fertilization, establishing cleavage stage ET as the norm [8]. The main reason behind this was the inadequate laboratory culture conditions, in line with the poorer culture media components, that failed to provide a proper environment for the extended development of the embryos. Thus, the number and quality of developing embryos was limited and perhaps compromised by the culture conditions, leading clinicians to proceed to ET [9]. Further to that, clinicians commonly opted for day-2 (D2) or day-3 (D3) ET, in an attempt to bypass the peril related to cleavage-arrested embryos. Studies reported that the majority of in vitro cultured embryos, presenting with four to eight cells, that do not continue to blastulation, may be attributed to several molecular and chromosomal abnormalities [10, 11].

Day-5 (D5) ET stands as the current trend among numerous IVF laboratories worldwide [2]. The advent of sequential culture media undoubtedly represents a determinant factor that challenged the previous practice. The wide pallet of components successfully sustains extended embryo culture, driving specialists to adopt blastocyst transfer with safety [2, 8]. The first major wave of the embryonic genome activation is noted on D3 between the four- and eight-cell stage, although the initial expression of the first paternal mRNAs occurs on D2 during the three- to four-cell stage [12]. The embryonic gene activation plays a pivotal role to the subsequent development of the embryo's morphology [13]. This is of paramount importance as only competent embryos could be adequately distinguished from those appearing with delayed or irregular division, which may lead to an accurate prediction of embryo quality and concurrently of the implantation potential [14, 15]. The latter goes hand in hand with the embryo's intrinsic selection permitting IVF specialists to transfer less—and at the same time—superior embryos [15].Concurrently, blastocyst transfer is synchronized with the endometrial receptivity that is supposed to be improved in order to favor the implantation process [16].

Remarkably, clinicians shifted their interest to day-4 (D4) ET, with Huisman and colleagues to be the forerunners of this approach [17]. Performing an ET at the morula stage may serve to facilitate and allow for flexible management of the cycle for both the patients and clinicians. A thorough literature search, yielded limited articles reporting on the alternative clinical practice of performing a D4 ET, while the majority of them were retrospective studies rather than prospective. According to a retrospective study on fresh IVF cycles, an impressive percentage of live birth rates was observed following a D4 ET [15].

The aim of this systematic review and network meta-analysis is to evaluate the available data regarding D4 ET in relation to results of D2, D3, D5, and D6 ET respectively, under the umbrella of the clinical pregnancy outcome. Our attempt mainly focuses on shedding light to the question whether there is a true place for D4 ET safe implementation in an IVF laboratory or it impartially portrays a comforting practice serving a highly demanding IVF program.

Methods

Search strategy

A systematic search of the literature was conducted in the databases of PubMed/Medline, Embase, and Cochrane Central Library and it was limited to full-length articles published in peer-reviewed journals up to 15th January 2019 (Supplementary Appendix 1). The search strategy included the following keywords along with respective combinations: "in vitro fertilization," "IVF," "intracytoplasmic sperm injection," "ICSI," "assisted reproduction," "assisted reproduction techniques," "medical-assisted reproduction", "cleavage stage embryo,", "morula," "blastocyst," "day 2," "day 3," "day 4," "day 5," "day 6," and "embryo transfer." The search was further refined by employing the filter "humans.". The initial search yielded 2362 studies from the three databases (PubMed/Medline: $n = 1235$, Embase: $n = 527$, Cochrane Central Library: $n = 600$). From the total yield, 141 studies were duplicates and thus removed, while 88 studies from Cochrane database were directly excluded, as they were referred as reviews ($n = 69$), protocols ($n = 11$), and editorial answers ($n = 8$). Following initial screening of titles and abstracts of all records, $n = 2044$ studies were removed in an attempt to obtain only the relevant articles. Thereafter, full texts were screened seeking for ultimately relevant articles, as well as the reference lists of selected relevant publications were assessed for final inclusion. This thorough screening

