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COMMENTARY

Should we be promoting embryo transfer at blastocyst stage?

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Abstract Improved laboratory standards and better culture media have made extended culture to blastocyst stage a reality to identify embryos with maximum implantation potential. The strategy of extended culture has become more popular across the world at a time when regulatory bodies have emphasized the need to increase the uptake of elective single embryo transfer, minimize complications associated with multiple births and aim for a healthy singleton live-birth as the preferred outcome in IVF. New data on perinatal outcomes suggest that pregnancies after embryo transfer at blastocyst stage are associated with a higher risk of preterm delivery, large for gestational age babies, monozygotic twins and altered sex ratio compared with those following embryo transfers at cleavage stage. In addition, concerns have been raised of increased congenital anomalies and epigenetic modifications with embryo transfer at blastocyst stage. Twenty-four years on from the first embryo transfer at blastocyst stage, we examine the reasons for extended embryo culture, evaluate the risks and benefits of this strategy and suggest the need to reconsider this policy in the interests of fetal safety.

KEYWORDS: blasocyst, embryo transfer, preterm delivery, IVF

Introduction

Advances in our knowledge of in-vitro culture conditions have led to the development of stage-specific or sequential media (Gardner et al., 1998), making it possible to conduct routinely extended culture of embryos to the blastocyst stage. The first reports of pregnancy and live birth from an embryo transferred at blastocyst stage (day 5–6 after egg collection) were published in 1985 (Cohen et al., 1985) and 1991 (Bolton et al., 1991), respectively. Since then, a constant increase in proportion of embryo transfers at blastocyst stage has been reported (from 1% in 2000 to 34% in 2012 in UK (http://www.hfea.gov.uk/104.html).

In this paper, we examine the reasons for extended embryo culture, evaluate the risks and benefits of this strategy and consider whether a policy of embryo transfer at blastocyst stage is still justified.

Reasons for choosing extended culture

Extended culture has been considered to be a better option than cleavage stage embryo transfer for a number of reasons.

Physiological synchronization

Transfer of the embryo to the uterine cavity after 5 days of insemination or injection is thought to provide better embryo–endometrium synchrony, and therefore higher chances of implantation as it mimics more closely the sequence of events in natural conception. Unlike the situation in a natural cycle, however, ovarian stimulation during IVF leads to supraphysiological levels of oestrogen and progesterone, which enhances the endometrial development, i.e. the endometrial milieu at day 3 after egg collection (in a stimulated

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environment) could be similar to the endometrial milieu on day 5 (after ovulation) in a natural cycle owing to the effect of ovarian stimulation (Kolibianakis et al., 2002).

Embryo selection

Activation of the embryonic genome occurs at the eight-cell stage (day 3). In the absence of activation, the embryo is unlikely to survive or implant. An obvious way to ensure this is to extend the duration of culture to blastocyst stage – a process that allows identification of embryos that have managed to activate their embryonic genome.

If embryo development between days 3 and 5 and 6 were solely based on inherent survival potential and embryonic activation, this would have been the ideal way to screen out poor-quality embryos. This is not necessarily the case, as in-vitro survival does not equate to in-vivo survival, which depends on the culture system used, e.g. medium, number and types of incubators and oxygen tension. By committing to embryo transfer at blastocyst stage, there is a risk of losing some embryos, which might not survive the challenge of extended culture but might have, if transferred to the uterus, survived *in vivo*, implanted and resulted in a pregnancy.

Blastocyst transfer improves the odds of transferring a viable embryo (Harton et al., 2013) but does not guarantee euploidy. Morphological scoring, either at blastocyst or cleavage stage, is not an accurate way of identifying chromosomal abnormalities, and recent studies have shown that chromosomally abnormal embryos can become blastocysts (Fragouli et al., 2014).

Improved live birth rates

A meta-analysis of 12 randomized controlled trials (RCT) showed a significant increase in live birth rate per started treatment when embryo transfer was carried out at blastocyst stage compared with cleavage stage (odds ratio [OR] 1.40, 95% confidence interval [CI] 1.13 to 1.74). Meta-analysis of four RCT showed that cumulative pregnancy rates, i.e. number of births from one egg collection, are significantly higher (OR 1.58, 95% CI 1.11 to 2.25) in embryo transfers carried out at cleavage stage compared with those at blastocyst stage (Glujovsky et al., 2012). The possible reason for decreased cumulative live birth rate with extended culture is that a number of embryos that do not reach blastocyst stage are discarded and not eligible for transfer. If these are frozen at cleavage stage and transferred after successful thawing, pregnancies can be achieved. This means that minimizing embryo wastage in in-vitro culture could lead to the possibility of more pregnancies and therefore higher cumulative live birth rate even in those with good prognosis.

