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Transferring Mosaic Embryos in Assisted Reproductive Technology: Balancing Opportunities and Challenges

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The Pros of Transferring Mosaic Embryos

Preimplantation Genetic Testing for Aneuploidy (PGT-A) has revolutionized assisted reproductive technology (ART) by enabling the selection of embryos with the correct chromosomal number for transfer, thereby increasing the chances of successful pregnancies.

However, the identification of mosaic embryos—those containing both euploid (normal) and aneuploid (abnormal) cells—has introduced a clinical dilemma. Historically, mosaic embryos were often excluded from transfer due to concerns about viability and potential adverse outcomes.

Recent advances in genetic testing and a deeper understanding of embryonic development have challenged this perspective.

The Cons of Transferring Mosaic Embryos

Over the past decades, our field has achieved tremendous milestones and witnessed breakthrough developments that have significantly advanced both the efficiency and safety of our daily practices. One such milestone is Preimplantation Genetic Testing (PGT), a technique pioneered by Handyside in 1989 (31), which allows us to assess the genetic profile of embryos before transfer. PGT marked the beginning of a new era, giving patients at risk the option to screen their embryos before implantation. Since its introduction into clinical practice, significant efforts from various research groups have shaped PGT into its current form. Today, despite the 35 years of continuous progress, challenges remain, particularly regarding the application of PGT for aneuploidy screening (PGT-A) and its impact on the overall prognosis. These concerns led to the 2024 Practice Committee opinion (32), which recommended against the routine use of PGT-A.

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Debate

Increased Chances of Pregnancy and Live Birth

Pros: Viability of Mosaic Embryos

Emerging evidence suggests that mosaic embryos possess significant developmental potential. In 2015, Greco et al. were the first to report successful pregnancies and healthy births following the transfer of mosaic embryos previously deemed unsuitable. In their study, six out of eighteen patients with only mosaic embryos available achieved healthy live births. The birth of healthy infants from these embryos indicates that mosaicism detected in preimplantation genetic testing may not always predict negative outcomes. The authors proposed that some mosaic embryos have the capability to develop normally, particularly when the mosaicism involves certain chromosomes or occurs at low levels (1).

In 2018, Spinella et al. further supported these conclusions by analyzing 100 mosaic embryo transfers. They observed an implantation rate of 37% and an ongoing pregnancy rate of 28%, suggesting that mosaic embryos can contribute significantly to successful ART outcomes. The study highlighted that excluding mosaic embryos from transfer decisions might unnecessarily reduce the chances of pregnancy for many patients (2).

Similarly, in 2020 Munné et al. analyzed the outcomes of patients who underwent PGT-A. They found that transferring mosaic embryos resulted in a live birth rate of 40%, which is significant considering these embryos were previously deemed unsuitable. The study also noted no increase in congenital anomalies among the infants born from mosaic embryos compared to those from euploid embryos (3).

Viotti and colleagues conducted a large-scale study involving over 1,000 mosaic embryo transfers. The researchers found that live birth rates for low-level mosaic embryos were comparable to those of euploid embryos, challenging the traditional view that mosaicism inherently reduces viability. Specifically, the implantation rate for low-level mosaic embryos was 42.4%, closely mirroring the 47.2% observed for euploid embryos. Live birth rates were 36.6% for low-level mosaics versus 41.4% for euploid embryos, a difference that was not statistically significant (4). These findings suggest that the presence of low-level mosaicism may not adversely affect the embryo's potential to result in a healthy live birth.

Mosaic Embryos Demonstrate Lower Reproductive Performance

One of the biggest challenges in PGT-A is managing mosaic embryos. Embryo mosaicism refers to the presence of two or more cell populations with different genotypes within a single embryo (33). Embryos with intermediate results after next-generation sequencing (NGS), falling between the ranges of euploidy and aneuploidy, have traditionally been classified as “mosaic embryos.” Mosaic embryos have historically been deselected from transfer and were grouped with uniformly aneuploid embryos as 'abnormal.' However, in recent years, numerous groups have reported the intentional transfer of mosaic embryos in the absence of uniformly euploid embryos, largely observing births of healthy babies. To date, more than 2,700 mosaic embryos have been transferred (34), showing that mosaic embryos can produce viable pregnancies and healthy babies. However, their reproductive performance is reported to be lower compared to euploid embryos. Several studies have reported reduced implantation, pregnancy, and clinical pregnancy rates, along with higher miscarriage rates, when mosaic embryos are transferred (30,35,4). Despite their lower overall reproductive performance, neonatal outcomes between euploid and mosaic embryo transfers have been found to be comparable (36). This suggests the potential presence of natural corrective processes that may eliminate cells with abnormal chromosome sets, allowing healthy cell lines to prevail. However, in few cases, persistence of mosaicism after transferring mosaic embryos has been reported (37–39).

