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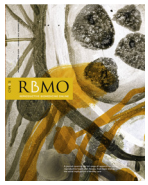

COMMENTARY

The eternal dilemma: are embryos better nurtured *in utero* or *in vitro*?

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ABSTRACT

One of the most debated topics in reproductive medicine is the optimal timing of embryo transfer. While blastocyst-stage transfer has become widely adopted due to the higher blastocyst implantation potential and possibility of synchronizing the transfer with endometrial receptivity, it may not offer universal benefits. A significant proportion of embryos fail to reach the blastocyst stage *in vitro* despite being chromosomally normal, raising concerns that extended culture may lead to unnecessary euploid embryo loss. This paper challenges the assumption that in-vitro culture conditions adequately mimic the uterine environment, and proposes the use of a more individualized approach to the timing of embryo transfer. Probability calculations have been developed to estimate an embryo's live birth potential at the cleavage stage, assessing whether earlier transfer improves the likelihood of live birth compared with extended culture. This is particularly critical for poor-prognosis patients (PPP), who typically produce fewer embryos and risk having no viable blastocysts for transfer. Cleavage-stage transfer may provide a valuable alternative for PPP, allowing the uterine environment to support continued development and rescue of embryos that might otherwise become arrested *in vitro*. These findings emphasize the need for personalized IVF strategies and highlight the clinical relevance and rationale for day 3 transfers in PPP.

INTRODUCTION

One of the most debated topics in reproductive medicine is whether the developmental competence of embryos cultured *in vitro* is similar to that of embryos developing *in utero*. This is reflected in the eternal dilemma of embryo transfer at the cleavage or blastocyst stage. Over the past decade, significant effort has been dedicated to optimizing embryo culture conditions to enhance the success rates of IVF treatments. As a result, the effectiveness of assisted reproductive technology (ART) has markedly improved, resulting in more high-quality embryos cultured *in vitro* from each cycle (*Bartolacci et al., 2024*).

For this reason, there is a clear shift towards prioritizing blastocyst-stage embryo transfer. This approach is motivated by the higher implantation potential, better alignment with the endometrial receptivity window and enhanced embryo selection (*Massimiani et al., 2019*). Consequently, an increasing number of fertility clinics worldwide are opting to transfer embryos on day 5 after fertilization rather than on day 3. However, despite these advancements in culture conditions, a proportion of chromosomally normal embryos still fail to reach the blastocyst stage during in-vitro embryo culture (*Orvieto et al., 2022*). Thus, while many IVF centres worldwide routinely perform day 3 transfer, the ideal timing for embryo transfer continues to be a controversial topic.

A recent randomized controlled trial (RCT) demonstrated that, following fresh embryo transfers in good-prognosis patients, the live birth rate (LBR) was higher in the blastocyst group (37.0%) compared with the cleavage-stage group (29.5%) [risk ratio (RR) 1.26, 95% confidence interval (95%CI) 1.00–1.58] (*Cornelisse et al., 2024*). However, the authors did not find a significant difference in the cumulative LBR (CLBR) between the two embryo transfer strategies (58.9% for blastocyst-stage transfer versus 58.4 for cleavage-stage transfer; [RR 1.01, (95%CI) 0.84–1.22] within 12 months of randomization. Furthermore, obstetric and neonatal outcomes were comparable, with similar birthweights, gestational ages at delivery and rates of small- or large-for-gestational-age births.

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KEYWORDS

Blastocyst stage
Cleavage stage
Cumulative live birth
Embryo transfer
Poor-prognosis patients

It has been estimated that between one-third and one-half of chromosomally normal human embryos generated through IVF fail to reach the blastocyst stage *in vitro* (*Ruangvutilert et al., 2000*), which may potentially reduce the cumulative chances of success. A more recent study indicated that the arrest of embryos before reaching the blastocyst stage cannot be attributed only to aneuploidy (*Orvieto et al., 2022*). These findings suggest that in-vitro culture might reduce the chance of certain embryos progressing to the blastocyst stage and further resulting in pregnancy. This argument becomes even more significant when considering a poor-prognosis patient (PPP) population, such as poor ovarian responders (POR) or those with low fertilization rates and/or limited embryo developmental potential. These subpopulations typically produce fewer embryos, which may face greater challenges surviving prolonged in-vitro culture. For them, cleavage-stage transfer on day 3 could offer a meaningful advantage by enabling the uterine environment to contribute and support a more physiological path to continued development.

Since a prolonged embryo culture to blastocyst stage does not seem to offer an advantage in CLBR and a blastocyst transfer policy may not offer equal benefits for all patient populations, in particular for PPP, we propose that re-evaluating the timing of embryo transfer in carefully selected patient populations could help optimize ART outcomes and minimize unnecessary embryo loss.