One can argue that this could be attributed to different media and culture conditions, as optimal culture conditions should ensure that the most embryos survive culture between days 3 and day 5. The data presented above, however, are from a meta-analysis of four RCT reporting on cumulative pregnancy rates. None of the trials compared the cost of extending the culture or extra freezing on day 3.

Comprehensive chromosome screening

Other reasons to extend embryo culture to day 5 include a strategy of comprehensive chromosome screening after day 5 biopsy. Embryos are frozen in these situations with a view to deferred embryo transfer, as it is difficult for biopsy results to be returned in time for embryo transfer. Increasing evidence from RCT show that comprehensive chromosome screening at the blastocyst stage improves implantation and pregnancy rates (Forman et al., 2013; Scott et al., 2013; Yang et al., 2012). These, however, are yet to become incorporated into routine clinical practice in most centres and will not be discussed further in this commentary.

Deferred embryo transfer

Electively freezing all embryos with routine use of frozen and thawed embryo transfer has been much debated (Maheshwari and Bhattacharya, 2013). This is being evaluated in a number of ongoing clinical trials, and has yet to find universal acceptance. In this commentary, we therefore focus on the pros and cons of day 5 versus day 3 embryos in conventional IVF where fresh embryo transfer is the norm and the rationale for extended culture is to select the best embryo.

Obstetrics and perinatal outcome of pregnancies

As good-quality embryos from women with the best prognosis tend to be selected for extended culture, one would expect perinatal outcomes to be better in pregnancies as a result of blastocyst transfer. This does not, however, seem to be the case.

Preterm delivery

Two separate meta-analyses have confirmed that IVF pregnancies resulting from embryo transfer at blastocyst stage were associated with a higher relative risk (95% CI) of preterm (<37 weeks) (1.27 [1.22 to 1.31]) and very preterm (<32 weeks) delivery (1.22 [1.10 to 1.35]) compared with with those resulting from the transfer of cleavage stage embryos (Dar et al., 2014; Maheshwari and Bhattacharya, 2013; Maheshwari et al., 2013). Both meta-analyses were based on published observational data, hence were unable to adjust for confounders, although, in individual studies, adjusted odds ratio was used. Conclusive evidence for increase in preterm delivery can only be provided by adequately powered RCT.

Risk of monozygous twins

Existing data suggest an increased chance of monozygotic twins (Luke et al., 2014) associated with blastocyst transfer with a pooled odds ratio of 3.04 (95% CI 1.54 to 6.01) (Chang et al., 2009) compared with embryo transfer at cleavage stage. The exact reason for this increase is not known, but alteration in

the zona pellucida induced by extended culture is thought to be responsible.

Large for gestational age babies

Data from animal as well as human studies have suggested that extended culture leads to large for gestation offspring (Zhu et al., 2014). The authors of this study admit that a key limitation of their study was that extended culture was mainly offered to poor-prognosis patients (unsuccessful IVF cycles or with uterine malformations), resulting in potential selection bias. Even gender-adjusted birth weight (Z scores) are higher for newborns after embryo transfer at blastocyst stage when compared with those after day 2 or 3 embryo transfers (Mäkinen et al., 2013), albeit only a small number had embryo transfer at blastocyst stage.

An increased proportion of large for gestational age babies may be the reason that meta-analysis (Maheshwari and Bhattacharya, 2013; Maheshwari et al., 2013) suggests a decreased risk of intrauterine growth retardation with blastocyst stage embryo transfer compared with those at cleavage stage. It could be argued that the type of culture media used in extended culture could be responsible for large for gestational age babies; however, a recent study seems to refute this (De Vos et al., 2015).

Congenital anomalies

A recent meta-analysis (Dar et al., 2014) reported that the odds of congenital anomalies were significantly higher for babies born after embryo transfer at blastocyst stage compared with those born after embryo transfer at cleavage stage (1.29, 95% CI 1.03 to 1.62).