Currently, our knowledge and experience do not allow us to accurately predict the reproductive outcomes of mosaic embryos, nor the genotype or phenotype of the infants born. These uncertainties make it extremely difficult to fully inform and adequately prepare patients regarding the potential risks associated with transferring a mosaic embryo. Only additional data from mosaic embryo transfers and paired prenatal tests will allow us to accurately estimate the expected prognosis for a viable pregnancy per type and level of mosaicism and accurately appreciate the incidence of mosaicism persistence and assess the short and long-term health risks involved in the respective babies born.

Cons: Mosaicism: Beyond the Embryo’s Biology

Embryo mosaicism is known to be a post-zygotic event and is the result of errors during mitotic divisions mainly in the early stage of development (40–42). The frequency of mosaic blastocysts is reported to be 6.1% (43), although the

Pros: Subtypes of Mosaicism and Their Impact

Research has shown that the type and degree of mosaicism play critical roles in determining embryo viability. Grati et al. (5) conducted a review highlighting that embryos with segmental mosaicism (involving parts of chromosomes) or low-level whole-chromosome mosaicism often have better clinical outcomes than those with high-level mosaicism. They found that transferring embryos with less than 30% abnormal cells resulted in live birth rates comparable to euploid embryos (5).

Munné et al. developed a comprehensive scoring system to assess the viability of mosaic embryos based on the level and type of chromosomal abnormalities. Their findings indicated that embryos with low-level mosaicism or involving chromosomes with less impact on development (e.g., sex chromosomes) had higher implantation and live birth rates. This nuanced understanding allows for more informed decision-making regarding embryo selection (3).

Pros: Meta-Analyses and Systematic Reviews

A systematic review and meta-analysis by Zhang et al. (6) evaluated the outcomes of mosaic embryo transfers. The analysis included data from multiple studies and found that the overall implantation rate of mosaic embryos was 30%, with a live birth rate of 20%. While these rates are lower than those for euploid embryos, they are significant for patients who lack euploid embryos. The study concluded that mosaic embryo transfer can be a viable option, especially when no euploid embryos are available (7).

Pros: Self-Correction Potential

The ability of embryos to self-correct chromosomal abnormalities adds another layer of support for transferring mosaic embryos. Bolton et al. demonstrated in a mouse model that embryos might eliminate abnormal cells through corrective mechanisms. The study showed that aneuploid cells in early embryos can undergo apoptosis or be allocated to the trophectoderm (which forms the placenta), while euploid cells contribute to the inner cell mass (which develops into the fetus). This self-correction capacity suggests that some mosaic embryos could develop into healthy fetuses despite initial chromosomal abnormalities (8).

Starostik et al. expanded on this concept by analyzing human embryos. They found that chromosomal mosaicism is relatively common in early human embryos but that many can progress to form healthy post-implantation frequency of mosaicism is lower, at about 2% (44,45) and drops to less than 0.2% in newborn infants.

Several factors may potentially determine an embryo's predisposition for mitotic errors. In particular, biological factors such as maternal (46) and paternal age (47,48) and severe oligozoospermia (39,48,49) have been reported. Moreover, clinical and lab factors have also been reported to be linked to mosaicism such as the stimulation daily dosage (50), the fertilization technique (51,52), the culture conditions (53), the day of biopsy (46), the biopsy technique (54), the blastocyst quality, and day of blastocyst (55).

Lastly, technical factors may also be involved, which creates a significant concern for our current diagnostic efficiency when it comes to embryo mosaicism. Different reproductive genetic labs use different diagnostic platforms with varying diagnostic accuracy for mosaicism and, as a result, a huge range of mosaicism in trophectoderm biopsies, from 2–40% (12,56–59), has been reported. This creates additional uncertainty for the results reported and whether they accurately represent the embryos' genetic status or are the result of technical artifacts.