THE HYPOTHETICAL SCENARIO OF THE FALLACY OF IN-VITRO SELECTION

Current knowledge supports extended embryo culture to the blastocyst stage to facilitate the self-selection of viable embryos. However, this paradigm assumes that in-vitro conditions adequately mimic the uterine environment — a notion that is increasingly being challenged.

A recent study by Orvieto and co-workers examined (through biopsy on day 3) the ploidy status of arrested embryos versus those reaching the blastocyst stage and found no significant difference in euploidy rates between the two groups (*Orvieto et al., 2022*). This finding suggests that developmental arrest *in vitro* is not driven only by aneuploidy, and the authors proposed that developmental arrest may be influenced by suboptimal culture conditions. Nevertheless, key performance indicators exist to standardize, monitor and maintain the quality of embryo culture conditions in IVF laboratories (*ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine, 2017*). Thus, if embryos that fail to reach the blastocyst stage are still chromosomally normal and are cultured following standard conditions, then a question arises: would some of the arrested embryos have survived and developed successfully *in utero* if transferred at the cleavage stage?

Although in-vitro embryo culture takes place under controlled conditions inside incubators designed to simulate conditions from the uterine environment, the latter is a highly dynamic biological system that provides complex biochemical cues absent in artificial culture systems. Moreover, embryos in extended culture are exposed to oxidative stress originating from the ex-vivo environment, which is further intensified by the increased metabolic demands as embryos progress toward the blastocyst stage. Stressors from in-vitro culture can contribute to disturbances in gene expression, metabolism and epigenetic regulation, resulting in the initiation of adaptive responses to cellular repair and a reduced embryonic survival rate (*Ramos-Ibeas et al, 2019*). Additionally, processes occurring during the early first-cell stages may influence embryo quality, even if they do not prevent progression to the blastocyst stage (*Liang et al., 2023*). These observations support the hypothesis that the uterine environment may offer a more favourable setting potentially rescuing embryos that might otherwise arrest *in vitro*. This consideration is particularly relevant for individuals with a limited number of embryos, for whom optimizing every opportunity for successful development is critical.

From a physiological perspective, however, a cleavage-stage embryo (day 2–3 post-fertilization) naturally resides in the Fallopian tube, not in the uterus. *In vivo*, fertilization occurs in the ampulla, and the embryo remains in the tube until it reaches the morula or early blastocyst stage around day 4–5, only then entering the uterus for implantation (*Ojosnegros et al. 2021*). Transferring a day 3 embryo directly into the uterus bypasses this critical tubal phase and may place the embryo in a hormonally and metabolically suboptimal environment.

While day 5 blastocyst transfer more closely mimics natural embryonic development, for PPP extended culture may increase the risk of cycle cancellation (*Glujovsky and Farquhar, 2016*). In such cases, cleavage-stage transfer may be more practical, ensuring the possibility of embryo transfer while minimizing exposure to in-vitro conditions, which, despite technological improvements, still differ from the dynamic in-vivo environment.

Ultimately, the timing of embryo transfer should reflect a balance between physiological accuracy and clinical pragmatism: blastocyst transfer is preferred when feasible, but cleavage-stage transfer could remain appropriate in selected cases.

IMPLICATIONS OF DAY 3 TRANSFERS FOR PPP

PPP represent a specific population for whom blastocyst culture may not be the optimal strategy. If fewer than four embryos are available, the balance between blastocyst- and cleavage-stage transfers may shift. Extending culture to the blastocyst stage increases the risk of having no viable embryos for transfer (*Glujovsky and Farquhar, 2016*). In such cases, cleavage-stage transfer may offer a higher chance of having at least one embryo available, potentially improving the likelihood of implantation and pregnancy.

One potential bias in comparing CLBR between day 3 and day 5 embryo transfers lies in the duration of follow-up in the available studies. For instance, the manuscript by Cornelisse and colleagues limits follow-up to 12 months, typically ending the analysis after the birth of the first child (*Cornelisse et al., 2024*). An alternative situation can be used to illustrate this conundrum. If, in an archery competition, the archer were to stop after the first hit on the bull's eye of the target without allowing competitors to use all their remaining arrows (with varying numbers of arrows left for each participant), it would be difficult to determine a clear winner or to distinguish differences in skill and success among the competitors, as most participants would probably hit the centre at least once given a sufficient number of attempts. This

difference in the abilities of the participants would probably become more evident if there was an analysis of how many times each competitor hit the bull's eye.