Altered male: female ratio

Several reports have suggested that blastocyst transfer leads to an altered sex ratio with a male–female ratio of 1.29 (95% CI 1.10 to 1.51) (Chang et al., 2009).

Reasons for adverse perinatal outcomes after extended culture

Although extended culture is usually offered to good-prognosis women in whom better perinatal outcomes are expected, the results of some of the existing studies seem to suggest the opposite. A possible explanation could be that extended culture may trigger genetic and epigenetic changes in trophodermal cells that can lead to abnormal placentation and implantation, and hence increased risk of preterm delivery. Results from animal studies support this hypothesis (Rizos et al., 2002).

Why do we still continue with extended culture?

Despite the concerns highlighted above (**Figure 1**), extending culture to blastocyst stage seems to be the preferred strategy among many IVF clinics, and its popularity has grown exponentially in recent years. The key drivers for this approach are the importance clinics, commissioners and patients attach to the realization of short-term goals (higher pregnancy rates per embryo transfer episode) and their position in national league tables based on success rates. This is mainly due to the fact that single embryo transfer at blastocyst stage leads to a significantly higher pregnancy rate per embryo transfer compared with those at cleavage stage.

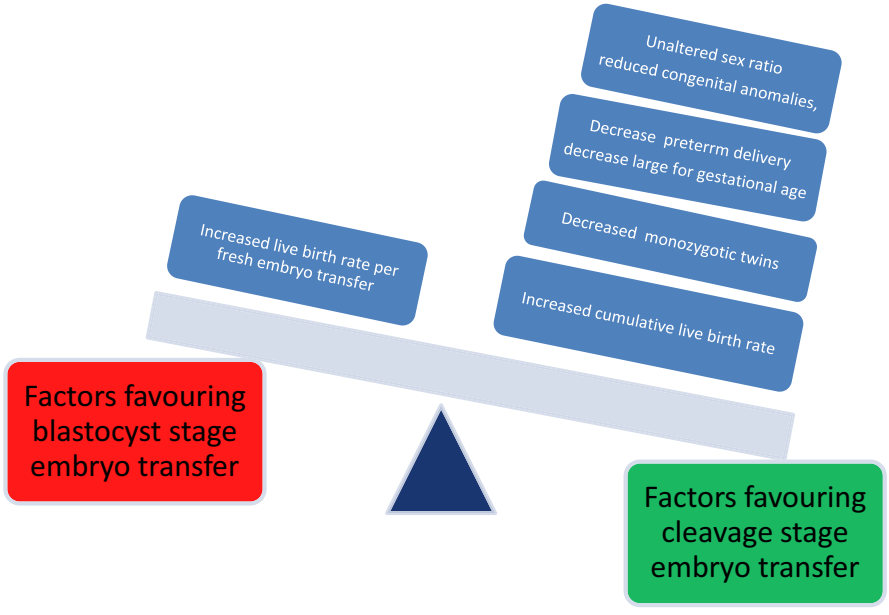

Figure 1 Balance between blastocyst and cleavage stage embryo transfer.

Do we need to change our focus?

As clinicians, our focus has shifted from live birth as the sole measure of treatment success to outcomes which reflect feto–maternal safety, especially when evidence is emerging that birth outcomes can be predictive of diseases in later life. It is increasingly becoming clear that the goal of assisted reproduction should be to achieve a healthy live baby with the potential to develop into a healthy adult.

Pregnancies achieved through IVF, even in singletons, are associated with higher rates of preterm deliveries compared with those conceived spontaneously. This risk is further increased in pregnancies after embryo transfer at blastocyst stage compared with embryo transfer at cleavage stage. If one adds the risks associated with monozygotic twins, large for gestational age and congenital anomalies there are further concerns about safety of future generations.

Moreover, it is clear that, although we go to extreme lengths to select the 'best' embryo, current tests of embryo quality lack precision, and pregnancies can arise from non-top quality embryos as well as those which never make it to blastocyst stage. Extended culture in women with very few embryos incurs the risk of either having no embryos for transfer in a fresh cycle or cryopreservation for future use. Hence we do need to consider alternative strategies.

What needs to be done to change the status quo?

In order for change to occur, a number of processes will need to be instituted.