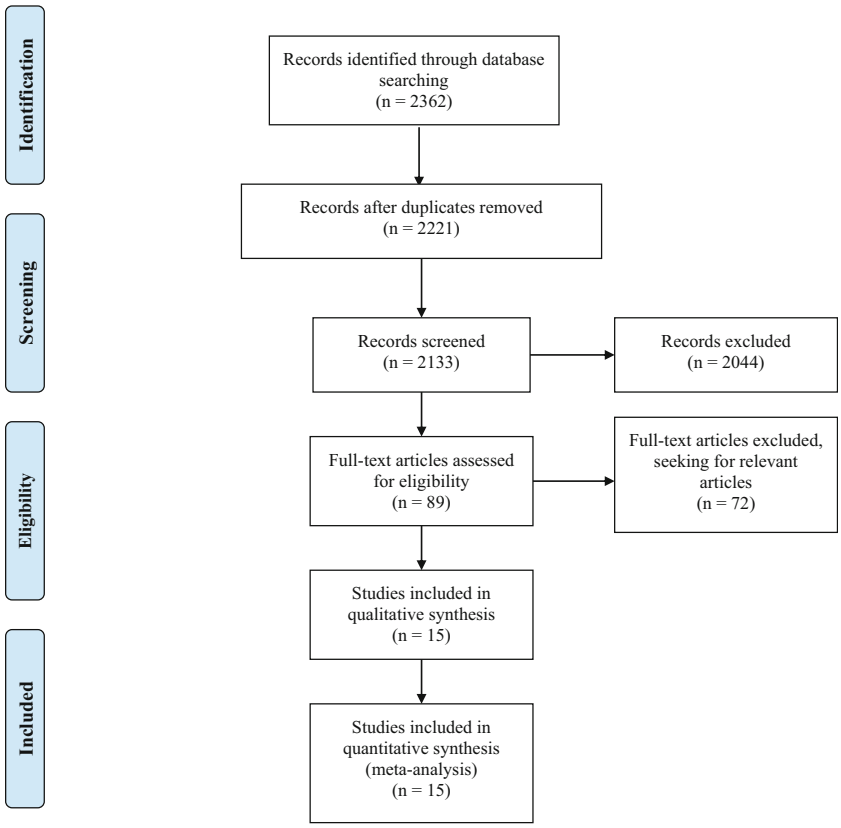

Fig. 1 PRISMA flowchart regarding the search results

resulted in a total of 15 studies [5, 6, 8, 13, 17–27] that are employed for the present meta-analysis. In Fig. 1, the flow chart of Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) is depicted. Screening and studies' selection were performed by three independent authors (PT, AR, and MS). Any disagreements between the authors were resolved by an arbitration mediated by the senior author. No protocol was submitted to the PROSPERO International Prospective Register of Systematic Reviews, providing details on conducting of this study.

Study selection

In the present meta-analysis, the authors concurred on including prospective, along with retrospective cohort studies, which were conducted in humans and published in English. The population included women undergoing IVF/ICSI cycles employing a D4 ET procedure compared with ET performed on D2, D3, and D5, following fertilization. Only studies that included D4 as a reference group were included. This systematic review and network meta-analysis uniquely brings to literature data sourced by the comparison of D4 ET with D2, D4 with D3, and D4 with D5 ET, respectively. This design served the purpose of an attempt to focus our findings on the direct effect of this intervention, limiting the indirect effect originating from the comparison of cleavage and blastocyst stage ET. This was a requirement ensuring a foolproof design, as the majority of studies report on cleavage versus blastocyst stage ET rather than D4 ET.

Excluded studies

Two studies were excluded—albeit presenting relevant to our study's scope—as they were published in Spanish [28] and Czech [29], respectively. Other exclusion criteria were on the grounds of the intervention of PGD [30] or due to the fact that studies failed to present the results of D4 and D5 ET as a separate group [31, 32]. Moreover, studies that presented the developmental stage of morula, as early blastocyst, were excluded on the grounds of failing to categorize developmental stage conclusively [33, 34]. Additionally, two studies were excluded as the outcome measure of pregnancy rate referred to biochemical pregnancy, rather than clinical pregnancy, the latter being our primary outcome [35, 36].

Data extraction

Studies' data were extracted independently by two authors (EM and SG) based on the selection criteria.

Outcomes

The reported primary outcome measure of interest was clinical pregnancy rates (CPR) per ET cycles. The reported secondary

outcome measures were ongoing pregnancy/live birth rates (OPR), clinical pregnancy rates per patient (PR), cancelation rates (CR), miscarriage rates (MR), and preterm birth rates (PBR).

Statistical analysis

D4 ET was assessed in comparison with different days of ET procedure during IVF/ICSI cycles. Risk ratio with 95% confidence intervals was employed for the analyses of the included studies. Risk ratio greater than 1 represented a safety benefit favoring the control group, namely ET procedure performed on days other than D4. Either the fixed effect or the random effects model was employed for combining the results according to heterogeneity. Heterogeneity of the exposure effect was evaluated through I^2 statistic. An I^2 value 80% or greater indicated high heterogeneity and thus the meta-analysis was not performed, while an I^2 value 60% or greater represented significant heterogeneity, leading to the employment of random effects model. A chi-squared test for heterogeneity was also conducted and the p values were provided. Funnel plots for potential publication bias were performed. The network was performed employing frequentist methods via the "netmeta" package of the R programming language for statistical purposes.

Results

Study characteristics

Fifteen studies were identified to be eligible for network meta-analysis [5, 6, 8, 13, 17–27]. Six prospective and nine retrospective studies were included, published up to January 2019. Specifically, the study conducted by Prapas et al. was discerned into two arms for this statistical analysis, with the first referring to ET procedure employing ultrasound guidance, while the second one referring to ET procedure employing clinical feel [25]. The evaluated characteristics of each included study in this meta-analysis are presented in Table 1. Characteristics include: study design, total number of ET cycles, study group referring to D4 ET, groups of comparison referring to D2, D3, and D5 ET, and outcome measures. It is notable that, following screening of the studies, no study that compared D4 with D6 ET was identified.

Assessment of risk of bias

Three authors (PG, AP, and MS) independently performed the assessment of risk of bias for the included prospective and retrospective studies, employing the tool "Risk of Bias In Non-randomized Studies-of Intervention" (ROBINS-I), which was developed by Sterne and his colleagues for non-randomized studies [37]. The evaluated parameters were bias due to confounding, selection of participants, classification of intervention, deviations from intended intervention, missing data, measurement of outcomes, and reporting selection of result. Each parameter of included studies was classified as "low," "moderate," "serious," "critical," or "no information" risk of bias. The assessment of risk of bias for each included study is presented in Fig. 2, while the summary of risk of bias assessment regarding each item for each study included in the meta-analysis is presented in Fig. 3. Any disagreements were resolved by discussion including the senior authors to achieve a consensus.

Primary outcome

Clinical pregnancy per ET

Fifteen studies [5, 6, 8, 13, 17–27] reported results on clinical pregnancy rates per ET cycle. Five different study designs (D4 vs. D3; D4 vs. D5; D4 vs. D2/3; D4 vs. D3/5; D4 vs. D2/3/5) comparing a total of four different days including 11,034 ET cycles were evaluated. Thirty-five pairwise comparisons were evaluated. According to funnel plot assessment, publication bias was present (Supplementary Fig. 1). Heterogeneity among the studies was reported to be significantly high ($I^2 = 62.2\%$), thus the random effects model was employed. No statistically significant difference was observed in any of the comparisons as presented in Fig. 4.