For many reasons, the close monitoring of the mosaic embryo rate is essential for a successful PGT program. The most important being that the increased prevalence of mosaicism observed in the in vitro embryos during PGT-A may highlight their role as stress factors for the in vitro cultured embryos, while the mitotic errors may represent embryos' response mechanism to these stress factors. A sound knowledge of the list of clinical and laboratory factors linked to mosaicism may help us implement the relevant stress mitigation strategies while creating an ideal environment for the embryos where mitotic errors are minimized. Moreover, a close collaboration and clear communication with all the departments involved in a PGT program, followed by regular internal and external audits, is essential, as minor changes in their daily practices may have a great impact on the rate of mosaic embryos.

Cons: Managing Mosaic Embryos Escalated the Complexity of a PGT-A Program

The management of the increasing number of mosaic embryos, previously discarded as "abnormal," represents another modern challenge for our field. More specifically, the Practice Committee and Genetic Counseling Professional Group (GCPG) of the American Society for Reproductive Medicine, along with PGDIS and ESHRE societies (59–61),

pregnancies. The researchers observed that mechanisms such as cell arrest, apoptosis of abnormal cells, and preferential growth of normal cells contribute to self-correction (9).

In clinical settings, Victor et al. (10) reported on the outcomes of 100 mosaic embryo transfers and found that the live birth rates were encouraging. Importantly, comprehensive chromosomal testing of the newborns showed normal karyotypes, suggesting that embryos initially diagnosed as mosaic had undergone self-correction during development. There were no significant differences in birth weights or neonatal complications between babies born from mosaic versus euploid embryos (10).

In 2019, Popovic et al. (11) utilized advanced genetic techniques, including next-generation sequencing and comprehensive chromosomal screening, to analyze the prevalence and nature of mosaicism in embryos. The study revealed that chromosomal mosaicism is more common than previously thought, occurring in a significant proportion of embryos assessed during IVF cycles. Importantly, the researchers found that many mosaic embryos possess the potential to develop into healthy pregnancies.

Pros: Molecular and Genetic Insights

Recent advancements in genetic analysis have significantly enhanced our understanding of self-correction. Treff et al. (15) used single-cell sequencing to track the fate of aneuploid cells in human embryos. They observed that aneuploid cells were often eliminated or became quiescent, allowing normal cells to dominate development (15). McCoy (16) proposed that the selective pressure during early development favors euploid cells, leading to the depletion of aneuploid cells. This natural selection process enhances the likelihood of normal development from mosaic embryos (16).

Pros: Clinical Outcomes and Long-Term Follow-Up

Fragouli et al. (13) investigated the developmental competence of mosaic embryos by analyzing their gene expression profiles. The study found that mosaic embryos that implanted successfully had gene expression patterns similar to those of euploid embryos, suggesting that they possess similar developmental potential (13).

Li et al. (14) performed a prospective cohort study involving 200 patients who received mosaic embryo transfers. The study reported a cumulative live birth

recommend that every individual who undergoes IVF with PGT-A should receive genetic counseling sessions before and after PGT-A. Within this context, patients should be adequately informed about the meaning of mosaicism, its biological mechanism, the origin of the embryonic cells analyzed during PGT-A, the expected in-house rate of mosaicism, and all the related technical and clinical limitations. While patients contemplating mosaic embryo transfer, a post-test genetic counseling session (62) should be provided. During this session, the possible explanations for the mosaic PGT-A result should be discussed, highlighting the expected clinical outcomes from the transfer of a mosaic embryo based on the most current evidence (32).

Moreover, all the embryos described as mosaic are currently accumulating in our cryobanks until their fate is decided. This creates an additional demand for capacity in our expanding cryobanks for embryos which are considered of lower priority and won't be thawed unless they become the patients' last resort or won't be discarded in the hope that they may be used "sometime" in the future. This represents an additional complexity in the management of our cryobanks while further stretches their capacity for long-term storage (63,64).