Reporting of IVF success rates will need to change

As scientists, healthcare providers and public health professionals, we have an obligation to ensure that the data we present on efficacy is consistent with our overarching objectives of promoting safe motherhood and the birth of healthy children. It is only when safety is a prominent part of reporting that the clinics and clinicians will accept strategies, which may not give instant results but are better for the long term. Although the currently favoured outcome 'Live birth per cycle' is understood and accepted by stakeholders, it is outdated and needs updating to reflect both the risks as well as the effectiveness of the treatment. The most important parameter for the couple is the ultimate cumulative healthy baby rate per started cycle. Success rates based on cumulative healthy baby rates will provide patients with a direct measure that will better enable them to make informed decisions about whether and how to undergo IVF treatment in a way that maximizes their chance for a healthy infant from one episode of egg collection.

Change the perceptions of stakeholders

No data have been published on patients' preferences on embryo transfer at cleavage stage or blastocyst stage. Studies are urgently needed to establish which reproductive outcomes couples value, i.e. instant gratification (higher live birth per fresh embryo transfer episode) versus delayed realisation of goals (higher cumulative live birth, i.e. all live births from one egg collection episode, with fewer complications for mother and baby).

A planned, multifaceted strategy to educate all relevant stakeholdersis is needed to change the prevailing culture. Although this may seem to be an impossible task, there are precedents in this field. For example, a similar situation was faced when elective single embryo transfer was promoted over double embryo transfer. Although initial progress was slow, most clinics, healthcare funders, as well as patients, understand the importance of achieving one baby at a time in the interest of feto–maternal safety, even though this results in a delay in having a second baby.

Change the way IVF treatment is funded

No data are available to support the choice between extended culture as a cost-effective option from a societal perspective compared with embryo transfer at cleavage stage. In most clincs, the charge for IVF treatment only includes fresh embryo transfer. If couples were to freeze spare embryos and use them later they need to pay more. This way of costing IVF encourages couples to focus on short-term gains. Guidance in UK by National Institute of Health and Care Excellence in 2013 (CG 156, NICE, UK) recommends that fresh and all associated frozen and thawed transfer cycles should be counted as part of one IVF cycle but, even in the UK, this strategy has only been followed by a few clinics for publicly funded treatments.

Follow-up studies

The oldest baby born after embryo transfer at blastocyst stage is just under 20 years old. Although, adverse perinatal outcomes have been reported, long-term outcomes are still to be revealed. More follow-up studies on long-term health of children born by embryo transfer at both cleavage and blastocyst stages are needed throughout the world to determine the final recommendations for clinical practice. Such collaborative studies should be encouraged now that most countries have registries for those having treatment using assisted reproduction techniques.

Better selection on day 3

Investment should be made on evaluating new technology such as time lapse, to select the best embryo on day 3 to give maximum number of pregnancies per treatment by increasing the cumulative pregnancy rate and reducing pre-term delivery.

Conclusion

Embryo transfer at blastocyst stage has become the strategy of choice for most clinics worldwide, with the aim of

achieving a healthy singleton live-birth and so minimizing the number of multiple births and their associated complications, while still maintaining pregnancy rates per transfer. This has been achieved by carrying out a single blastocyst transfer instead of single embryo transfer on day 3. Although this leads to higher live birth rates per embryo transfer episode, it ultimately results in lower cumulative live birth rates per couple, higher risk of preterm birth, large for gestational age, monozygotic twins and congenital anomalies compared with embryo transfer at cleavage stage.

The evidence presented in this commentary clearly highlights reasons for concern, with the possibility of epigenetic changes resulting from extended culture and potential increased risks to fetal health. The available data at present are weak and do not justify stopping extended culture but, in the interest of long-term outcomes, it is perhaps time to rethink the current policy of blastocyst transfer. Larger RCT of day 3 versus day 5 embryo transfer, involving highly experienced laboratories and including longer term follow-up data on offspring outcome are necessary to provide conclusive evidence for or against blastocyst transfer.

The main drivers for extended culture include clinics' keenness to secure a favourable position in national league tables and an intuitive desire to maximise short term reproductive success. It is therefore of utmost importance that league tables reflect safety as well as efficacy. In addition, appropriate strategies are needed to inform all stakeholders about the value of appreciating long term reproductive outcomes. Once all the information about the short- and long-term implications is available, it is only then that information should be discussed with a couple and a decision should be made which is right for them rather than solely promoting the policy of embryo transfer at blastocyst stage.

As providers of IVF treatment, we have an obligation to minimize complications associated with IVF and safeguard the long-term health of future generations.

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