Secondary outcomes

Ongoing pregnancy/live birth

Six studies [8, 19–21, 23, 24] reported results on ongoing pregnancy/live birth rates. Four different study designs (D4 vs. D3; D4 vs. D5; D4 vs. D3/5; D4 vs. D2/3/5) comparing a total of four different days including 3319 ET cycles were evaluated. Thirteen pairwise comparisons were evaluated. According to funnel plot assessment, no publication bias was present (Supplementary Fig. 2). Heterogeneity among the studies was reported to be insignificant ($I^2 = 0\%$), thus the fixed effect model was employed. No statistically significant difference was observed in any of the comparisons as presented in Fig. 5.

Clinical pregnancy per patient

Four studies [13, 22–24] reported results on clinical pregnancy per patient. Two different study designs (D4 vs. D3; D4 vs. D3/5) comparing a total of three different days including 3873 ET cycles were evaluated. Ten pairwise comparisons were evaluated. According to funnel plot assessment, publication bias was present on D3 to D5 comparisons (Supplementary Fig. 3). Heterogeneity among the studies was reported to be

Table 1 Principal characteristics of studies included in the meta-analysis

Included studies	Study design	Total and cycles	Study group	Groups of comparison			Outcome measures
			D4 ET	*D2 ET*	*D3 ET*	*D5 ET*	
Feil et al. [18]	Retrospective	317	124	–	–	193	CPR
Goto et al. [19]	Retrospective	207	46	54	79	28	CPR, OPR, MR
Huisman et al. [17]	Prospective	1112	350	872	1084	–	CPR
Huisman et al. [13]	Prospective	1078	488	–	590	709	CPR, PR, CR
Holschbach et al. [8]	Retrospective	599	124	–	–	475	CPR, OPR, MR
Kang et al. [20]	Retrospective	605	130	–	–	475	CPR, OPR, MR, PBR
Kiltz et al. [6]	Retrospective	354	44	–	272	38	CPR
Lee et al. [5]	Retrospective	747	440	–	–	307	CPR
Li et al. [21]	Retrospective	427	107	–	–	320	CPR, OPR, MR, PBR
Montag et al. [22]	Prospective	273	95	–	90	88	CPR, PR, CR
Nada et al. [23]	Prospective	1467	159	–	1224	84	CPR, OPR, PR, MR
Pantos et al. [24]	Prospective	346	173	–	173	–	CPR, OPR, PR, CR, MR
Prapas et al. [25] (1)	Prospective	433	85	–	261	87	CPR
Prapas et al. [25] (2)	Prospective	636	98	–	315	223	CPR
Saadat et al. [26]	Retrospective	525	239	–	286	–	CPR
Tao et al. [27]	Retrospective	339	242	–	97	–	CPR

CPR, clinical pregnancy rates per cycle; *OPR*, ongoing pregnancy rates; *PR*, pregnancy rates per patient; *CR*, cancelation rates; *MR*, miscarriage rates; *PBR*, preterm birth rates

significantly high ($I^2 = 64.8\%$), thus the random effects model was employed. No statistically significant difference was observed in any of the comparisons as presented in Fig. 6.

Cancelation rate

Three studies [13, 22, 24] reported results on cancelation rates. Two different study designs (D4 vs. D3; D4 vs. D3/5) comparing a total of three different days including 2406 ET cycles were evaluated. Seven pairwise comparisons were evaluated. According to funnel plot assessment, no publication bias was present (Supplementary Fig. 4). Heterogeneity among the studies was reported to be insignificant ($I^2 = 0\%$), thus the fixed effect model was employed. No statistically significant difference was observed in any of the comparisons as presented in Fig. 7.

Miscarriage rate per clinical pregnancy

Six studies [8, 19–21, 23, 24] reported results on miscarriage rate per clinical pregnancy. Four different study

