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Day 1 versus Day 2 Intracytoplasmic Sperm Injection: Comparison of Fertilization and Embryo Development Outcomes in IVF Patients

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Abstract

Background: Intracytoplasmic sperm injection (ICSI) has become an essential assisted reproductive technology for overcoming male-factor infertility and improving fertilization in in vitro fertilization (IVF) cycles. Although ICSI is commonly performed on the day of oocyte retrieval after mature metaphase II (MII) oocytes are identified, immature oocytes may undergo in vitro maturation (IVM) and be injected on the following day. The influence of delayed ICSI on embryological and clinical outcomes remains controversial, with inconsistent findings among previous studies.

Objective: To compare fertilization rate, embryo quality, and pregnancy outcomes between Day 1 and Day 2 ICSI following oocyte retrieval in IVF patients.

Methods: A retrospective observational study was conducted using medical records of IVF patients who underwent ICSI at Tiara IVF Clinic, Graha Amerta, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, between 2018 and 2025. Seventy-two eligible IVF cycles were included, consisting of 48 Day 1 ICSI cycles and 24 Day 2 ICSI cycles. Fertilization rate was classified according to laboratory performance indicators, while embryo quality was evaluated using cleavage rate and blastocyst rate. Pregnancy outcome was determined by serum β-human chorionic gonadotropin (β-hCG). Comparisons between groups were performed using the Chi-square test or Fisher's exact test, with statistical significance defined as $p < 0.05$.

Results: No significant difference was observed in fertilization rate between Day 1 and Day 2 ICSI groups ($p = 0.474$). Cleavage-stage embryo quality was significantly better in the Day 1 ICSI group than in the Day 2 group ($p = 0.001$). Blastocyst rate could not be statistically compared because no blastocyst data were available for the Day 2 group; therefore, this outcome was reported descriptively. Among Day 1 ICSI cycles, 32 of 48 embryos (66.7%) developed into good-quality blastocysts. Pregnancy rate was significantly higher in the Day 1 ICSI group than in the Day 2 group ($p = 0.001$).-

Conclusion: Day 1 ICSI was associated with superior cleavage-stage embryo quality and higher pregnancy rates compared with Day 2 ICSI, whereas fertilization rate was comparable between groups. The effect of ICSI timing on blastocyst development could not be determined because of the absence of blastocyst data in the Day 2 group. Larger prospective studies are warranted to confirm these findings.

Keywords: ICSI; IVF; Embryo quality; Fertilization; Pregnancy rate

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1. Introduction

Infertility is a major global health problem affecting approximately 10–15% of reproductive-aged couples worldwide and has substantial medical, psychological, and socioeconomic consequences. Assisted reproductive technology (ART), particularly in vitro fertilization (IVF), has significantly improved the management of infertility by enabling fertilization outside the human body. Since the birth of the first IVF baby in 1978, continuous advances in laboratory techniques have markedly improved fertilization, embryo development, implantation, and live birth rates.

Intracytoplasmic sperm injection (ICSI) is one of the most important developments in ART. Initially introduced to overcome severe male-factor infertility, ICSI is now widely applied in IVF cycles for various indications, including previous fertilization failure, diminished oocyte quality, unexplained infertility, and the use of surgically retrieved sperm. During ICSI, a single spermatozoon is injected directly into the cytoplasm of a mature metaphase II oocyte, thereby bypassing many natural barriers to fertilization and increasing the likelihood of successful fertilization.

The timing of ICSI following oocyte retrieval has attracted increasing attention. Conventionally, mature oocytes are inseminated on the same day as oocyte retrieval (Day 1). However, some retrieved oocytes remain immature at retrieval and may complete maturation through short-term in vitro maturation (IVM). These oocytes may subsequently undergo ICSI on the following day (Day 2). Although delayed ICSI allows additional mature oocytes to be utilized, prolonged incubation before fertilization may expose oocytes to post-maturation aging (oocyte aging), which has been associated with mitochondrial dysfunction, spindle abnormalities, oxidative stress, chromosomal instability, and reduced developmental competence.