Similarly, when comparing the CLBR between cleavage- and blastocyst-stage transfers, the common approach of considering only cycles that stop at the birth of the first child overlooks the potential for subsequent pregnancies. Expanding the definition of CLBR to include these additional pregnancies could offer a more comprehensive assessment of the true differences between day 3 and day 5 transfer strategies.

However, as highlighted by *Awadalla (2021)*, a well-designed RCT comparing planned fresh transfer at the cleavage stage with planned fresh transfer at the blastocyst stage in POR is very unlikely to be conducted, for two main reasons. The decision to proceed with cleavage- or blastocyst-stage transfers is often made dynamically during embryo culture, limiting the feasibility of strict randomization; additionally, the required sample size would be prohibitively large. Given the difficulty of recruitment and the costs related to an RCT, this approach becomes very unlikely. This limitation underscores a significant gap in the current comparisons of CLBR between these two transfer strategies. However, it is still not possible to exclude the idea that day 3 embryo transfer has its advantages and that it might not be possible to address them in the near future.

Additionally, several studies have highlighted that the cleavage-stage transfer still has acceptable outcomes. Neblett and co-workers reported that in women with a limited number of embryos, the LBR following cleavage-stage transfer remained within acceptable clinical practice, particularly for individuals at high risk of having no transferable embryos on day 5 (*Neblett et al., 2021*). Given that PPP typically produce fewer oocytes and/or embryos, prioritizing cleavage-stage embryo transfer may be a more effective approach.

It is becoming evident that, in good-prognosis patients, a blastocyst-stage transfer results in a lower mean number of embryo transfers required to achieve a live birth (*Cornelisse at al., 2024*; *Ma et al., 2024*). While this strategy offers a clear advantage in shortening the time to pregnancy, its effectiveness may be lost in individuals with fewer embryos. Moreover, blastocyst transfers have been associated with a lower cumulative pregnancy loss rate, but also with a higher incidence of moderate preterm birth (32 to < 37 weeks) (*Cornelisse et al., 2024*; *Ma et al., 2024*). This becomes particularly relevant in the clinical setting when choosing between the two transfer strategies. Such decisions should not be based solely on the number of embryos available, but should rather consider a broader set of factors, including the patient's characteristics and medical history — an approach that aligns with the principles of personalized medicine.

PROBABILITY CALCULATIONS OF EMBRYO VIABILITY AND LIVE BIRTH OUTCOMES FOLLOWING EXTENDED EMBRYO CULTURE

The current authors have developed probability calculations to estimate a hypothetical embryo's live birth potential at the cleavage stage during extended culture. This approach helps in considering whether embryos transferred at the cleavage stage are more likely to result in an live birth than those cultured further and transferred at the blastocyst stage.

Based on data from the work of Cornelisse and colleagues, the authors report an embryo utilization rate — calculated by dividing the number of fresh transferred embryos plus the number of cryopreserved embryos by the total number of embryos available on day 2 after oocyte retrieval — of 55.3% in the blastocyst transfer group (*Cornelisse et al., 2024*). This can be interpreted as the blastulation rate of embryos that, at the cleavage stage on day 2, were randomized and assigned to the blastocyst transfer arm; if this is then multiplied by the LBR per single blastocyst transfer in that group (37%), the average probability of a live birth for those embryos at day 2, at the time of randomization, can be obtained:

Average probability of live birth per day 2 embryo
= (Blastulation rate)
× (LBR per single blastocyst transfer)

Applying this formula to Cornelisse and colleagues' data gives
$0.553 \times 0.370 = 0.2046$, which results in an average probability of live of 20.46% per embryo at the cleavage stage.

In contrast, the embryos transferred at the cleavage stage in the other randomization arm resulted in a probability of live birth of 29.5%. This difference would suggest that the blastocyst transfer group experienced a loss of 9.04% in live birth potential (a 30.6% reduction) for each embryo left in extended embryo culture. Essentially, this hypothetical calculation indicates that approximately one out of every three arrested embryos could have resulted in a live birth if it had been transferred at the cleavage stage.

CONCLUSION

The assumption that an extended embryo culture policy to blastocyst stage universally improves outcomes must be re-evaluated. While blastocyst transfer remains the standard for many patients, it may not be the optimal choice for all patient populations. In particular, PPP may benefit from cleavage-stage transfer, allowing embryos to complete their development in a more natural setting. Our hypothetical probability calculations support the idea that embryos are better nurtured *in utero* than *in vitro*. Acknowledging the potential of the uterine environment to rescue embryos that might otherwise arrest *in vitro* could pave the way for more personalized and effective IVF strategies. It is time to move beyond a one-size-fits-all approach and adopt a more nuanced, individualized framework for selecting the optimal timing of embryo transfer.

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