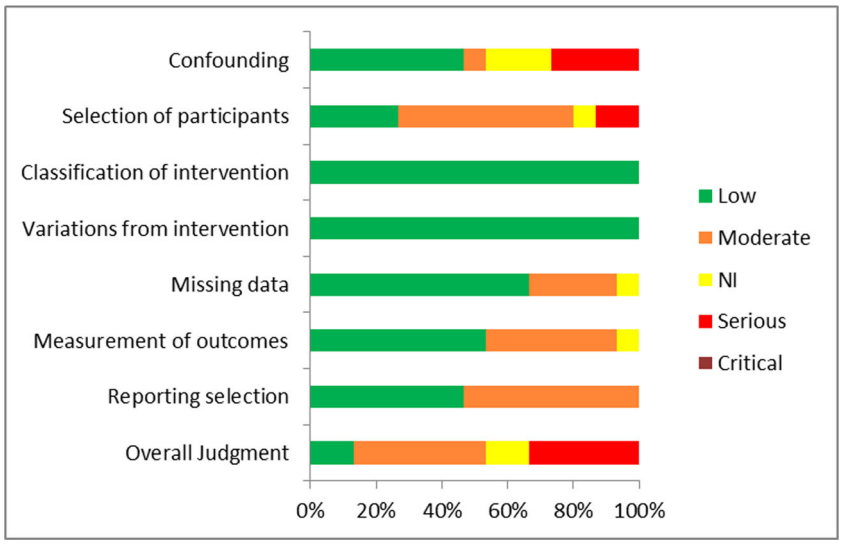

Fig. 2 Assessment of risk of bias of studies included in the meta-analysis

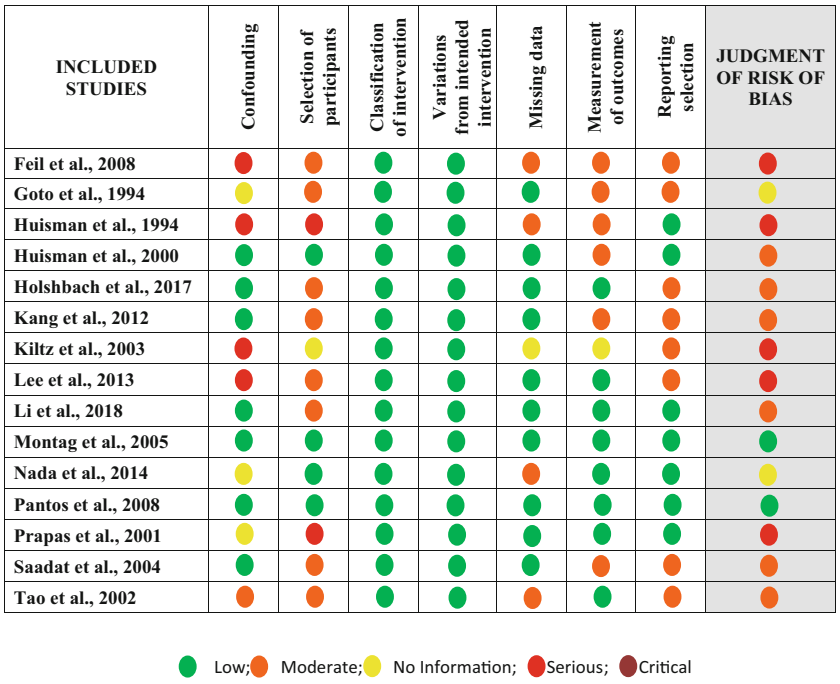

Fig. 3 Summary of risk of bias assessment regarding each item for each study included in the meta-analysis

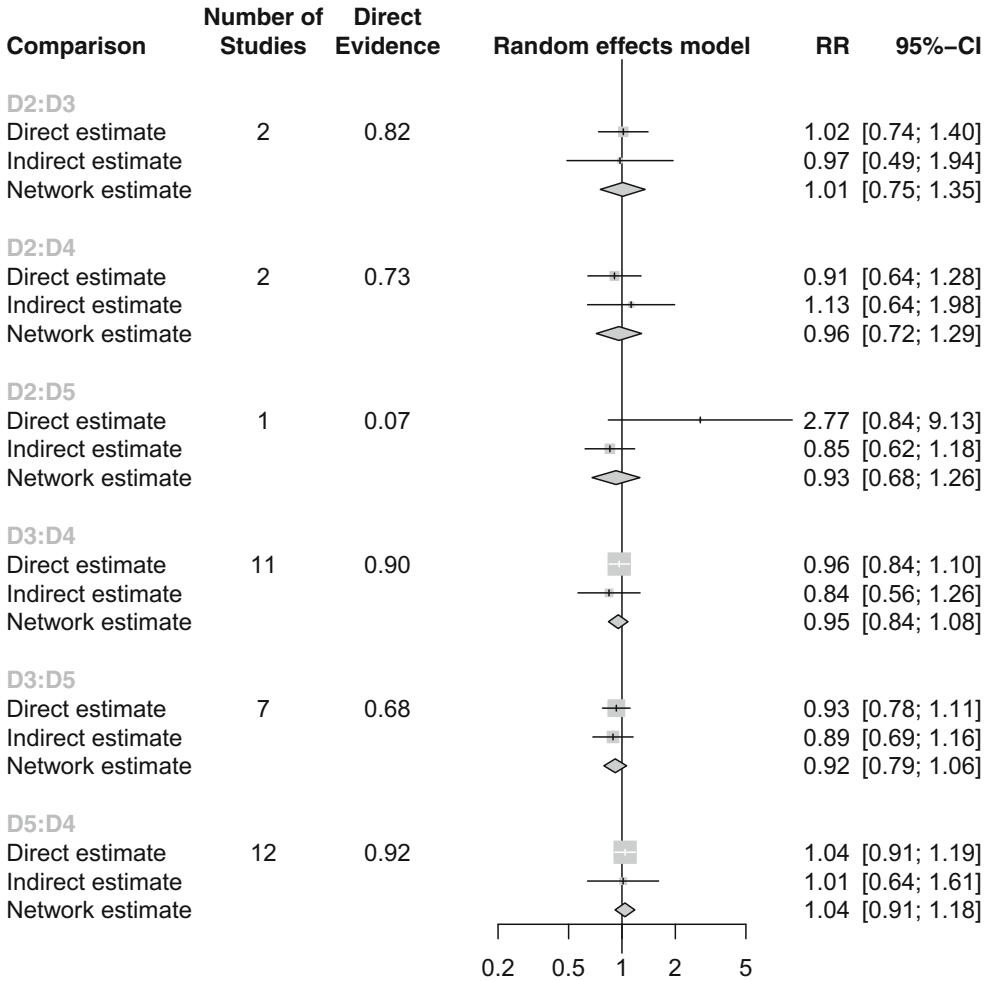

Fig. 4 Forest plot of clinical pregnancy rates per ET cycle resulting from the comparison of D4 ET with D2, D3, and D5 ET respectively

