REPRODUCTIVE POTENTIAL AND PLOIDY COMPARISON OF 5, 6, AND 7-DAY BLASTOCYSTS: AN ANALYSIS OF IMPLANTATION, CLINICAL PREGNANCY AND LIVE BIRTH RATES

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SUMMARY

Extended culture to the blastocyst stage has become a widely adopted practice in assisted reproductive technologies (ARTs), offering significant improvements in embryo selection and supporting single embryo transfer. Conventionally, the selection of viable blastocysts occurs on Days 5 and 6 of embryo culture, with embryos deemed unsuitable for transfer, biopsy, or cryopreservation after Day 6 being routinely discarded. However, a subset of embryos exhibits slower development, reaching the blastocyst stage on Day 7. Remarkably, Day 7 blastocysts possess the potential for viability, as they can attain top morphological grades, display euploidy, and ultimately lead to healthy live births. Unfortunately, the prevailing practice in most clinics involves terminating culture on Day 6, which may result in prematurely discarding viable Day 7 blastocysts. While Day 7 blastocysts constitute only 5% of the total viable blastocysts, those that meet the criteria for cryopreservation or biopsy hold significant clinical value. Culturing embryos for an additional day extends the window of opportunity, allowing for identifying more usable embryos per the in vitro fertilization (IVF) cycle. This approach is particularly advantageous for patients with limited numbers or lower-quality blastocysts, providing enhanced prospects for successful pregnancies.

Keywords: Day 7 blastocyst, slow-growing blastocyst, delayed embryo development, live birth rate, euploidy, aneuploidy, clinical pregnancy rate

INTRODUCTION

Embryo selection is a crucial factor in the success of assisted reproductive technology (ART) treatments. The development of advanced embryo culture media has revolutionized in vitro fertilization (IVF) by enabling the extended culture of embryos to the blastocyst stage, resulting in improved embryo selection and higher implantation rates.1, 2, 3, 4, 5, 6 Furthermore, advance-

ments in cryopreservation techniques and preimplantation genetic testing for aneuploidy (PGT-A) have enhanced the selection of euploid embryos for transfer, leading to improved IVF cycle outcomes.[7, 8, 9, 10, 11, 12, 13]

While traditionally, embryo culture has been halted on day 6, recent studies have shed light on the potential of day 7 blastocysts and their viability for transfer.[14, 15, 16, 17, 18, 19] Early studies suggested lower pregnancy and live birth rates for day 7 blastocyst transfer, leading to a reluctance to culture embryos beyond day 6.[20,21] However, more recent investigations have reported viable pregnancies and live births with day 7 blastocysts, providing an opportunity for patients who lack day 5 or 6 embryos to achieve pregnancy.[19]

Despite the growing interest in extended embryo culture, data on outcomes associated with culture beyond day 6 are limited. Questions arise regarding the impact of prolonged embryo culture on embryo quality, the window of blastulation, and the range of time during which normal embryo blastulation occurs.[22, 23, 24, 25, 26] Additionally, the balance between optimizing the number of usable blastocysts and the risk of discarding potentially viable embryos needs to be addressed.[27]

This review article explores the outcomes and developmental potential of day 7 blastocysts. Through a comprehensive analysis of studies that include biopsy or cryopreservation of day 7 blastocysts, several vital clinical questions will be addressed, including the incidence of usable day 7 blastocysts, rates of euploidy, implantation and pregnancy rates, potential mechanisms for delayed blastocyst development, strategies for clinical implementation, and recommendations for future studies.

RESULTS

Association Between Embryo Euploidy and Biopsy Time

„What is the reproductive potential of day 7 euploid embryos?“

Day 7 blastocysts have a significantly lower euploidy rate than day 5 and day 6 blastocysts, indicating a higher likelihood of aneuploidy. A comprehensive analysis of IVF cycles from a single academic center, which included 25,775 embryos undergoing trophectoderm biopsy and preimplantation genetic testing for aneuploidy (PGT-A), revealed that only 40.5% of day 7 blastocysts were euploid. In contrast, the euploidy rates were notably higher for day 5 blastocysts at 54.7% and day 6 blastocysts at 52.9%. After accounting for potential confounding factors such as maternal age, anti-Müllerian hormone levels, BMI, embryo quality, and the number of embryos biopsied, the significant association between aneuploidy and day 7 biopsied embryos compared to day 5 and day 6 was evident.

„Day 7 blastocyst euploidy supports routine implementation for cycles using preimplantation genetic testing.“

Despite the lower euploidy rate, day 7 blastocysts can still be used in cycles with preimplantation genetic testing, as they have shown potential to develop into viable euploid embryos, providing additional opportunities for successful in vitro development and live births. A study investigating single, euploid frozen embryo transfers (FET) found that day 7 blastocysts, though accounting

for only 6.6% of all biopsied blastocysts, exhibited a substantial implantation potential of 35.9%. This finding indicates that even with a lower euploidy rate, day 7 blastocysts can contribute to successful pregnancies when selected for transfer in the context of PGT-A.