Previous studies investigating the impact of delayed ICSI have reported inconsistent results. Several investigators have demonstrated that delayed fertilization adversely affects embryo development, implantation, and pregnancy outcomes, whereas others found no significant differences when oocytes reached adequate nuclear maturation before fertilization. These discrepancies may be attributable to differences in patient characteristics, laboratory protocols, embryo culture conditions, and definitions of delayed insemination.

Although delayed ICSI is occasionally performed in clinical practice for oocytes that mature after retrieval, evidence regarding its influence on embryological and clinical outcomes remains limited, particularly in Indonesia. Furthermore, data from Southeast Asian IVF centers are scarce. Understanding whether the timing of ICSI affects fertilization, embryo quality, and pregnancy outcomes is important for optimizing laboratory practice and maximizing IVF success.

Therefore, this study aimed to compare fertilization rate, embryo quality, and pregnancy outcomes between Day 1 and Day 2 ICSI among IVF patients treated at Tiara IVF Clinic, Graha Amerta, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

2. Methods

This study was a comparative analytic study with a cross-sectional design using secondary data from medical records. The study aimed to compare fertilization outcomes, embryo quality, and pregnancy rates between intracytoplasmic sperm injection (ICSI) performed on day 1 and day 2 after oocyte retrieval.

The study was conducted at Tiara IVF Clinic, Graha Amerta, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, from patients undergoing IVF/ICSI treatment during the period 2018–2025.

A total of 72 IVF patients were included in this study. The sample consisted of 48 patients in the day 1 ICSI group and 24 patients in the day 2 ICSI group, selected based on inclusion and exclusion criteria using proportional random sampling for the day 1 group and total sampling for the day 2 group.

The variables analyzed included fertilization rate, embryo quality (cleavage rate and blastocyst rate), and clinical pregnancy rate. Fertilization and embryo quality outcomes were categorized into high and low/ good and poor based on predefined clinical cut-off values.

Statistical analysis was performed using the Chi-square test, and when expected cell counts were less than 5, the Fisher's exact test was applied. A p-value of <0.05 was considered statistically significant.

This study had obtained ethical approval from the Health Research Ethics Committee of Dr. Soetomo General Academic Hospital, Surabaya (Ethical clearance number: 2160/LOE/301.4).

2.1. Results

Table 1 Comparison of fertilization rate, embryo quality, and pregnancy outcomes between Day 1 and Day 2 ICSI groups

Variable	Day 1 ICSI (n=48)	Day 2 ICSI (n=24)	p-value
Fertilization Rate			
High Fertilization	14 (29.2%)	9 (37.5%)	0.474
Low Fertilization	34 (70.8%)	15 (62.5%)	
Embryo Quality – Cleavage Rate			
Good	36 (75.0%)	8 (33.3%)	0.001
Poor	12 (25.0%)	16 (66.7%)	
Embryo Quality – Blastocyst Rate			
Good	32 (66.7%)	0 (0.0%)	
Poor	16 (33.3%)	0 (0.0%)	
Pregnancy Outcome			
Positive	24 (50.0%)	1 (4.2%)	0.001
Negative	24 (50.0%)	23 (95.8%)	

3. Discussion

This study compared fertilization outcomes, embryo quality, and pregnancy rates between patients undergoing intracytoplasmic sperm injection (ICSI) on the first and second day after oocyte retrieval. The findings demonstrated that delaying ICSI until the second day did not significantly affect fertilization rates but was associated with significantly poorer embryo quality and lower pregnancy rates. These findings suggest that although delayed-matured oocytes retain the capacity for fertilization, their developmental competence appears to be compromised during subsequent embryogenesis.

The absence of a significant difference in fertilization outcomes between the two groups indicates that additional in vitro maturation may allow immature oocytes to complete nuclear maturation sufficiently for successful fertilization. Previous studies have shown that immature oocytes collected during ovarian stimulation can resume meiosis and reach the metaphase II stage after short-term culture, thereby maintaining acceptable fertilization potential following delayed ICSI. Fertilization mainly reflects successful sperm incorporation, pronuclear formation, and activation of the oocyte, processes that may remain intact despite delayed maturation. Consequently, fertilization alone may not accurately represent the developmental potential of the resulting embryos.