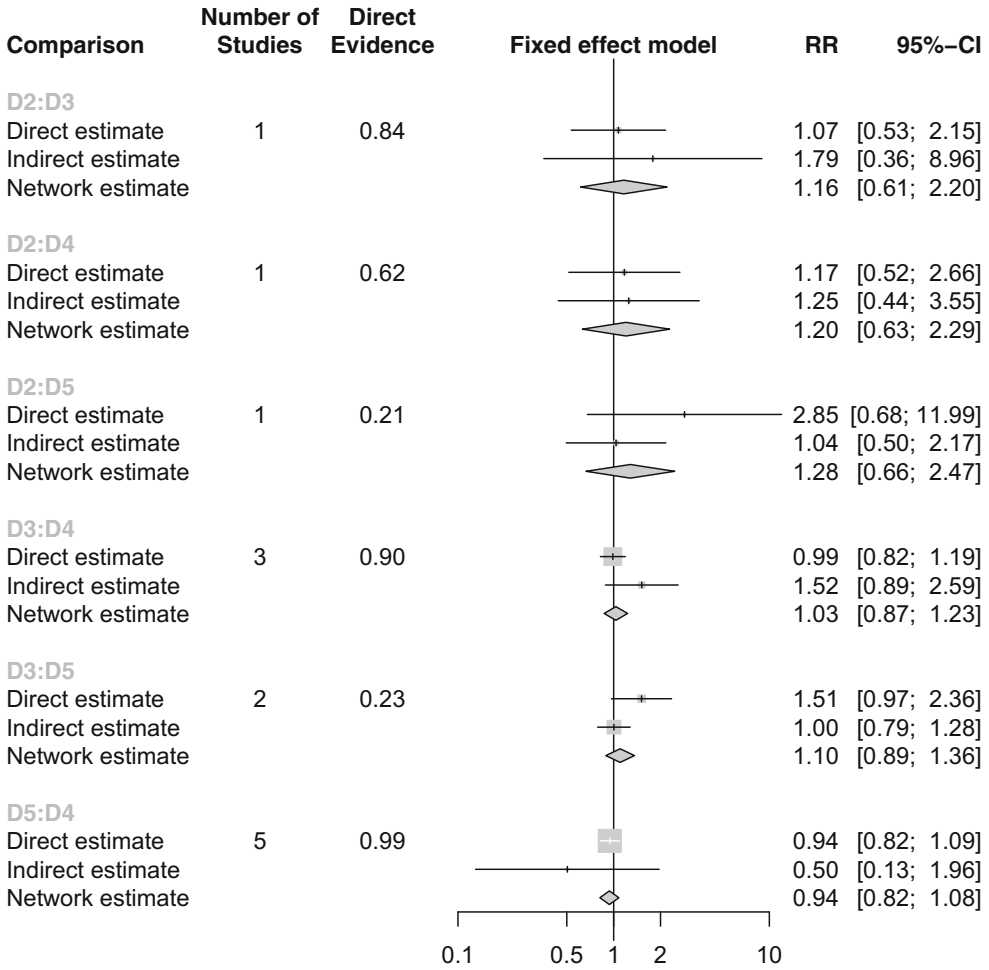

Fig. 5 Forest plot of ongoing pregnancy/live birth rates resulting from the comparison of D4 ET with D2, D3, and D5 ET respectively

designs (D4 vs. D3; D4 vs. D5; D4 vs. D3/5; D4 vs. D2/3/5) comparing a total of four different days including 1378 ET cycles were evaluated. Thirteen pairwise comparisons were evaluated. According to funnel plot assessment, no publication bias was present (Supplementary Fig. 5). Heterogeneity among the studies was reported to be insignificant ($I^2 = 0\%$), thus the fixed effect model was employed. No statistically significant difference was observed in any of the comparisons as presented in Fig. 8.

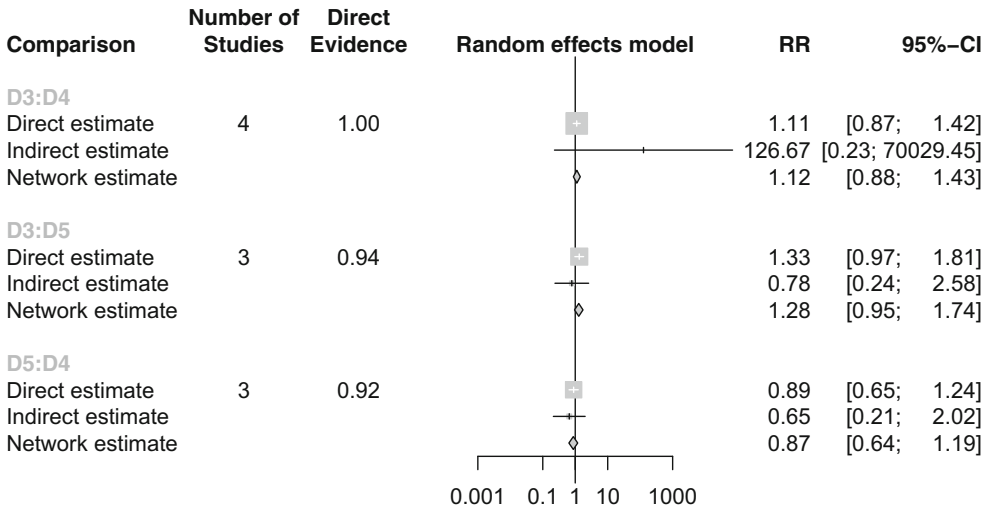

Fig. 6 Forest plot of clinical pregnancy rates per patient resulting from the comparison of D4 ET with D3 and D5 ET, respectively