Cons: Litigation Aspects of Managing Mosaic Embryos

Lastly, the litigation aspects involved in the transfer of mosaic embryos must not be overlooked. Mosaic embryos are still linked to many uncertainties and lack of knowledge when it comes to their ability to establish a viable pregnancy and determine the infant's genotype and phenotype. As these fronts are still in progress, allowing the transfer of mosaic embryos means that our systems are faced with escalating risks. Risks that need to be addressed and linked to risk mitigation measures which will safeguard our system from future litigation threats. This can only be achieved by ensuring that all the support is available, provided, and documented to the patients to help them make informed decisions for their mosaic embryos, followed by detailed consent forms where the risks and limitations related to the mosaic embryo transfer are clearly outlined and explained before they are signed by the patients.

In this new era of PGT-A, the management of mosaic embryos and our risk management plans urgently need to be re-evaluated while additional measures and procedures need to be added, such as the upgrade of training of all the personnel involved in the management of mosaic embryos, the provision of additional consultation time for these patients, the

rate of 35% and found no significant differences in obstetric complications or neonatal outcomes compared to euploid embryo transfers. Furthermore, the infants were followed up until the age of two years, and no developmental delays or chromosomal abnormalities were detected (14).

Long-term health and development of children born from mosaic embryos are essential to assess the safety of this practice. Maxwell et al. (17) conducted follow-up studies on 50 children born from mosaic embryo transfers up to the age of five years. The study found normal growth parameters, cognitive development, and no increased incidence of health issues compared to children born from euploid embryos (17). Gleicher et al. (18) emphasized the need for registries and longitudinal studies to monitor outcomes, which will provide valuable data to guide future clinical practice (18).

Ethical Considerations and Patient Autonomy

Pros: Respecting Patient Choices

The decision to transfer mosaic embryos extends beyond clinical outcomes to encompass ethical considerations, particularly regarding patient autonomy. Muñoz et al. (19) argue that patients should be fully informed about the option to transfer mosaic embryos. Providing comprehensive information allows patients to make decisions aligned with their values and reproductive goals, thereby respecting their autonomy and promoting shared decision-making in clinical practice (19).

Gleicher et al. (20) emphasize that denying patients the choice to transfer mosaic embryos may infringe upon their reproductive rights, especially when no euploid embryos are available. They contend that withholding this option limits patients' ability to pursue parenthood and may not be ethically justifiable given the emerging evidence supporting the viability of mosaic embryos (20).

Pros: Ethical Use of Embryos

The ethical use of embryos is a critical consideration in ART. Harper and SenGupta (21) discuss the moral implications of discarding embryos based solely on PGT-A results. They advocate for cautious interpretation of genetic testing outcomes to prevent the unnecessary loss of potentially viable embryos, suggesting that mosaic embryos should not be automatically excluded from consideration (21).

De Wert et al. (22) highlight the ethical duty to avoid discarding embryos that could develop into healthy extension of long-term cryostorage, and the thorough revision of the PGT consent forms.

Cons: Conclusion

Transferring mosaic embryos presents notable challenges and uncertainties in assisted reproductive technology (ART). Studies have shown that mosaic embryos have lower reproductive performance compared to euploid embryos, with reduced implantation and pregnancy rates and higher miscarriage rates. There is also the risk of persistent mosaicism in offspring, making it difficult to fully inform patients about potential outcomes and associated risks.

Mosaicism is influenced by various factors beyond the embryo's biology, including parental age, clinical practices, laboratory conditions, and technical aspects of genetic testing. Inconsistencies in diagnostic methods across laboratories lead to variable reporting of mosaicism rates, raising concerns about the accuracy of these diagnoses.

Managing mosaic embryos adds complexity to PGT-A programs. The accumulation of these embryos in cryobanks increases storage demands and logistical challenges, as they are often stored indefinitely. Ethical and legal considerations also arise, as transferring embryos with uncertain outcomes may expose clinics to litigation risks. Comprehensive genetic counseling before and after PGT-A is recommended, necessitating additional resources and trained personnel.

In summary, while transferring mosaic embryos can offer hope to patients without euploid embryos, it introduces significant uncertainties and potential risks. Until more definitive data are available to accurately predict outcomes, caution is advised. Clinicians should provide thorough counseling to ensure patients are fully informed, obtain detailed consent, and carefully consider the ethical implications. Ongoing research and standardized protocols are essential to improve the management of mosaic embryos, ultimately enhancing patient care and safety in ART programs.

children. They promote the transfer of mosaic embryos under informed consent, emphasizing that patients should be given the opportunity to decide whether to proceed with such embryos based on a thorough understanding of the potential risks and benefits (22).