Despite comparable fertilization rates, embryo quality differed markedly between the two groups. Patients undergoing first-day ICSI exhibited significantly higher cleavage and blastocyst formation rates than those undergoing second-day ICSI. Notably, no good-quality blastocysts were observed in the second-day ICSI group. These findings indicate that although delayed-matured oocytes may achieve normal fertilization, they possess reduced developmental competence during subsequent embryonic development.

One possible explanation is the dissociation between nuclear and cytoplasmic maturation. While oocytes may complete meiotic maturation during extended culture, cytoplasmic maturation—which includes mitochondrial redistribution, accumulation of maternal messenger RNA, protein synthesis, calcium homeostasis, and organelle reorganization—may remain incomplete. Inadequate cytoplasmic maturation can impair early embryonic cleavage, genome activation, and blastocyst development despite apparently normal fertilization. Furthermore, prolonged culture before fertilization may expose oocytes to oxidative stress and metabolic alterations that adversely affect mitochondrial function and DNA integrity, ultimately reducing embryonic developmental potential.

The significantly lower pregnancy rate observed in the second-day ICSI group is consistent with the poorer embryo quality identified in this study. Embryo quality remains one of the strongest predictors of implantation and pregnancy success in assisted reproductive technology. Embryos derived from delayed-matured oocytes may exhibit impaired implantation potential because of suboptimal cytoplasmic maturation, mitochondrial dysfunction, or chromosomal instability acquired during prolonged culture. Consequently, although fertilization can still occur, the probability of achieving an ongoing pregnancy appears substantially reduced.

These findings are consistent with previous reports indicating that delayed ICSI or rescue maturation of immature oocytes may preserve fertilization capability but often results in inferior embryo development and lower reproductive outcomes. Studies evaluating in vitro matured oocytes have similarly demonstrated that embryo developmental competence and implantation rates remain lower than those achieved using oocytes that reach metaphase II at the time of oocyte retrieval. Collectively, these observations support the concept that fertilization should not be considered the sole indicator of treatment success, as embryo quality and pregnancy outcomes provide more clinically meaningful measures of reproductive competence.

From a clinical perspective, the present findings suggest that first-day ICSI should remain the preferred strategy whenever mature oocytes are available. Nevertheless, delayed ICSI may still serve as a rescue option for immature oocytes that subsequently achieve maturation during short-term culture, thereby maximizing oocyte utilization in selected patients. Clinicians should, however, counsel patients that although delayed ICSI may increase the number of fertilized oocytes, the likelihood of obtaining good-quality embryos and achieving pregnancy appears lower than with conventional first-day ICSI.

Several limitations should be acknowledged. First, the retrospective cross-sectional design limits causal inference. Second, this was a single-center study with a relatively small number of patients undergoing second-day ICSI, potentially limiting statistical power and external validity. Third, several important confounding variables, including maternal age, body mass index, ovarian reserve, infertility etiology, sperm quality, ovarian stimulation protocols, and endometrial characteristics, were not evaluated separately. In addition, pregnancy outcome was assessed using positive serum β-hCG rather than clinical pregnancy or live birth, which represent more comprehensive indicators of reproductive success. Future prospective multicenter studies with larger sample sizes are warranted to validate these findings and investigate the biological mechanisms underlying the reduced developmental competence of delayed-matured oocytes.

Overall, this study demonstrates that delaying ICSI until the second day after oocyte retrieval does not significantly compromise fertilization but is associated with significantly poorer embryo quality and lower pregnancy rates. These findings support the current clinical practice of performing ICSI on the first day whenever possible to optimize embryo development and improve reproductive outcomes.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Statement of ethical approval

This study was approved by the Health Research Ethics Committee of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (Ethical Clearance No. 2160/LOE/301.4).

Statement of informed consent

The requirement for informed consent was waived by the Health Research Ethics Committee because this retrospective study used anonymized medical record data.

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