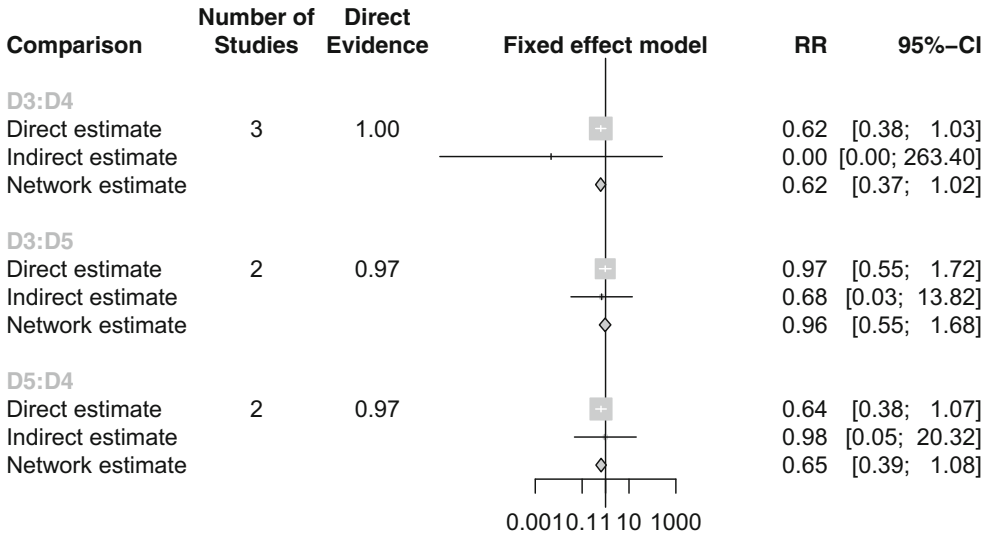

Fig. 7 Forest plot of cancelation rates resulting from the comparison of D4 ET with D3 and D5 ET, respectively

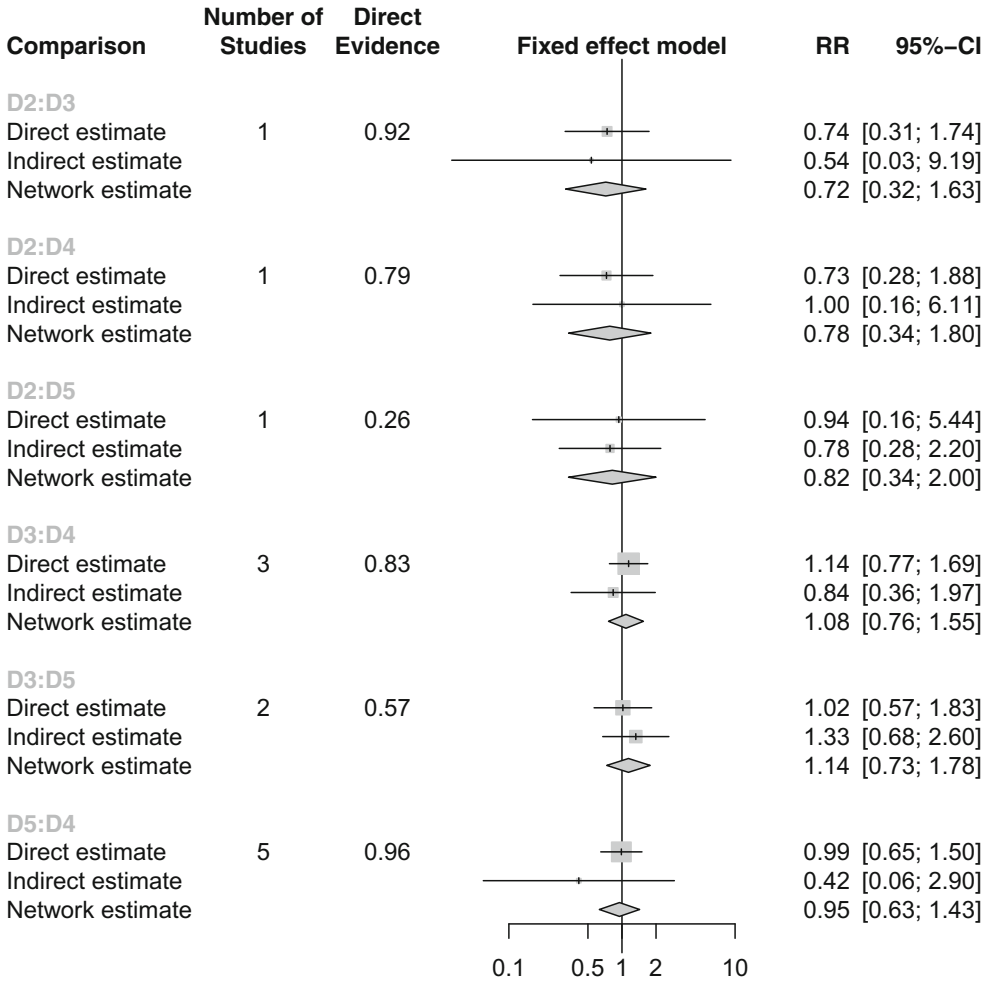

Fig. 8 Forest plot of miscarriage rate per clinical pregnancy resulting from the comparison of D4 ET with D2, D3, and D5 ET respectively

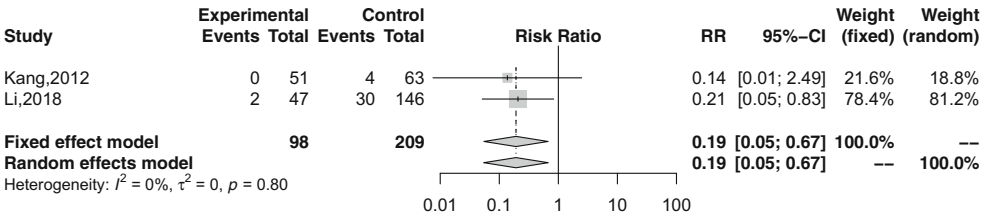

Fig. 9 Forest plot of preterm birth rates resulting from the comparison of D4 with D5 ET

Preterm birth

Two studies [20, 21] reported on preterm birth rates. Only one study design (D4/D5) comparing two different days including 307 ET cycles were presented, thus conventional meta-analytic methods were employed. Heterogeneity among the studies was reported to be insignificant ($I^2 = 0\%$), thus the fixed effect model was employed. A statistically significant lower preterm birth rates was presented in D4 ET (RR, 0.19; 95% CI, 0.05–0.67; $p = 0.01$) as presented in Fig. 9.