„Correlation between aneuploidy, standard morphology evaluation and morphokinetic development in 1730 biopsied blastocysts: a consecutive case series study."

The study considered the impact of biopsy timing on blastocyst development and aneuploidy rates. Among the 1730 biopsied blastocysts, there were differences in euploidy rates based on the biopsy day. Specifically, 67.7% of blastocysts biopsied on day 5 were euploid, while only 52.1% and 43.1% of blastocysts biopsied on day 6 and day 7, respectively, were euploid (Day 5 versus Day 6: odds ratio (OR) 0.7, $P < 0.001$; Day 5 versus Day 7: OR 0.56, $P < 0.001$; Day 6 versus Day 7: OR 0.81, $P = 0.036$).

The results emphasize that the biopsy timing may influence the likelihood of obtaining euploid embryos. Additionally, the study found that aneuploidy risk increased with maternal age, with an approximate 10% increase in aneuploidy probability per year of maternal age (odds ratio (OR) = 1.1, $P < 0.001$).

„Earlier days of blastocyst development predict embryonic euploidy across all ages: essential data for physician decision-making and counseling patients."

Earlier blastocyst development is associated with a higher rate of embryonic euploidy, and this finding holds truth for both autologous and donor embryos. An extensive analysis of 2,132 biopsied blastocysts from 388 IVF cycles demonstrated a clear trend: blastocysts biopsied on days 5, 6, and 7 had euploidy rates of 49.5%, 36.5%, and 32.9%, respectively. This trend indicates that embryos biopsied at earlier stages of development have a higher likelihood of being euploid. Additionally, maternal age and the number of blastocysts biopsied per patient were identified as independent factors associated with a more significant percentage of euploidy, reinforcing the importance of considering these variables during embryo selection.

„Worth the wait? Day 7 blastocysts have lower euploidy rates but similar sustained implantation rates as Day 5 and Day 6 blastocysts."

The prevalence of euploidy decreased with increasing time to embryo blastulation, reinforcing the importance of considering blastocyst development timing during preimplantation genetic testing. The results showed that day 7 blastocysts had lower euploidy rates (43.1%) compared to day 5 (67.7%) and day 6 (52.1%) blastocysts. These findings suggest that embryos taking longer to reach the blastocyst stage are more likely to be aneuploid. The prevalence of euploidy decreased with increasing time to embryo blastulation, reinforcing the importance of considering blastocyst development timing during preimplantation genetic testing. Based on the research results regarding euploidy, aneuploidy, and PGT-A (preimplantation genetic testing for aneuploidy), it can be concluded that day 7 euploid embryos have a lower reproductive potential compared to day 5 and day 6 embryos. The rate of embryo euploidy is significantly lower in day 7 blastocysts, indicating that a higher proportion of embryos biopsied on day 7 are likely to be aneuploid. However, despite the lower euploidy rate, day 7 blastocysts can still support routine implementation for

cycles using preimplantation genetic testing, and the extended culturing to day 7 can lead to the development of viable euploid embryos that would have otherwise been discarded.

IMPLANTATION AND CLINICAL PREGNANCY RATE

After analyzing the results from multiple research studies, several important conclusions can be drawn regarding the clinical contribution and reproductive potential of poor-quality blastocysts (PQBs) and day 7 euploid embryos in assisted reproductive technologies:

„Looking past the appearance: a comprehensive description of the clinical contribution of poor-quality blastocysts to increase live birth rates during cycles with aneuploidy testing."

Poor-quality blastocysts (PQBs) have proven to contribute to an impressive 12.4% increase in cycles leading to at least one live birth (LB) during aneuploidy testing. Nevertheless, it is essential to note that PQBs display slower development rates compared to their non-PQB counterparts. On average, each cycle yielded 0.7 ± 0.9 PQBs for biopsy, of which 0.2 ± 0.4 were euploid. The prevalence of PQBs is influenced significantly by maternal age, with women over 42 years benefiting more from these embryos. Intriguingly, 18 women achieved their only live births thanks to PQBs. Furthermore, patients with limited or no sibling non-PQBs also experienced higher success rates in achieving successful pregnancies through PQBs.

„Cryopreserved embryo transfers suggest that endometrial receptivity may contribute to reduced success rates of later developing embryos."

Observations from research on cryopreserved embryo transfers suggest that endometrial receptivity might reduce success rates for later developing embryos. Clinical pregnancy rates (PRs) were comparable between blastocysts cryopreserved on day 5 and day 6 (32% vs. 28%). However, blastocysts cryopreserved on day 7 displayed a lower clinical PR (15%). Although this difference initially appeared significant after accounting for the number of embryos per transfer, statistical analysis showed that the discrepancy was not statistically significant (P = 0.15).