Psychological Benefits for Patients

Pros: Reducing Emotional Stress

Offering the option to transfer mosaic embryos can have positive psychological effects on patients undergoing ART. Maxwell et al. (23) reported that patients experienced reduced anxiety and feelings of hopelessness when given the option to transfer mosaic embryos. This empowerment enhances their overall treatment experience and may contribute to better emotional well-being during the challenging IVF process (23).

Gleicher et al. (24) found that patient satisfaction increased when they were actively involved in the decision-making process regarding mosaic embryo transfer. Allowing patients to participate in their care fosters a sense of control and may improve adherence to treatment protocols (24).

Financial Considerations

Pros: Cost-Effectiveness

Transferring mosaic embryos may also have financial benefits for patients. Fiorentino et al. (25) noted that utilizing mosaic embryos can reduce the need for additional IVF cycles, thereby lowering the overall financial burden. Each additional cycle involves significant costs, both monetary and emotional, so maximizing the use of available embryos is advantageous (25).

Murugappan et al. (26) conducted an analysis of the cost-effectiveness of transferring mosaic embryos. They concluded that in many cases, transferring these embryos is a financially viable option that can lead to successful outcomes without the expenses associated with further treatment cycles (26).

Contribution to Scientific Knowledge

Pros: Advancing ART Practices

Data gathered from mosaic embryo transfers contribute to the broader understanding of embryonic development and ART. Grifo et al. (27) emphasized that studying the outcomes of these transfers

enhances knowledge about mosaicism, potentially leading to improved protocols and success rates in ART (27).

Spinella et al. (3) suggested that long-term follow-up of children born from mosaic embryo transfers is essential. Such data can inform future guidelines, refine patient counseling, and ensure that practices evolve based on empirical evidence (3).

Pros: Conclusion

The transfer of mosaic embryos represents a significant advancement in assisted reproductive technology, offering hope to couples struggling to start a family. Emerging evidence indicates that mosaic embryos can result in healthy babies, challenging previous notions about their viability. This game-changing prospect has been substantiated by the latest data presented by Dr. Francesca Spinella at the 40th Annual Meeting of ESHRE in July 2024. The IRMET (International Registry of Mosaic Embryos Transfers – www.irmet.net) data collection (data from 3,704 mosaic embryo transfers) demonstrated that the transfer of mosaic embryos contributes positively to clinical outcomes, providing valuable opportunities for patients who might otherwise have limited options (29).

Avoiding the transfer of mosaic embryos may lead to the unnecessary loss of embryos with the potential for healthy development. Capalbo et al. (30) conducted a prospective, non-selection clinical trial that revealed the developmental potential of mosaic human preimplantation embryos. Their findings underscored that excluding these embryos from transfer decisions could deny patients a viable chance at pregnancy, emphasizing the importance of reconsidering current practices (30).

Mosaicism appears to be an inherent feature of human embryos, irrespective of oocyte age (33), arising during early embryonic stages. Human embryos possess autocorrection mechanisms, which contribute to the development of healthy fetuses even when mosaicism is detected after preimplantation genetic testing for aneuploidy (PGT-A). The trophectoderm biopsy performed during PGT-A captures this transient phenomenon, but reliance on this information alone may limit clinical decisions. By avoiding the transfer of mosaic embryos due to concerns over mosaicism, we may inadvertently lose potential positive outcomes based on unfounded fears.

It seems that the traditional binary classification of embryos into 'normal' and 'abnormal' is obsolete.

Viotti et al. (31) highlight that maintaining such a system risks misclassifying mosaic embryos, either overestimating or underestimating their developmental potential and therefore advocate for stratifying mosaic embryos into subgroups based on the level and type of mosaicism, allowing for better prioritization of embryos with the highest chances of clinical success (31).

Consequently, the detection of mosaicism should not be an indication to avoid embryo transfer. Instead, it should serve as a tool to prioritize the transfer of the most capable embryos, as emphasized by Muñoz et al. (28). By refining our understanding and categorization of mosaic embryos, we can enhance clinical outcomes and offer couples a greater opportunity to achieve successful pregnancies.

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