Sensitivity analysis

A sensitivity analysis based on prospective studies was performed, in order to statistically strengthen the validity of the sourced results. Six prospective studies were included [13, 17, 22–25] reporting on the primary outcome measure of clinical pregnancy rates per ET cycle. Three different study designs (D4 vs. D3; D4 vs. D2/3; D4 vs. D3/5) comparing four different days including a total of 7248 ET cycles were evaluated. Heterogeneity among the studies was reported to be significantly high ($I^2 = 71\%$), thus the random effects model was employed. No statistically significant difference was observed in any of the comparisons (D4 vs. D2 (RR, 0.96; 95% CI, 0.72–1.29); D4 vs. D3 (RR, 0.95; 95% CI, 0.84–1.08); D4 vs. D5 (RR, 1.04; 95% CI, 0.91–1.18)), as presented in Supplementary Fig. 6. A further analysis was attempted employing strictly studies with an overall low risk of bias [22, 24]. Heterogeneity of $I^2 = 80.2\%$ was observed and thus a sensitivity analysis could not be performed.

Regarding the number of embryos transferred only studies with two or less embryos transferred were selected for evaluation. Four studies were included in this sensitivity analysis [8, 13, 18, 20] reporting on the primary outcome of this meta-analysis, namely clinical pregnancy rate. Two different study designs (D4 vs. D3/5 and D4 vs. D5) comparing three different days including a total of 2974 cycles were evaluated. Heterogeneity among the studies was reported to be insignificant ($I^2 = 0\%$), thus the fixed-effects model was employed. No statistically significant difference was observed in any of the comparisons [D4 vs. D3 (RR, 1.01; 95% CI, 0.83–1.24); D4 vs. D5 (RR, 1.01; 95% CI, 0.9–1.14)]. In the cases where strictly single embryo transfers were evaluated, only two studies reported results on clinical pregnancy rates [18, 20] including a total of 588 cycles. Both studies presented with the same design (D4 vs. D5) thus conventional meta-analytic methods were employed. Heterogeneity was reported as insignificant ($I^2 = 0\%$) thus the fixed effects model was employed. No statistically significant difference was observed (RR, 1.07; 95% CI, 0.89–1.28).

Discussion

In IVF practice, selecting the embryos of the highest implantation potential to include for ET is probably the most crucial step in efficiently managing an IVF cycle. Commonly, the choice of the day of ET may dictate the outcome especially if a cycle could "perform" better employing a certain day. It is notable that recent meta-analyses reveal higher implantation and pregnancy rates regarding blastocyst transfer compared with cleavage stage ET, albeit of no statistical significance [14, 38]. Further to that, higher rates of transfer cancelation rates are correlated with prolonged blastocyst culture [14]. The quality and number of embryos along with the patient's choice, the legal framework and the IVF team's consultation based on reproductive history and etiology, all conspire towards selecting the best day. A vague hesitation may surround the option of D4 ET as not the standard practice. Clinical experience shows that sometimes an embryo may not be suitable to be subjected to extensive culture, and that developmental ability may become compromised [39]. It was a requirement for D4 ET to be introduced in IVF laboratory allowing for extra time for PGD, employing the advanced micro-array CGH [3]. Additionally, in the scope of cross border reproductive care, patients opt to travel abroad or more rarely, they may become unable—for personal reasons—to present to an assisted reproduction unit [40]. Thus, the option of arranging an ET procedure on D4 taking into consideration the patients' requests, even on account of avoiding weekends [6], may provide further flexibility to the demanding schedule of an IVF laboratory [8, 18]. Nonetheless, decision making within the context of an IVF laboratory requires data supporting 1 day versus another developmental stage. The present study focuses on delineating the approach of D4 ET as a potential "baladeur" option for IVF professionals, aiming to achieve an optimal personalized management during an IVF cycle.

Studies comparing D4 ET with D2, D3, or D5 ET, respectively, were included in this network meta-analysis and were further evaluated. Notably, no study comparing D4 with D6

ET was detected. Our results reveal no statistically significant difference in regard to clinical pregnancy rates per ET cycle, ongoing pregnancy/live birth rates, as well as clinical pregnancy rates per patient, following the comparison of D4 with D2, D3, and D5 ET. The sensitivity analysis performed did not alter the results sourced herein, neither when employing strictly prospective studies, nor when employing studies with two or less embryos transferred. Moreover, no statistically significant difference was reported regarding cancelation rates and miscarriage rates per clinical pregnancy, following the comparison of D4 with D3 and D5 ET. Remarkably, statistically significant lower preterm birth rates are associated with respect to D4 ET compared with D5 ET (RR, 0.19; 95% CI, 0.05–0.67; p value = 0.01). It should be noted that results originating from the comparison between D2, D3, and D5 ET should be interpreted with caution. The aim of the present study was to employ D4 ET as the reference group in order to delineate its true place in clinical practice. Thus, only studies performing D4 ET were included. The comparisons reported herein between D2, D3, and D5 are due to the network nature of the analysis for this study. Cleavage versus blastocyst transfer still rises a heated debate, fueling the conduction of meta-analyses dedicated to delineating this [14, 41].

The majority of concerns regarding management of a D4 embryo stem from the fact that morula physiology is considerably more complex to delineate in comparison with the two clearly distinct developmental stages namely cleavage and blastocyst. The D4 embryo or morula may be portrayed as a blurry mass of cells [42]. This may be associated to the fact that indeed, 4 days following fertilization, a "sensitive" period is triggered, characterized by intense DNA synthesis in order to enable cell division. Along with this event, the phenomenon of the embryo's contractions is documented, as the embryo is prepared for cell line differentiation coupled by a notable growth in volume [43]. The morula appears more vulnerable during this stage, remaining in "limbo" between two highly distinct developmental stages [44]. The process of the morula's compaction further indicates alteration of blastomeres' shape, loss of sphericity, along with changes regarding contact links [42]. Consequently, metabolic requirements during this embryonic stage are presented as considerably more demanding when compared with the cleavage stage. The heightened metabolic requirement indicates the intent to achieve gene expression along with the embryonic expansion [45, 46].