„What is the reproductive potential of day 7 euploid embryos?"

The analysis of the reproductive potential of day 7 euploid embryos revealed significant distinctions among cohorts in terms of implantation, clinical pregnancy, live birth, and clinical loss rates. Compared to day 5 and day 6 embryos, day 7 biopsied embryos exhibited decreased odds of implantation, clinical pregnancy, and live birth. The implantation rate for day 5 blastocysts – 65.4%, day 6 – 56,2%, day 7 – 30.1%. However, no significant association was observed with clinical loss or multiple pregnancy rates in patients utilizing day 7 embryos during treatment.

„Worth the wait? Day 7 blastocysts have lower euploidy rates but similar sustained implantation rates as Day 5 and Day 6 blastocysts."

Despite this lower euploidy rate, day 7 euploid embryos displayed a sustained implantation rate (SIR) similar to day 5 and day 6 embryos. For euploid single embryo transfers (SET), the SIR for day 5 and day 6 embryos was approximately 68.9% and 66.8%, respectively. Although day 7 euploid SET showed a slightly lower SIR at 52.6%, this difference did not reach statistical signifi-

cance. The collective research findings emphasize the positive clinical contribution of poor-quality blastocysts in increasing live birth rates during cycles with aneuploidy testing, particularly benefiting women over 42 years old and those with limited sibling non-PQBs. However, it is crucial to acknowledge that PQBs display slower development and lower euploidy rates than nonPQBs. Day 7 euploid embryos, despite their lower euploidy rates, exhibit comparable sustained implantation rates as day 5 and day 6 embryos. Considering these factors is vital for assisted reproductive technology clinics to optimize success rates when selecting and transferring blastocysts for their patients.

LIVE BIRTH RATE

„What is the reproductive potential of day 7 euploid embryos?“

A sub-analysis of 3824 single, euploid frozen embryo transfer (FET) cycles was conducted, classifying them based on the day of blastocyst development: day 5 (n = 2321 cycles), day 6 (n = 1381 cycles), and day 7 (n = 116 cycles). Significant differences were observed among these groups in implantation, clinical pregnancy, live birth (LB), and clinical loss rates. The odds of implantation, clinical pregnancy, and LB were significantly lower in patients who utilized day 7-biopsied embryos during treatment. The live birth rates for day 5, day 6, and day 7 groups were 56.4%, 45,8%, and 21.5%, respectively.

„Worth the wait?

Day 7 blastocysts have lower euploidy rates but similar sustained implantation rates to Day 5 and Day 6 blastocysts“ Day 7 blastocysts have lower live birth rates than Day 5 and Day 6 blastocysts. Although they offer viable euploid embryos that would have otherwise been discarded, the extended culture to Day 7 is associated with increased miscarriage rates. The live birth rates for Day 7 blastocysts were significantly lower at 43.8% compared to 67.4% and 77.2% for Day 5 and Day 6 blastocysts, respectively.

„Euploid day 7 blastocysts of infertility patients with only slow embryo development have reduced implantation potential.“

In this study, a total of 2966 women underwent single euploid FET using embryos that reached blastulation on either day 5 (n = 1880), day 6 (n = 986), or day 7 (n = 100). The results showed that day 7 embryos had significantly lower implantation and live birth rates compared to both day 5 and day 6 embryos ($P < 0.001$). The live birth rates for the day 5, day 6, and day 7 groups were 68.5%, 55.2%, and 36.0%, respectively. Furthermore, the day 7 group was older than the day 5 group ($P < 0.001$). Even after comparing age-matched cohorts, the day 7 group still exhibited lower implantation and live birth rates ($P < 0.0001$ and $P < 0.001$, respectively). Interestingly, the study found that the live birth rates were not influenced by embryo grade.

CONCLUSION

Maternal age, culture medium, blastocyst expansion time, and biopsy timing influence the prevalence of euploidy, implantation potential, and live birth rates. These findings highlight the im-

portance of considering extended culture and blastocyst development on Day 7 in ART practice. Day 7 blastocysts can be of high quality and genetically normal. They can potentially result in healthy live births following frozen embryo transfer. However, further research is needed to determine the success rates and live birth rates of Day 7 blastocysts, considering factors such as blastocyst grade, ploidy result, and patient age. Further research is needed to accurately define the Day 7 blastocysts' success rates and incorporate them into clinical practice. It is recommended to consider extended culture to Day 7, especially for embryos with early or borderline grades on Day 6, as it may increase the live birth rate per cycle. Additionally, the management of Day 7 blastocysts should involve setting patient expectations and considering individual patient characteristics.

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CONFLICT OF INTEREST

None declared.

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