Albeit providing promising results, D4 ET still remains a less-preferable approach, mainly due to the dynamic of embryonic development [42]. From an embryologists' point of view, at this developmental stage, one may be more inclined to minimize exposure attributed to removal from the incubator [44]. An interesting point worth considering in regard to day 4 ET implementation complication, may involve the difficulties and challenges in grading a morula stage embryo. The morula stage may present as neglected from an evaluation point of view in light of the fact that most laboratories fail to include morula morphology data in their final verdict in embryo grading. The embryo development on D4 being a dynamic process, renders accurate morphological assessment of the morula or of the compaction stage embryo, a complicated and challenging process lacking clear standardization. Hitherto, there is lack of an established morula grading system [8]. In fact, little is documented concerning the morula score [47], apart from several in-house scoring lines that are mainly based on the proportion of blastomeres' compaction, as well as the fragmentation rate [21, 27, 48]. Albeit grading may prove challenging, on the other hand, the perivitalline space of the morula is reported to be comparatively large and thus facilitate the practice of assisted hatching adding an advantage to opting for a D4 ET in the IVF laboratory [15].

Interestingly, various studies investigated the vitrification method on D4 stage embryos, providing good results. The team of Tao was the first to report live births following cryopreservation of morula stage embryos [49]. Moreover, the same group, in a retrospective study, observed promising results regarding morula's survival rates following the freeze–thaw procedure, with the majority of morula stage embryos continuing on to the compaction progress following the thaw reaching full compaction, whereas a small portion presented with proceeding backwards to the cleavage stage [50]. A point of paramount importance advocating the optimal results associated with morula vitrification versus the blastocyst stage is the structure characterizing the morula and blastocyst. The blastocoelic fluid filled cavity may render the vitrification procedure deleterious for its integrity, especially for the IVF laboratories practicing blastocyst vitrification excluding blastocoelic collapse [51]. Subsequently, D4 embryos may appear with comparable results concerning pregnancy rates following vitrification [52].

Time-lapse imaging provided the IVF specialists with the opportunity to ultimately evaluate the embryos' development, without exposing them to any variation in relevance to the well-provided culture conditions [53], limiting stress stimuli. Nonetheless, in clinical practice at the era of time-lapse, D4 ET is still argued as not being the first line option even though the argument of subjecting the embryo to a rollercoaster of variations is not part of the equation. It is notable that each IVF laboratory may adopt different set-up approaches for handling IVF cycles during clinical routine. In the poll of selecting the best ET day, the majority lean towards cleavage stage ET, namely D2 or D3 ET, or blastocyst stage ET, namely D5 or D6 ET, or even their combination, excluding D4 as an option [41]. It is noteworthy, that studies proposed that blastocyst transfer may positively favor the clinical pregnancy outcome [5, 14], along with decreasing either ectopic [38, 54] or multiple pregnancies [5, 13]. However, the foundation of the thought that Day 4 ET is not optimal could be disputed in light of lack of solid evidence supporting this notion.

The rationale behind D4 ET is similar to that of blastocyst transfer, regarding the improved uterine receptivity, in line with reduced contractility, resulting to higher chances of successful implantation [18, 23]. The advancement to the morula stage guarantees the switch from maternal to embryonic gene activation, a fact that could represent as an additional advantage [55]. The embryo's physiological "journey," traveling from the fallopian tube to the uterine cavity, between the end of D3 and on the onset of D4 has been acknowledged [55].Could it be that the implantation window may in fact be more responsive towards a morula stage embryo? Perhaps, the possibility of considering D4 ET in routine practice could allow for such questions to be further investigated.

A finding of statistical significance resulting from the present meta-analysis regarding the increased preterm birth following blastocyst transfer in comparison with morula transfer may reflect a statistical verdict failing to be buttressed by any clinical or physiological theory or hypothesis. Such findings may remind us of other studies reporting a significant association between frozen–thawed cycles and increased placenta accrete prevalence, along with pregnancy induced hypertension [56, 57]. Nonetheless, the observation stands. Findings reporting on perinatal and neonatal data are of heightened significance especially as the interest of the scientific community on assisted reproduction has shifted from implantation rates to the pediatric follow data of the children born through ART [58]. This fact renders such observations meriting further investigation. The retrospective nature of the two included studies reporting on preterm delivery association [20, 21], along with the small sample of reported births should be acknowledged as a limitation en route to drawing definite conclusions. Interestingly, a retrospective cohort study conducted by Kalra and her colleagues observed a relation between prolonged embryo culture and preterm birth. However, similarly this study failed to reach a conclusion regarding a possible mechanism [59].

Concerning limitations of the current study, the variation in the number of transferred embryos in conjunction with their quality as assessed employing various grading systems among studies, could stand as confounders in this study. Additionally, several included studies published decades ago are understandably reporting on out of date culture condition systems at a time when embryo culture was more challenging and less efficient.

It should be highlighted that this network meta-analysis concerning D4 ET is a first entry in the current literature. Our results suggest an affirmative answer to the initial question posed, strengthening D4 ET in IVF clinical practice, as a valid option claiming an equal right with respect to the standard suggested ET days, as no significant difference was reported in comparison. This may lead to transform the IVF laboratory set-up, enabling greater flexibility, and options with regard to the optimal cycle's management. The authors refrain from making statements based on findings with respect to a change in practice, especially in light of the fact that with the exception of preterm delivery, other outcome measures evaluated presented with no statistical difference between days of ET. Large randomized controlled trials (RCTs) along with basic research on the physiology of the morula in comparison with that of the cleavage and blastocyst stage embryo are imperative in order to provide strength in decision making and proceed with a possible horizontal clinical application.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

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